

Waters[®] Xevo[®] TQ-S

Installation Manual

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Introduction

This document is designed to aid a qualified Waters Field Service Engineer (FSE) to carry out a full installation of a Xevo TQ-S ACQUITY UPLC, system at a customer site.

The customer must have completed the [Xevo TQ-S Site Preparation Guide](#) (715002440) before the instrument was delivered. A completed copy of the site preparation guide must be sent to the local Waters office before the installation can commence. The engineer must confirm that these requirements are met before unpacking the crates.

The [Xevo TQ-S ACQUITY UPLC System Installation Checklist](#) (715002460) must be completed during the course of the installation. If the qualification option has been purchased then the installation checklist need not be completed but instead the *Installation Qualification* (715002207) and an *Operational Qualification* (715002208) documents must be completed instead. The customer training section of the installation checklist must be performed on all installations.

NOTE: The [Xevo TQ-S ACQUITY UPLC System Installation Checklist](#) (715002460) contains the Xevo TQ-S and ACQUITY UPLC installation checklists and specifications. There is no need to complete the [ACQUITY UPLC System Installation Checklist](#) (715000877) when installing a Xevo TQ-S with ACQUITY UPLC.

At the end of the installation, the instrument must be signed off using the final page of the installation checklist. A copy of this and the front page must be faxed/mailed to the local Waters subsidiary.

The installation time for the Xevo TQ-S is estimated at between five to seven days. This depends on the options purchased and includes approximately two days for customer training.



Figure 1 - Xevo TQ-S

Useful information and contacts

Global Services Support (GSS) individuals are available for technical assistance.

Toll-free numbers are available for contacting GSS Wilmslow during office hours (7:45 to 17:15 GMT):

USA and Canada	800 887 0874
France	0800 902 342
Italy	0800 979 283
Sweden	0207 99 639
United Kingdom	0800 345 7521
Germany	0800 181 4899
Spain	9008 12 173

Outside these countries call the Wilmslow MSHQ switchboard +44 (0) 161 946 2400.

CAUTION: *These numbers are to be used by engineers only and must not be given to customers.*

Startup procedures

The startup procedure in the [Xevo TQ-S ACQUITY UPLC System Installation Checklist](#) (715002460) details the basic steps required to set up the instrument, and the tests an FSE must demonstrate to ensure system performance. The installation checklist is supplied with the instrument, or is available from Service Assist on the Portal.

Site preparation

The Xevo TQ-S requires specific laboratory facilities that must be in place prior to the installation commencing. These prerequisites are detailed in the [Xevo TQ-S Site Preparation Guide](#) (715002440) which is available from the Waters Service Knowledge Center. The local Waters office is responsible for ensuring that the customer has satisfied the criteria within this document, and that a signed copy of this document is returned to the relevant organization.

System startup kits

- Instrument Spares Kit (289005829)
- Installation Kit (289005827)
- Source Spares Kit (205000534)
- ESI Probe Spares Kit (205000530)
- Chemical Kit (700005472)

In addition to the items above, the Xevo TQ-S includes several consumables spares kits.

Customer training

Initial customer training will take approximately two days and covers basic operation, familiarity with the instrument, cleaning, and basic maintenance. The Installation Checklist contains a series of customer training topics that must be acknowledged by the customer as they are covered. Applications training can be requested and will be performed after the installation by a local, regional, or global Waters applications specialist.

Checking the sales order

It is important that the contents of the crates are examined and are checked against the sales order. Any shortages must be reported to the local service manager and GSS immediately. The sales order can be obtained from the local manager or from GSS in Wilmslow.

CAUTION: *Ensure the source and backing pump options selected by the customer have been supplied.*

Chemicals

Performing the installation specifications requires use of the Xevo TQ-S Sample Kit (700005472), which includes a set of instructions (715002796) for preparing the required standard solutions. This kit ensures the required chemical compounds are available during the installation.

To prepare the test samples for TUV and PDA detector tests, use the Waters ACQUITY UPLC MS Startup Solution Kit (700002741).

The customer is required to supply LC/MS grade solvents (see note below), these requirements are detailed in the [Xevo TQ-S Site Preparation Guide](#) (715002440).

NOTE: Refer to [Controlling Contamination in Ultra Performance LC/MS and HPLC-MS Systems](#) (715001307) for more detail on solvent selection.

Waters cannot provide certification for the chemical kit. If this information is required for the customer's own validation procedures, then they must purchase the required samples prior to the installation commencement.

Unpacking the instrument

The Xevo TQ-S is delivered in a number of crates/boxes. The largest crate contains the instrument and installation kit, and has a length of 1210 mm (47.7 inch), a depth of 830 mm (32.7 inch) and a height of 1230 mm (48.5 inch). The gross weight of the instrument box is approximately 230 kg (507 lbs). The net weight of the Xevo TQ-S is approximately 160 kg (353 lbs).

The pumps ship in two smaller crates. Other boxes contain peripheral items such as the data systems and source options. The large crate contains the instrument, and must be unpacked according to the instructions below.

Read through the instructions before beginning to dismantle the crate.

Unpacking the transport crates

CAUTION: *It is essential that the customer provides suitable equipment for transporting and lifting the instrument. The installation cannot be implemented unless this equipment is made available. The engineer will require assistance moving, lifting and positioning the instrument.*

1. Check all shock and tilt watchers.
2. Perform a visual check to ensure there is no other damage to the packaging.
3. If any watchers have turned red or there is any damage to the packaging, report to the local service manager and GSS before progressing any further.
4. Carefully remove the packaging and perform a visual check to ensure there is no damage to the packaging or instrument.



Figure 2 - Unpacking the instrument

NOTE: Refer to the [ACQUITY UPLC System Configuration Guide \(715001701\)](#) for recommended layouts of systems with other ACQUITY UPLC options.

5. Unpack the instrument and any other equipment.

WARNING: *To avoid injury, at least two people must lift the rotary pump and place it on the floor.*

Moving the instrument

WARNING: *The net weight of the Xevo TQ-S is approximately 160 kg (353 lbs). Take great care whenever attempting to move or lift the instrument.*

The instrument must only be moved and lifted using suitable equipment capable of handling the equipment safely, with the harness supplied with the instrument. The instrument must not be lifted manually. The lifting equipment employed must be capable of raising the instrument to the same height as the bench on which it is to be located.

1. Using the harness supplied, lift the Xevo TQ-S onto a flat bench (preferably one that is movable).
2. Ensure 150 mm (6 inch) minimum clearance behind the instrument for service access and ventilation, see [Figure 3](#).
3. If installing the sample organiser, use the Chassis Height Adjustment Kit (289004491) to adjust the height of the instrument.
4. Plug the shipped 2.5 m (8 ft) power cord into the rear of the chassis.
5. Install the external roughing pump on a level surface, underneath or behind the instrument, and within 1.5 m (5 ft) of the rear of the instrument chassis.

CAUTION: *To prevent overheating it is not recommended that the external pump be installed behind closed doors. It is recommended that the external pump be elevated 150 to 200 mm (6 to 8 inch) above the floor to provide easy access during routine maintenance, for example, changing the pump oil.*

NOTE: *If the external pump is positioned under the instrument bench, it may be necessary to cut an access slot (diameter 76 mm, 3 inch) in the bench top to allow the tubing to be passed through to the instrument ([Figure 3](#)).*

The access slot must be positioned outside the footprint. Connect the right angle fittings supplied with the system to route the vacuum hoses straight down from the rear of the instrument; the vacuum tubes must not be pinched or kinked or stressed in any way.

There must be no less than 150 mm (6 inch) space behind the instrument.

At least 225 mm (9 inch) additional clearance is required at the top of the UPLC system to allow for solvent bottles. The height of the UPLC system is based on a standard system; see the [ACQUITY UPLC Site Preparation Guide](#) (715000878) for further details.

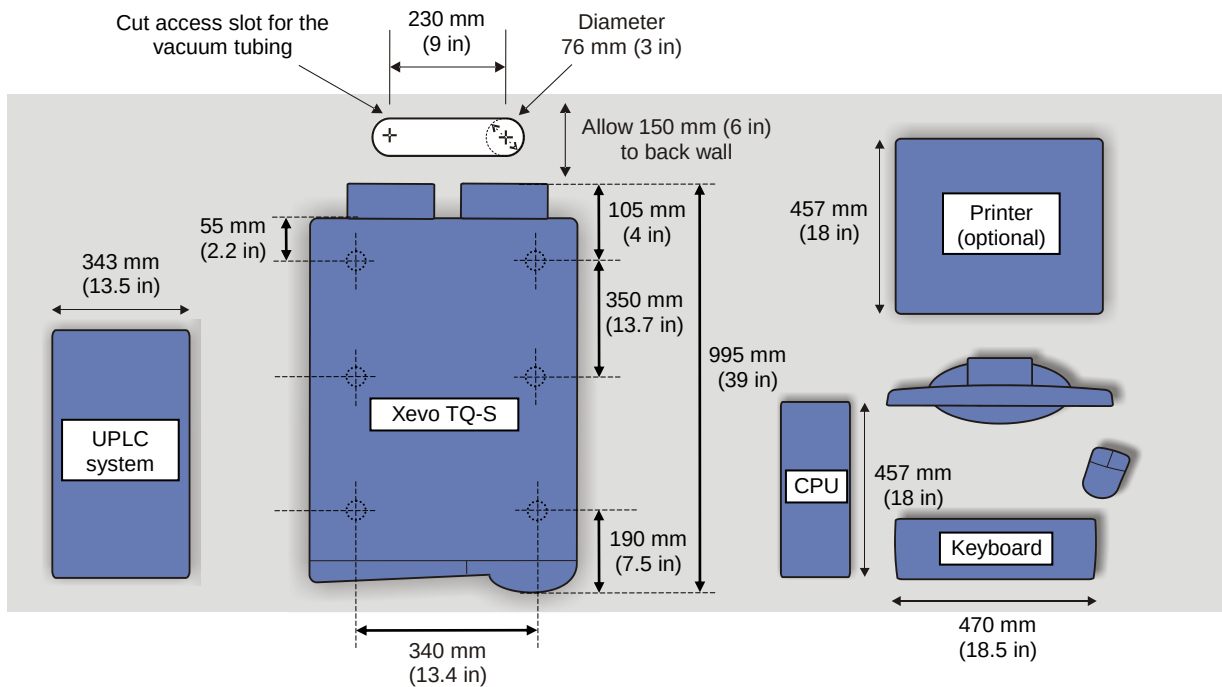


Figure 3 - Plan view, showing space requirements

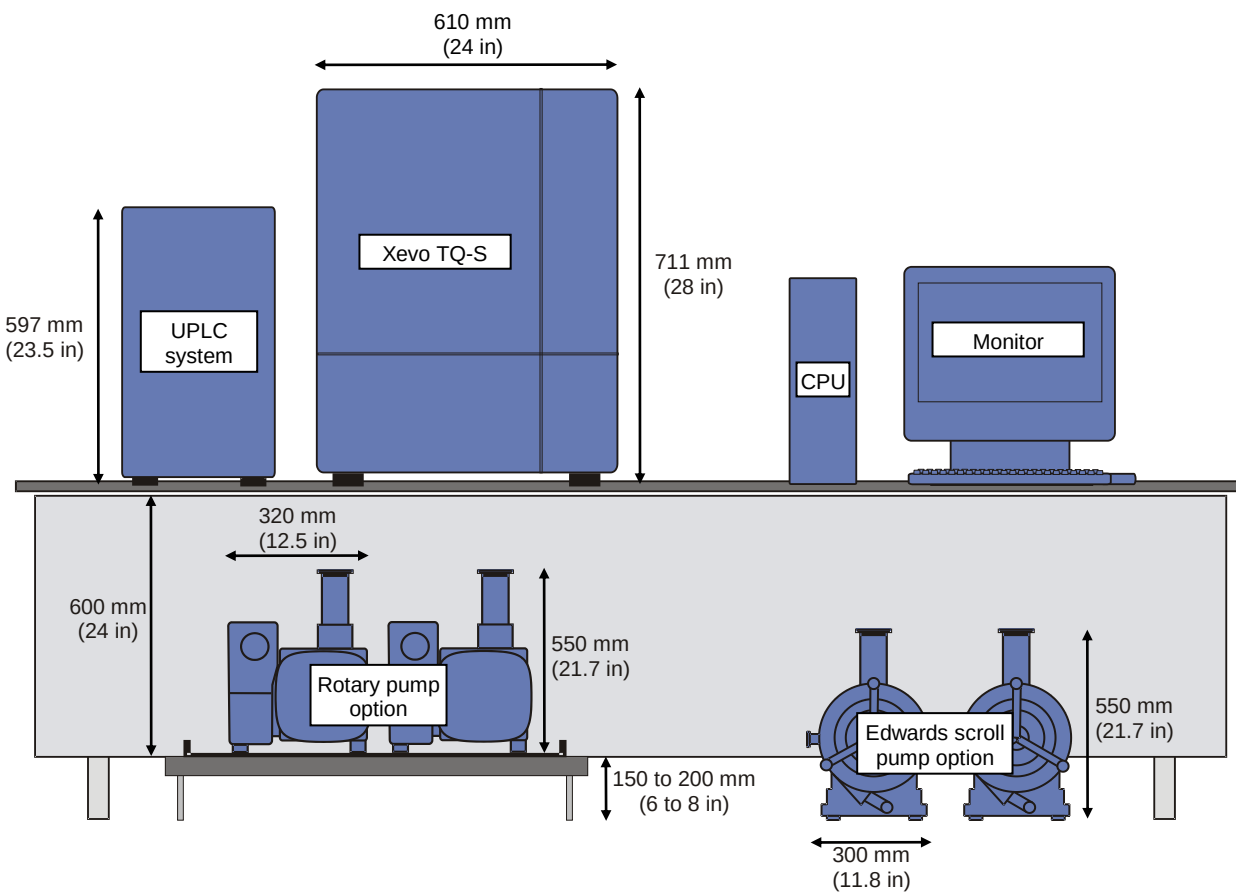


Figure 4 - Front view, showing space requirements (initial configuration)

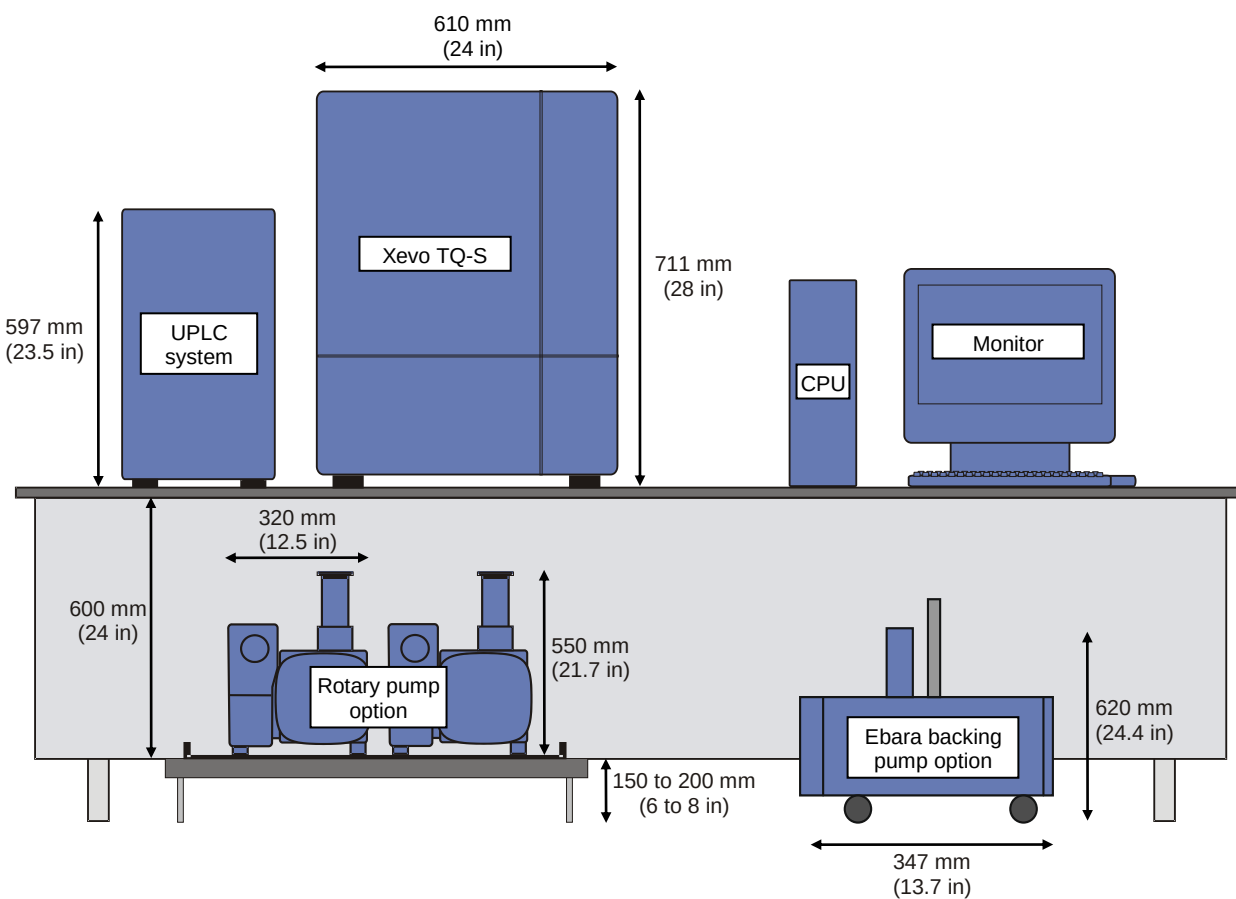


Figure 5 - Front view, showing space requirements (current configuration)

Instrument setup

Rear panel connections

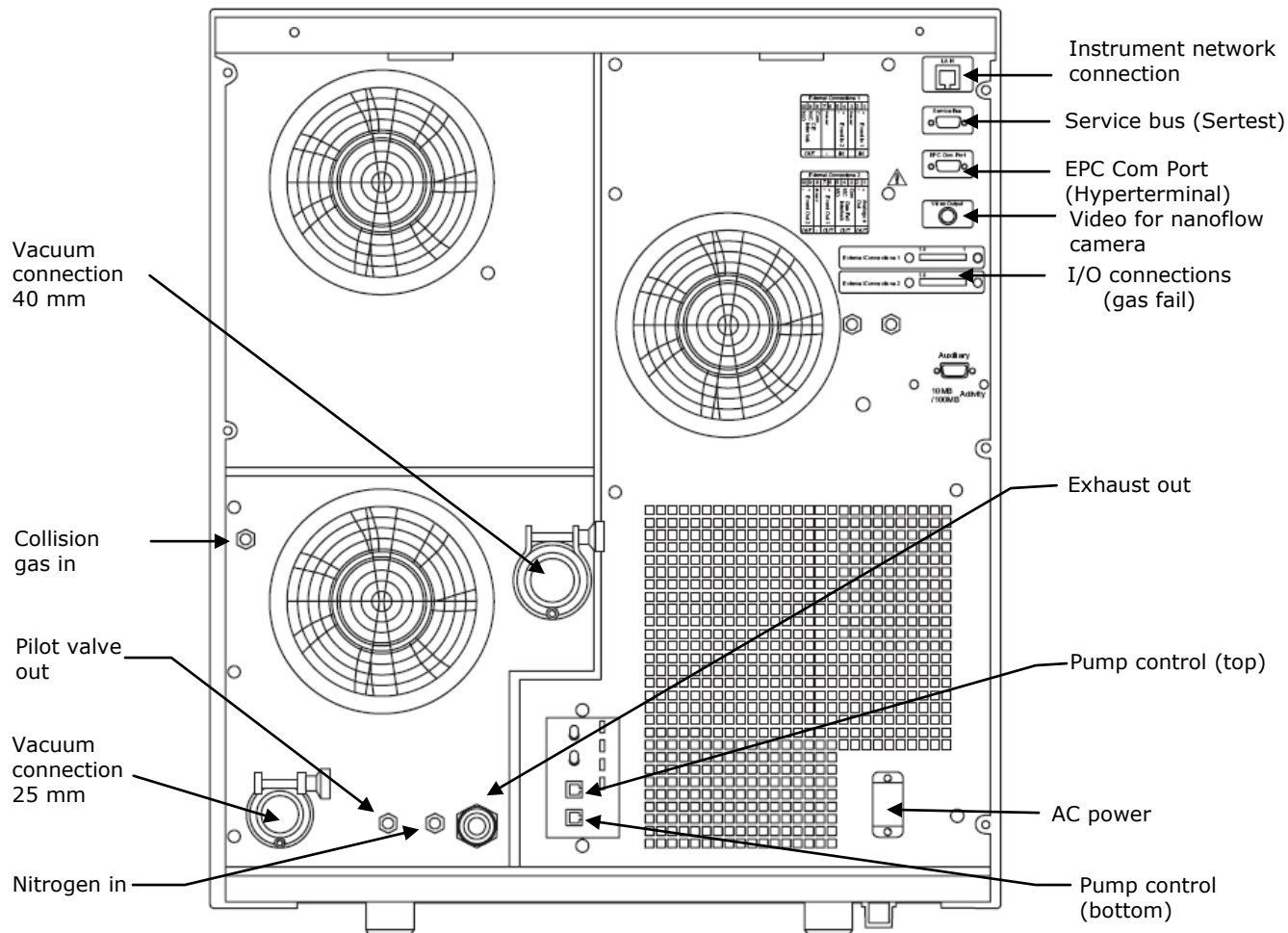


Figure 6 - Rear panel connections

CAUTION: When positioning the instrument, ensure that the cooling fans are not blocked.

Connecting the fluidics waste line

1. Connect the waste fitting (located under the instrument) to a suitable waste container.
2. Slide the waste line over the barbed fitting of the drain located at the bottom of the instrument.

CAUTION: *To avoid distorting the drip tray or causing the drain cup to leak, support the drain cup when attaching or removing the waste line.*



Figure 7 - Liquid waste line connections

3. Perform the following procedure to avoid fluid backup and ensure proper drainage of waste:
 - a. Place the waste container below the instrument.
 - b. Ensure that the drain tubes do not crimp or bend.
 - c. Ensure the exit of the drain tube is not covered by waste solvent.

NOTE: *If necessary, shorten the waste tube so that no section drops below the top of the waste container.*

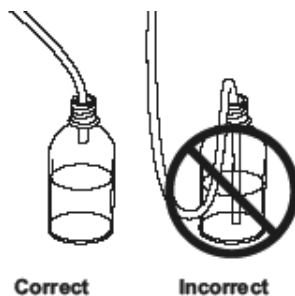


Figure 8 - Waste container connection

Connecting the instrument exhaust line

The source waste on the instrument rear panel is used to vent the source gases and must be connected to a suitable extraction system through the waste bottle (see below). This waste system comprises a single continuous waste line between the source and the source waste outlet on the instrument rear panel which connects directly to a solvent waste trap bottle assembly.

1. Connect the bottle to the 12-mm source exhaust port on the instrument rear panel.
2. Connect the pilot valve on the waste bottle to the instrument with 4-mm PTFE tubing.
3. Connect the outlet of the pilot valve to the laboratory exhaust system, using the 10-mm PTFE tubing.
4. Place the exhaust bottle in the protective bottle stand.

WARNING: *The active exhaust vent must provide a minimum vacuum of 2 mbar below atmospheric pressure (negative pressure). It must be capable of supporting a maximum instrument exhaust gas load of 2000 L/hour. Failure to provide the specified exhaust system will cause the source pressure test to fail.*

The source exhaust line must be connected to either a laboratory fume hood or an active exhaust system.

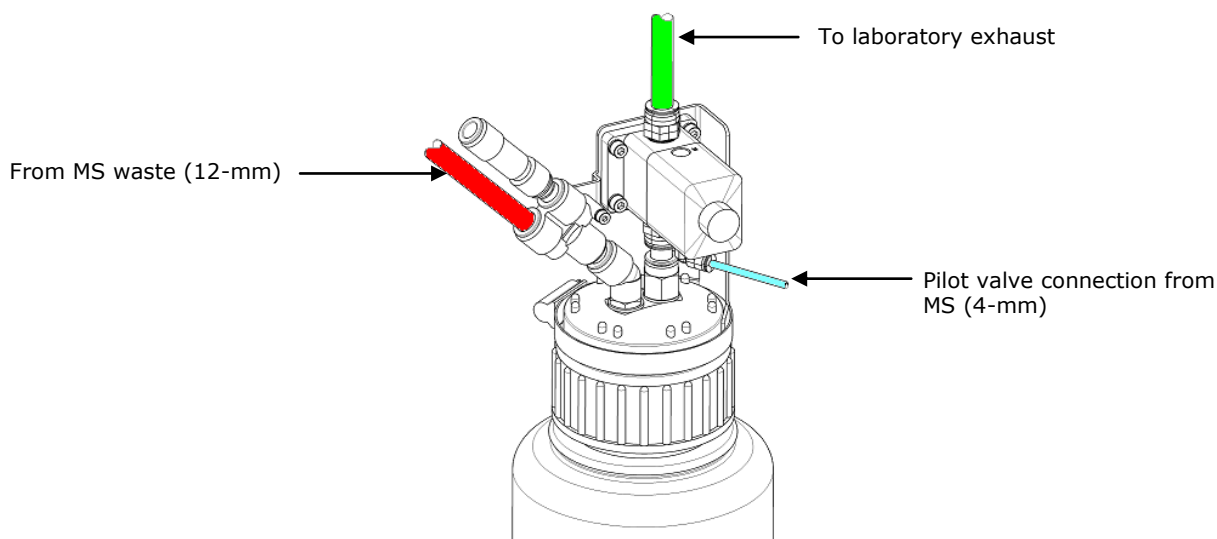


Figure 9 - Source exhaust and the vent exhaust system

CAUTION: *Severe contamination of the instrument may result if the source exhaust line is connected to the rotary pump exhaust line. The damage will occur when the nitrogen supply is turned off or when the nitrogen runs out, as any rotary pump oil vapor will migrate through the source exhaust to the ion source and through the sample cone into the analyzer.*

Source exhaust tubing kinks very easily, causing variable pressure and source pressure errors. Use 12-mm 90-degree elbows (700005020) to improve the flow of the tubing around bends.

Setting up the external pumps

The Xevo TQ-S currently ships either with two Leybold SV65BI pumps, or with one Ebara EV-SA20 dry backing pump. The Xevo TQ-S initially shipped either with two Leybold SV65BI pumps, or with Edwards XDS35i and XDS100B scroll pumps.

Follow the appropriate procedure to suit the configuration on site.

CAUTION: *To avoid damage to the instrument, it is critical that the external backing pumps are configured correctly for your instrument. Refer to the relevant documentation for details on setting up the pumps.*

Table 1: Xevo TQ-S pump configurations

Configuration	Documentation
Ebara	"Systems using Ebara EV-SA20 dry backing pump"
Leybold	"Systems using rotary pumps (2 x Leybold SV65BI)"
Edwards	"Systems using scroll pumps (Edwards XDS35i and XDS100B scroll pumps)"

Systems using Ebara EV-SA20 dry backing pump

WARNING: *The Ebara pump is very heavy. The pump must only be lifted using lifting equipment capable of raising the pump's weight safely. The pump must not be lifted manually.*

Where it is necessary to install an external Ebara pump away from the instrument, a 3-meter vacuum hose extension kit is available (700011268). The 3-meter vacuum hose variant comes pre-assembled with the pump control cable and is a direct replacement for the 1.5-meter variant.

CAUTION: *Do not use the 3-meter vacuum hose in conjunction with the standard hose provided in the installation kit.*

1. Unpack the pump (Figure 10).

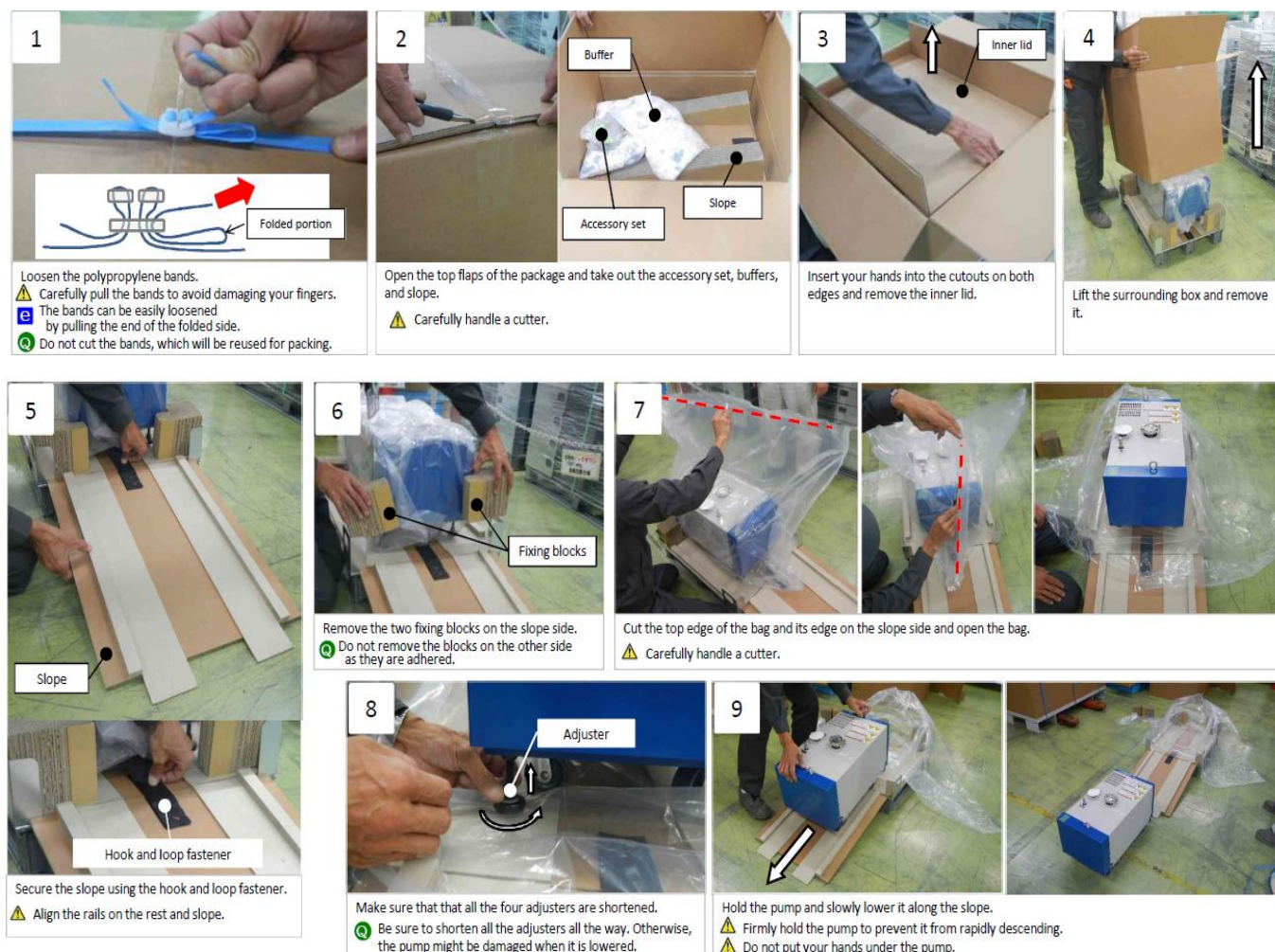


Figure 10 - Unpacking the Ebara pump

2. Position the pump on a surface that is level to within ± 1 degree in any direction.
3. Ensure the instrument backing control switches are set to Automatic and electronics off.

4. Connect the filter to the exhaust port on the pump (Figure 11).

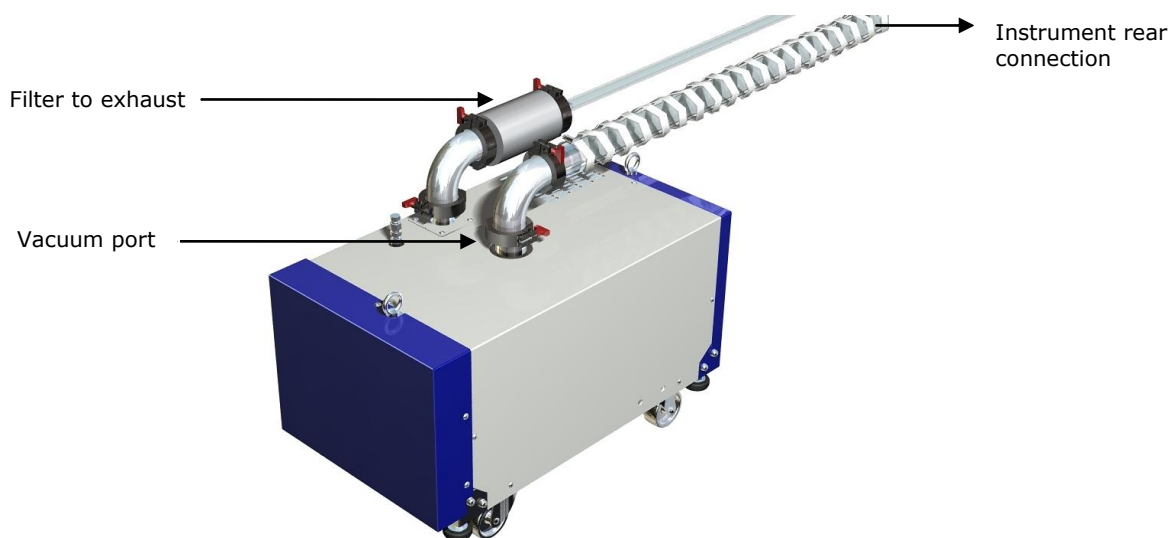


Figure 11 - Backing pump and vacuum connections

5. Add the barbed fitting to the filter and secure the 19-mm PVC waste hose using a jubilee clip and place in a suitable air-extraction system.

WARNING: For EMC compliance reasons, the vacuum hose must be connected to the pump with the grounding fasten tab attached to the MS rear panel (Figure 12).

6. Remove the bottom M3 screw from the backing control PCB.
7. Using the M3 screw, fit the grounding fasten tab to the backing control PCB (Figure 12).

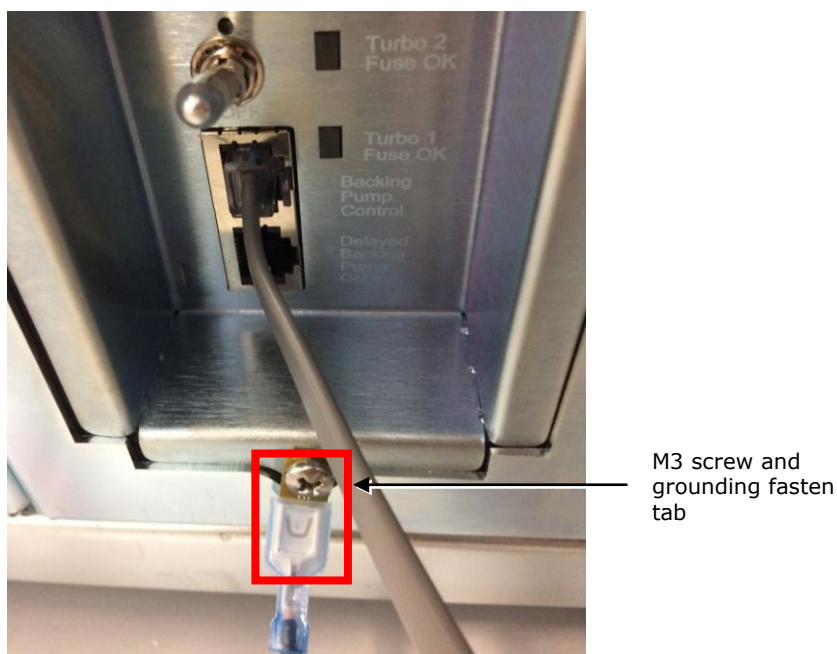


Figure 12 - Backing control PCB on the rear panel

8. Connect the instrument backing hose between the instrument rear panel.
9. Connect the pump control cable to the top port labeled "Backing Pump Control".
10. Fit the grounding wire from the pump control cable to the previously attached grounding fasten tab (Figure 13).

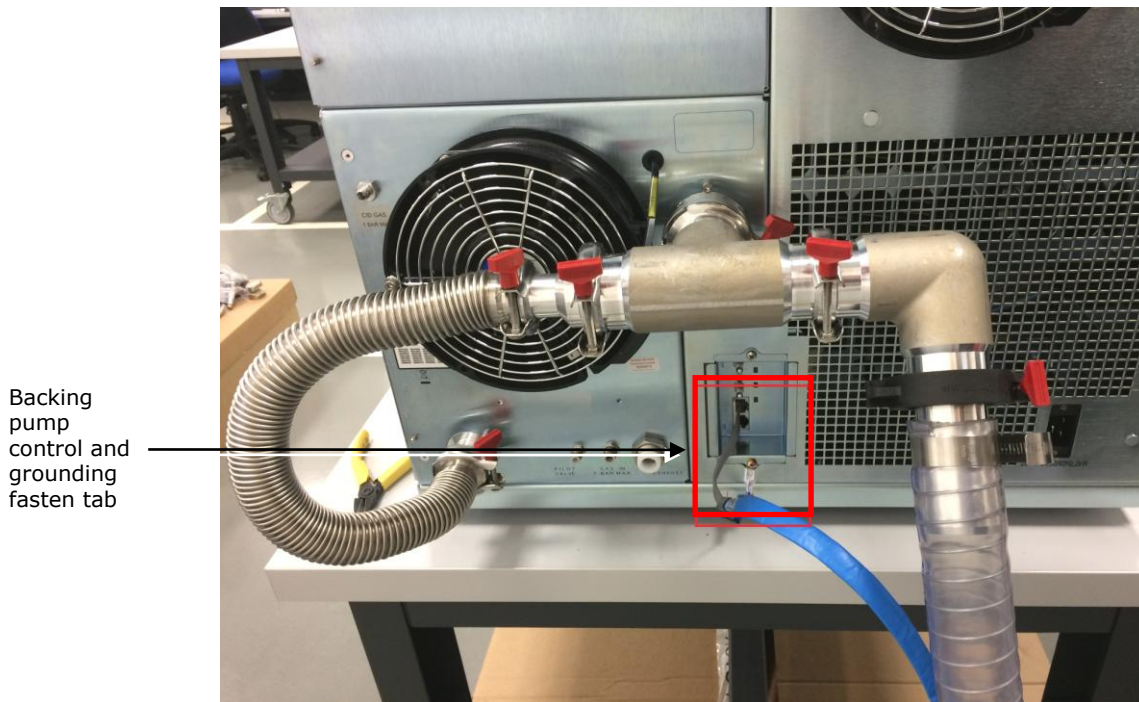


Figure 13 - Backing pump configuration

11. Ensure the green LED is not lit on the Local/Remote button,  to signify the pump is in Remote mode, and ensure the pump power switch is up, in the on position.

CAUTION: *The Ebara pump must be in Remote mode to allow the instrument to be pumped using MassLynx.*

Systems using rotary pumps (2 x Leybold SV65BI)

WARNING: Always wear protective gloves when handling pump oil.

1. Unpack the pumps and place in the final location.
2. Ensure there is enough room around the pumps to allow for service access.
3. Wearing gloves, fill the pumps with oil to just below the maximum level.

CAUTION: After initial startup, the oil level may decrease. If so, top the oil level as required.

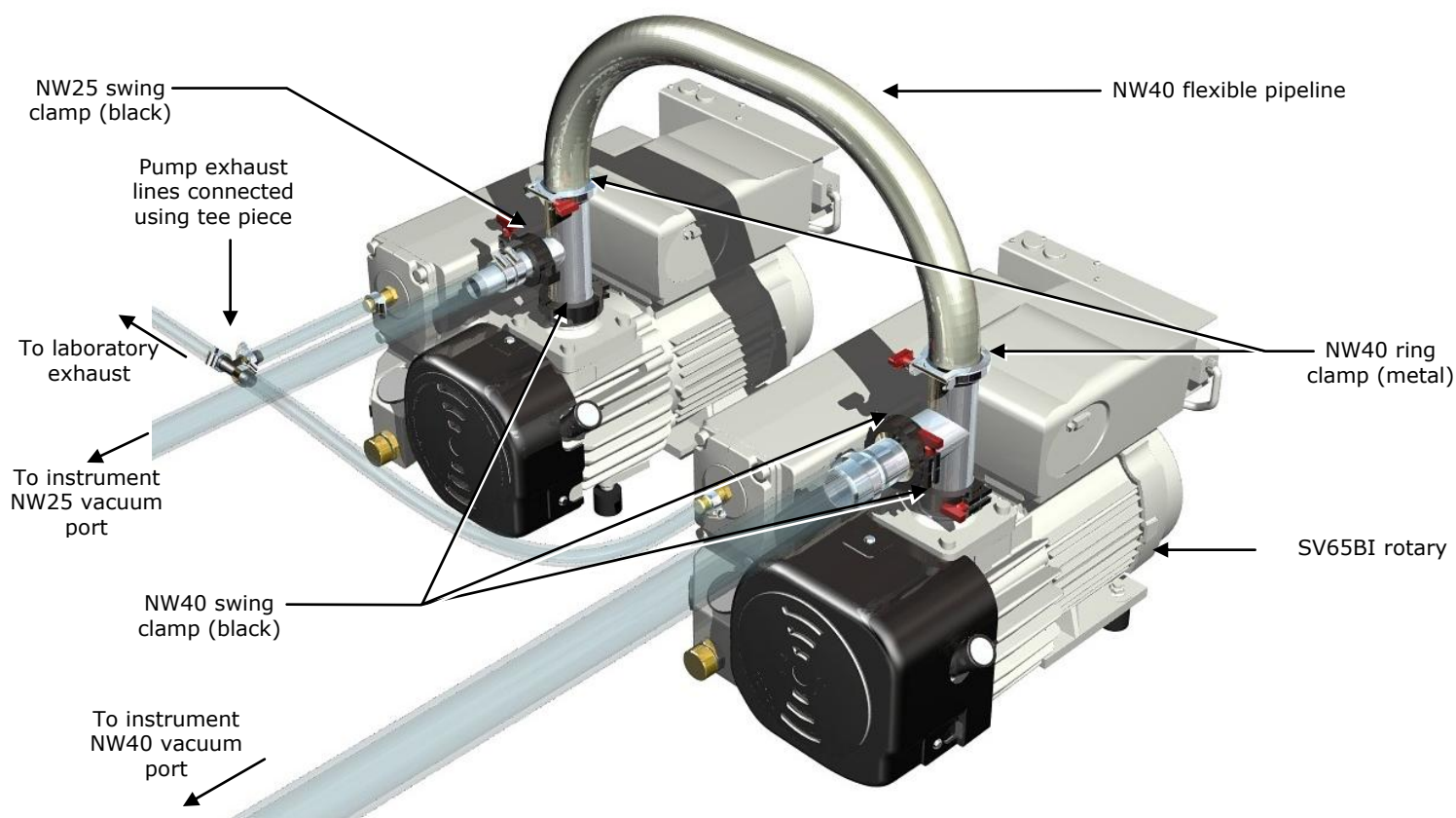


Figure 14 - Rotary pump/vacuum hose configuration

4. Configure the vacuum hoses using the appropriate components (Figure 14).
5. Connect the rotary pump exhaust to a suitable air extraction system using the 9-mm PVC hose supplied in the startup kit.
6. Connect the other end of the hose to the 25-mm nozzle on the pump.
7. Secure the hose using a hose clip supplied with the startup kit.

WARNING: The pump exhaust must be installed properly and must be monitored by the user.

CAUTION: *Continuous instrument operation over extended periods with sustained solvent flow may cause condensation to collect in the pump exhaust line.*

8. Connect the pump control cable (4300038BC1, blue label) to the top pump control socket on the rear panel of the instrument (Figure 15).

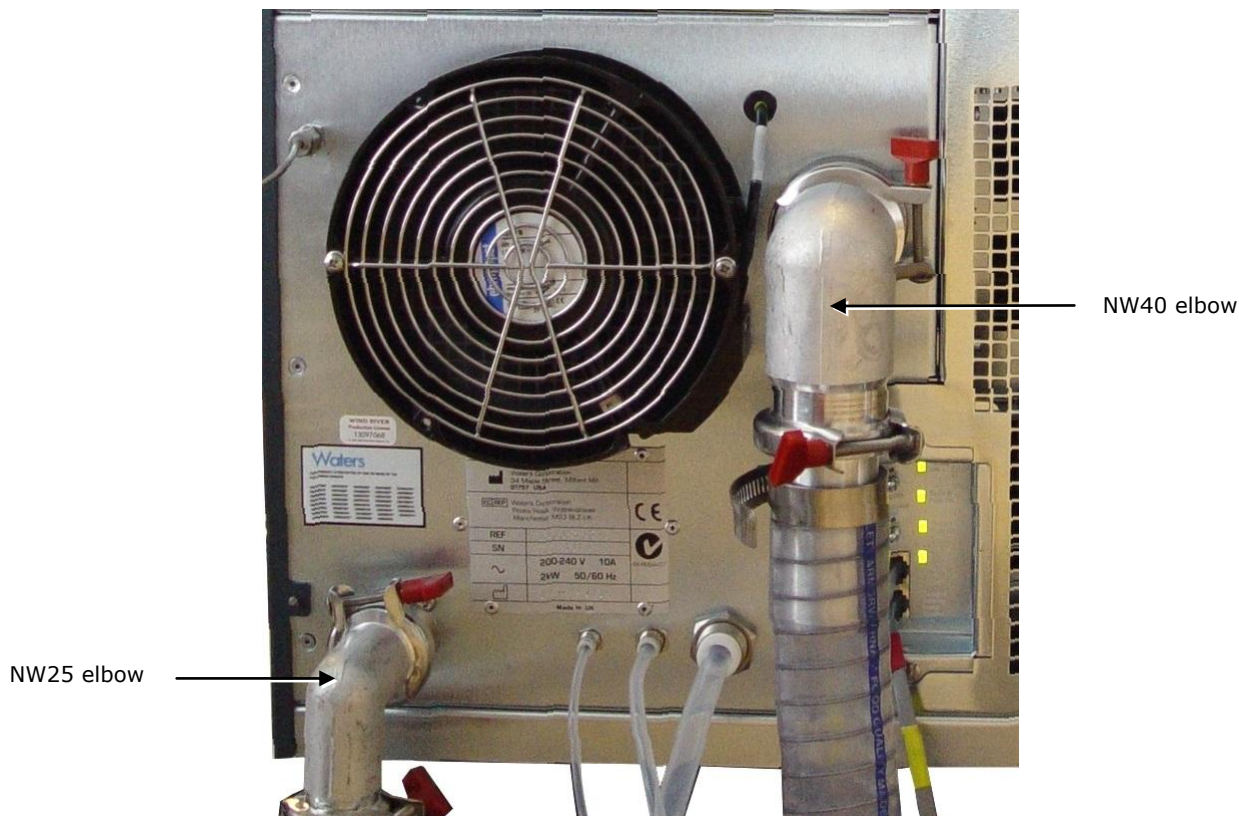


Figure 15 - Backing pump/vacuum hose configuration

9. Connect the other end of the cable to the pump control socket on the rotary pumps.

NOTE: *The lower pump control socket is not used with the rotary pump option.*

10. Ensure that the pumping override and maintenance toggle switches are in the upright position (Figure 16).

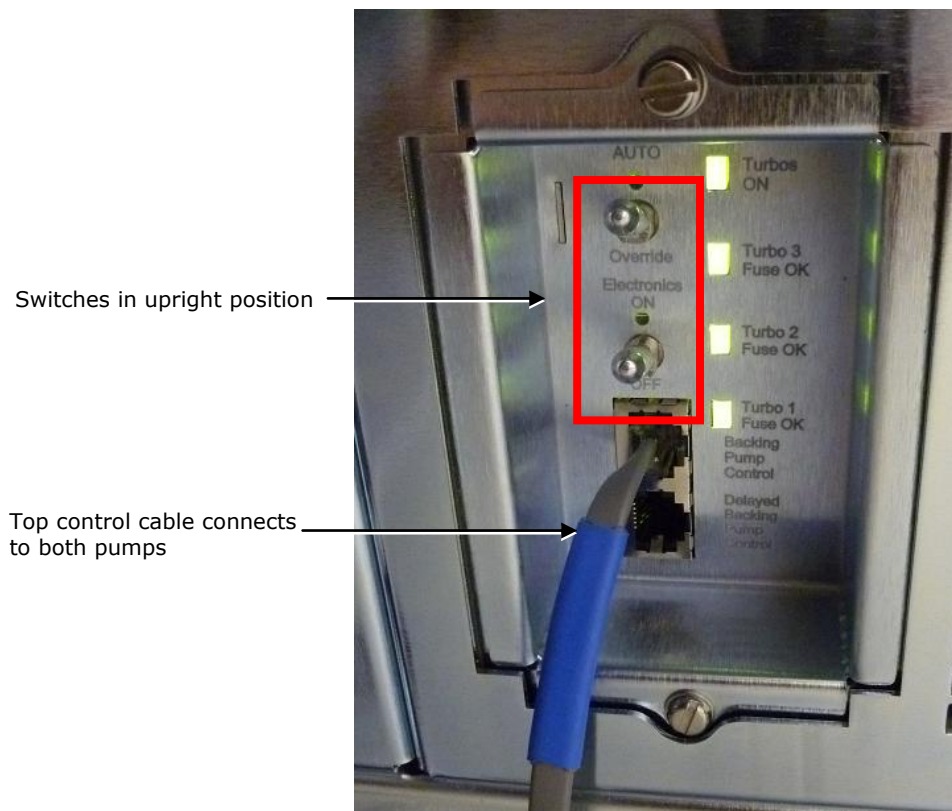


Figure 16 - Connecting the pump control cable (rotary pump configuration)

WARNING: Do not use the override switch to pump the instrument.

11. Connect the vacuum hoses to the appropriate ports on the instrument rear panel, using the additional NW25 and NW40 elbow fittings if required.
12. Connect the power cords from the pumps to the laboratory power supply.

Systems using scroll pumps (Edwards XDS35i and XDS100B scroll pumps)

WARNING: *The scroll pumps are very heavy.*

1. Unpack each pump, by rolling it out of the packaging to avoid unnecessary lifting.
2. Roll the pump onto its top, and remove the bottom section.
3. Roll the pump back onto its rubber feet and into its final location.

CAUTION: *Leave enough room around the pump to allow for service access.*



Figure 17 - Scroll pump and vacuum connections

4. Configure the vacuum components as shown in [Figure 17](#).

5. Connect the exhaust of the XDS35i scroll pump to a suitable air extraction system using the 12-mm PVC hose supplied in the startup kit (Figure 18).

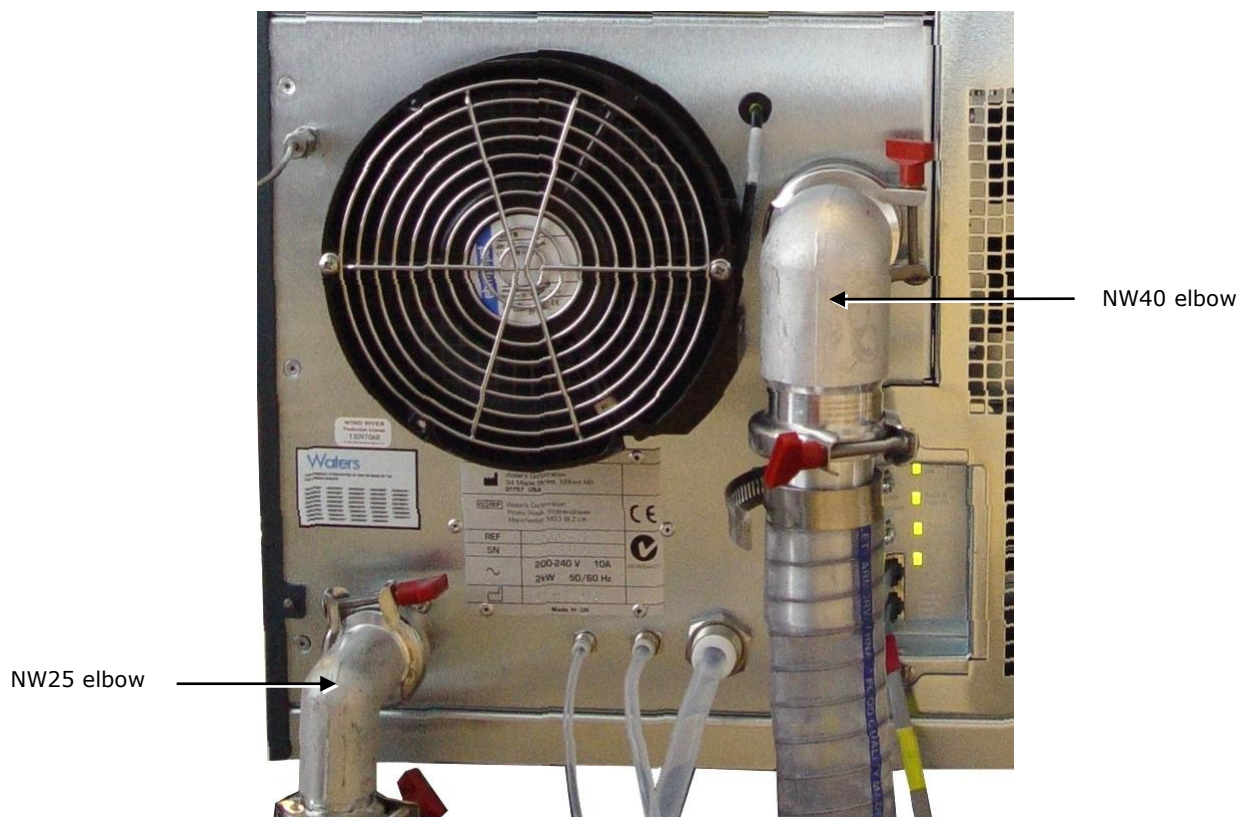


Figure 18 - Backing pump/vacuum hose configuration

6. Connect the other end of the hose to the 25-mm nozzle on the pump.
7. Secure the hose using a hose clip supplied with the startup kit.
8. Ensure that scroll pump exhaust gases are vented to the atmosphere outside the laboratory through a user-supplied fume hood or industrial vent.

WARNING: *The exhaust may be connected to an existing laboratory vent carrying other gases, but which is separate from the instrument source exhaust vent.*

The pump exhaust must be installed properly and must be monitored by the user.

9. Connect the XDS35i pump control cable (4300021BC1 - blue label) to the top pump control socket on the rear of the instrument.
10. Connect the other end of the cable to the XDS35i pump.
11. Connect the XDS100B pump control cable (4300021BC2 - red label) to the bottom (delayed pump) pump control socket on the rear of the instrument.
12. Connect the other end of the cable to the XDS100B pump.

13. Ensure that the pumping override and maintenance toggle switches are in the upright position (Figure 19).

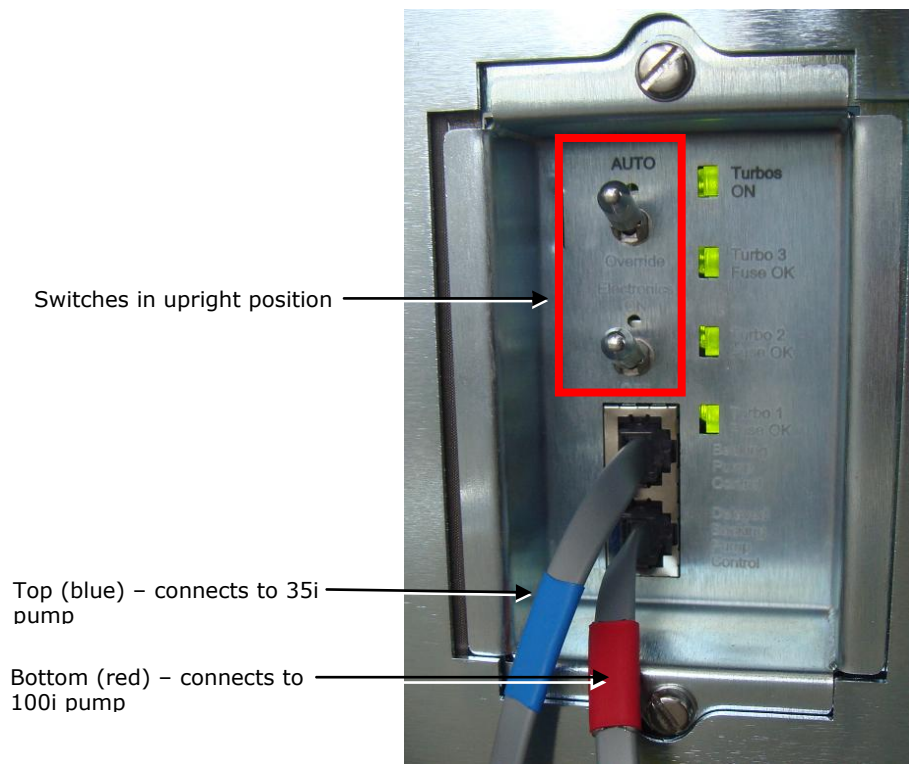


Figure 19 - Connecting the pump control cables (scroll pump configuration)

WARNING: Do not use the override switch to pump the instrument.

14. Ensure that the scroll pump switches are set to the off position so that the MassLynx software can control the pump.
15. Connect the power cords from the pumps to the laboratory power supply.

Vacuum hose extension kit

The installation kit provides a 1.5-meter hose and fittings as standard. Where it is necessary to install a Leybold or Edwards external pump away from the instrument, a 3-meter vacuum hose extension kit is available (205001227). The 3-meter extension kit includes two 40-mm hoses and fittings. This extension kit has been assessed to provide sufficient instrument performance and allows for the use of the existing electrical connections.

CAUTION: *The 3-meter vacuum hose should not be used in conjunction with the standard 1.5-meter hose, provided in the installation kit, because it is not a supported configuration.*

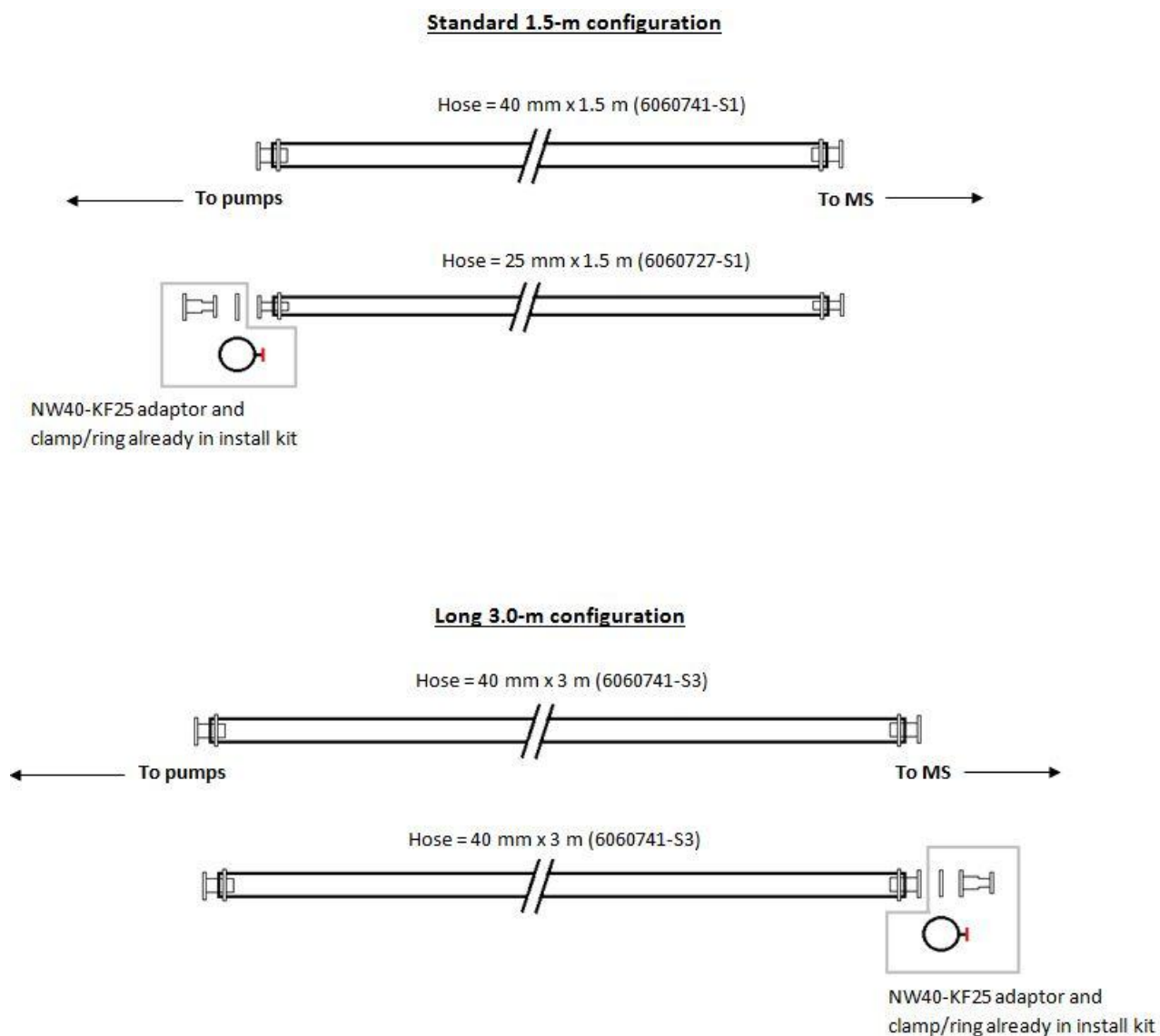


Figure 20 - Standard configuration compared with long 3-meter configuration

Connecting the API gas supply

The Xevo TQ-S requires a supply of dry, oil-free nitrogen with a purity of at least 99.9%.

1. Ensure the nitrogen is regulated at 6.75 ± 0.25 bar (98 ± 4 psi) outlet pressure.
2. Connect the nitrogen to the API gas port on the rear panel of the instrument using the 6-mm Teflon tubing supplied in the start up kit.

NOTE: *A regulator can be connected to the nitrogen line using fitting $\frac{1}{4}$ " MNPT x $\frac{1}{8}$ " COMP (700006668). This part may not fit all types of regulator and is not supplied in the installation kits.*

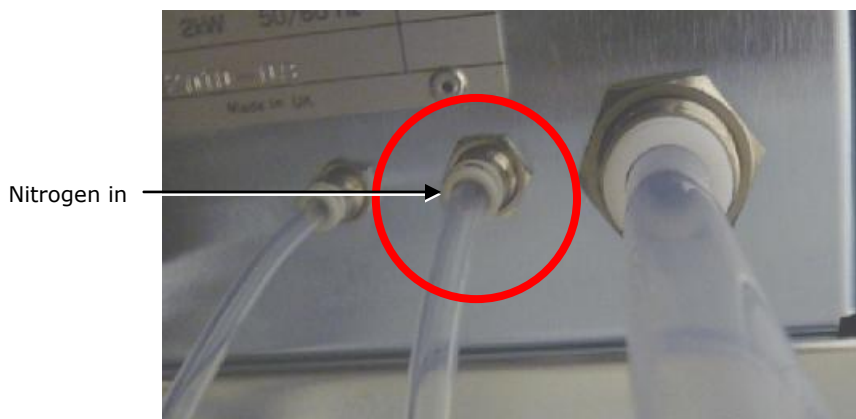


Figure 21 - API gas connection on the rear panel

Connecting the collision gas supply

The Xevo TQ-S requires a supply of dry, oil-free argon with a purity of at least 99.997%.

1. Ensure the argon is regulated at 0.5 bar (7 psi) outlet pressure.
2. Connect the argon to the collision gas port on the rear panel of the instrument using the $\frac{1}{8}$ -inch stainless steel tubing (6395995).

Connecting the instrument power

1. Connect the appropriate power cord from the power connector on the rear panel of the instrument to the laboratory mains supply.

WARNING: *Do not switch on the instrument power switch at this time.*

Making fluidic connections for infusion setup

1. Plumb the fluidic connections as shown in [Figure 22](#).
2. Use 70:30 acetonitrile/water for the wash solution.

WARNING: *It is very important to plumb the wash solution correctly otherwise the fluidics will not function.*

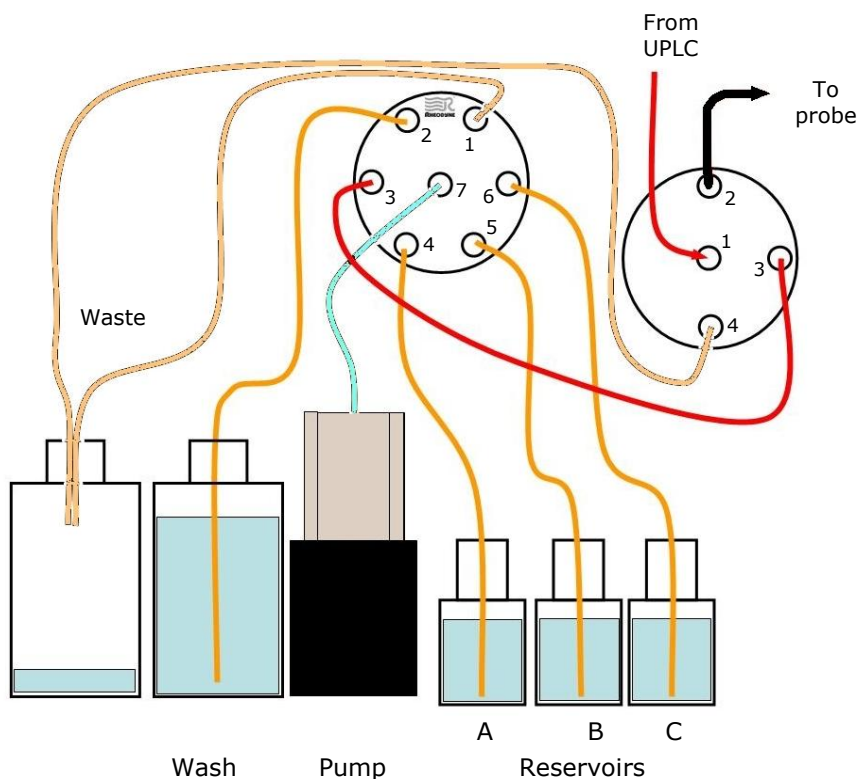


Figure 22 - Plumbing the fluidic connections

Table 2: Fluidics PEEK tubing sizes

Tubing	Description	Color	Size (I.D.)	Length (mm)
	Pump to select valve (7)	S. Steel	0.040 inch/1.0 mm	500
	Waste lines	Natural	0.040 inch/1.0 mm	1000
	Wash bottle to select valve (2)	Orange	0.020 inch/0.5 mm	1000
	Reservoirs to select valve (4, 5 and 6)	Orange	0.020 inch/0.5 mm	600
	Select valve (3) to diverter valve (3)	Red	0.005 inch/0.127 mm	200
	Diverter valve (2) to probe (not applicable to tool-free probes)	Black	0.004 inch/0.102 mm	360

NOTE: *Tubing lengths are very important for the operation of the fluidics. It is recommended that only Waters pre-cut tubing be used when replacing fluidic components.*

Basic host PC setup and installing MassLynx

1. Unpack the host PC, LCD monitor, peripherals, and place in the final location.
2. Connect the PC components together (keyboard/mouse).
3. Ensure the voltage selection switch on the PC tower is set to the correct voltage for the laboratory supply.
4. Ensure that similar selections are made for any peripheral items.
5. Connect the appropriate regional power cords from the host PC, monitor, and peripherals to the laboratory mains supply.
6. Switch on the host PC.
7. Login to the Microsoft Windows desktop, using the administrator account (password = serial number of the PC).
8. Install the MassLynx software from the supplied CD (see the release notes instructions).

CAUTION: *The TargetLynx and OpenLynx application managers must be installed on all systems. OpenLynx is required for the IntelliStart System QC function to operate.*

ACQUITY UPLC preparation (if applicable)

1. Ensure the ACQUITY UPLC system has been unpacked and placed in the final configuration.
2. Make all appropriate fluidic and electrical connections as appropriate for the ACQUITY UPLC options purchased.

NOTE: Refer to the ACQUITY UPLC installation documentation for further details.

Making communications connections to the instrument

Table 3: Xevo TQ-S communication connections

ACQUITY UPLC system	Connection
Yes	Connect the Ethernet cable from the instrument LAN port to the Ethernet switch on the rear panel of the high temperature column heater module to which the ACQUITY components are connected.
No	Connect the Ethernet from the instrument LAN port directly to the host PC network card.



Figure 23 - Router for ACQUITY UPLC connections

Powering on and pumping the instrument

CAUTION: *Ensure that the host PC is turned on, MassLynx software has been installed, and that all Ethernet cables have been connected before powering on the instrument.*

Turning instrument power on (without ACQUITY UPLC)

1. Switch on power to the Xevo TQ-S.
2. Allow up to three minutes for the EPC to successfully boot.

Turning instrument power on (with ACQUITY UPLC)

1. Switch on power to the ACQUITY module containing the Ethernet hub (for example, high temperature heater/cooler or the column manager).

CAUTION: *The ACQUITY UPLC module that contains the Ethernet hub must be switched on first to allow communications to be established.*

2. Switch on power to the Xevo TQ-S.
3. Switch on power to the binary solvent manager.
4. Switch on power to any additional UPLC modules.
5. Allow up to three minutes for the EPC to successfully boot.

Launching MassLynx software

1. Click **Start > Run** on the host PC.
2. Type **ping epc -t** to display a command prompt window showing the communications status of the instrument.

NOTE: *During a ping, the host PC sends a small data packet to the EPC and awaits a response. The EPC can be considered fully booted when a whole page of successful replies (reply from 192.168.0.X bytes = 32 time <1 ms TTL = 64) are received.*

The EPC address may change from system to system, as the values are controlled by the DHCP server. See "[Useful software tools](#)" for information on how to check this address.

3. Launch the MassLynx software.
4. Ensure that MassLynx successfully established communications with the instrument.

Pumping the instrument

The front panel vacuum LED displays information regarding the pump status of the instrument, see [Table 5](#).

1. Click the **MS Tune** button on the main MassLynx window to launch the MS Tune page.
2. Click **Vacuum > Pump** on the Tune page to start the instrument pumping.

NOTE: *The gas cell reaches 10E-3 mbar after approximately five minutes and the turbo pump speeds reach 100% in the same time. Once the turbo pump speed has passed 80%, the front LED turns steady green.*

It may take in excess of two hours for a new instrument to show a collision cell pressure of less than 10E-4 mbar.

Checking instrument serial number

1. Open the Target Registry editor.
2. Ensure the serial number for the instrument has been set.

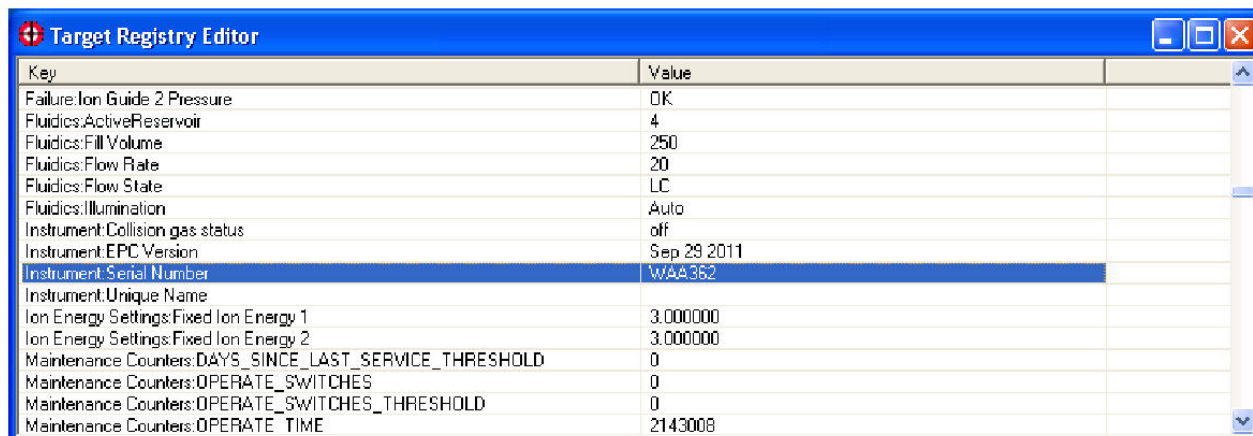


Figure 24 - Target registry

3. If it has not, set it through the target registry editor.

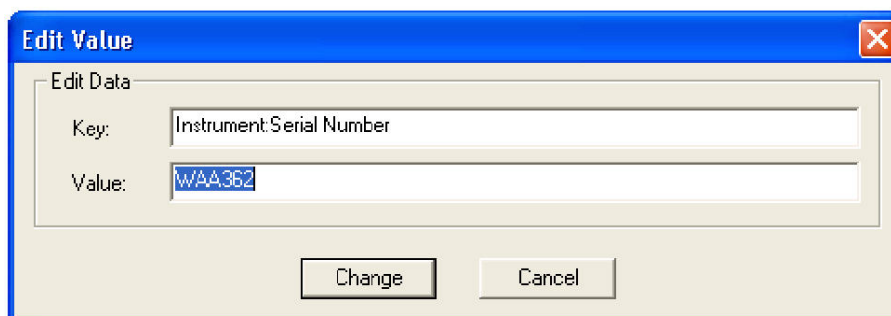


Figure 25 - Editing the target registry

NOTE: *Value is illustration only.*

Connecting the gas fail cable

- Using the gas fail cable, make connections as described below:

Table 4: Gas fail connections

Equipment	Connector	Function	Pins
Xevo TQ-S	2	Stop flow + Stop flow -	3 (black) 4 (red)
ACQUITY	II	Stop flow + Stop flow -	3 (red) 4 (black)

CAUTION: *Fit a stop flow cable to ensure the stop flow function still works in the case of loss of communications with the ACQUITY.*

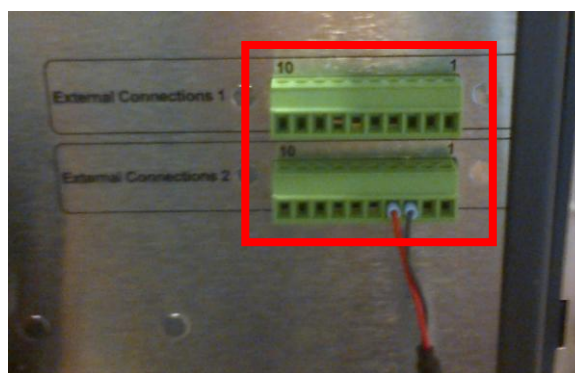
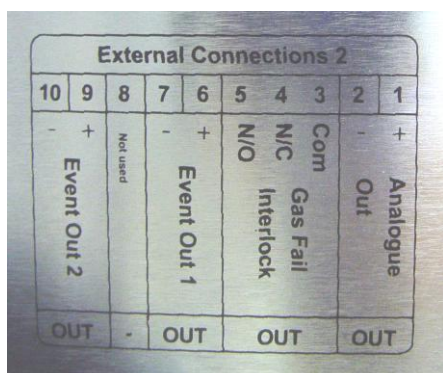


Figure 26 - Xevo TQ-S gas fail connections

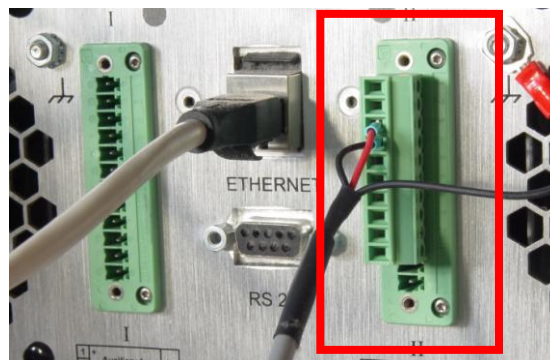
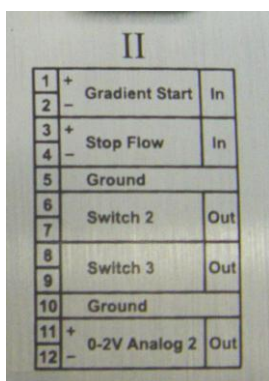


Figure 27 - ACQUITY gas fail connections

- Switch off the nitrogen gas supply at the regulator to check the stop flow function works correctly.
- Ensure the UPLC pump switches off when the nitrogen fail message appears in MassLynx.

Xevo TQ-S LEDs

The two front panel LEDs provide information about the instrument's activity.

Table 5: Status LEDs

LED Type	Mode	Color	Description
Power LED (Hardware controlled)	Green		DC power supply is on and is supplying power
	Off		Either the instrument is turned off, or there is a problem with the power supply
Status LED (Software controlled)	Off		The instrument is in Standby with voltages and temperatures disabled
	Red (for three minutes)		The instrument is waiting for the pump to initialize.
	Red (for more than three minutes)		Indicates a serious system failure that prevents further operation. Communications to the data system PC may also be stopped This LED state indicates that the problem exists within the instrument module itself and is not a software, PC, or network interface issue Power off the instrument, then power on. If the LED is still steady red for more than 3 minutes, contact GSS
	Flashing red		The system has stopped on an error and is no longer processing any samples or maintenance requests Further information regarding the error that created this failure is available in the data system's control panel or in the console software
	Flashing green		The system is waiting for an instrument component to reach operational conditions This could, for example, flash while the system waits for programmed temperature set point or vacuum levels to be attained The instrument is healthy and in Operate but not fully ready
	Green		The instrument is in Operate with temperatures and voltages on

Basic instrument setup

Samples required

The following sample is required to perform a basic instrument setup:

- Ion beam setup Setup Solution Xevo TQ-S

During the installation, the instrument must be tuned and tested to provide the customer with the following proof of performance:

- Documented evidence of the instrument performance

CAUTION: *Read this section carefully before progressing with the instrument performance tests.*

Installing the ESI probe

1. Unpack and install the ESI probe.

Loading the installation project

To load the installation project perform the following procedure:

1. Copy the project **Xevo TQ-S - Acquity UPLC System Specifications Project.PRO** into the C:\MassLynx directory.

NOTE: *The project can be found in [Reference Information](#) in Service Assist on the Portal.*

2. Open MassLynx and load the **Xevo TQ-S Acquity UPLC Installation Project.PRO**.
3. Open the sample list **WAAXX_DDMMYY.spl**.

NOTE: *This sample list also contains methods for the sensitivity tests that are used for non-ACQUITY systems.*

4. Save and rename the sample list, in the format **WAAXX_DDMMYY.spl** (where DDMMYY = date, and XXX is the serial number of the instrument).
5. Load the installation sample list format **XEVO_TQS_UPLC.fmt** from the C:\MassLynx\Xevo TQ-S - Acquity UPLC Installation Project.pro\SampleDB directory.

Spectrum Chromatogram Map Edit ▾ Samples ▾								
File Name	File Text	MS File	MS Tune File	Sample Type	Inlet File	Bottle	Inlet Volume	
1 MS1_MassScaleResolution_1	MS1 Mass scale and resolution setup	MS1_MassScaleResolution	Instrument_Setup				0.000	Mass scale and resolution setup
2							0.000	
3 MS2_MassScaleResolution_1	MS2 Mass scale and resolution setup	MS2_MassScaleResolution	Instrument_Setup				0.000	
4							0.000	Quad Resolution tests
5 MS1_HighMassVerification_1	MS1 High mass resolution verification	MS1_HighMassVerification	MS1_HMVerification				0.000	
6							0.000	
7 MS2_HighMassVerification_1	MS2 High mass resolution verification	MS2_HighMassVerification	MS2_HMVerification				0.000	
8							0.000	
9							0.000	
10 Column_Conditioning 2	Blank	Gradient	SYSTEM	Blank	Gradient	1:A,1	10.000	System-level tests
11 Gradient 1	Gradient	Gradient	SYSTEM	Blank	Gradient	1:A,1	10.000	
12 Gradient 2	Gradient	Gradient	SYSTEM	Standard	Gradient	1:A,2	10.000	
13 Gradient 3	Gradient	Gradient	SYSTEM	Standard	Gradient	1:A,2	10.000	
14							0.000	
15 ESI_Positive_Sensitivity 1	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	Standard	Gradient	1:A,2	10.000	
16 ESI_Positive_Sensitivity 2	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	QC	Gradient	1:A,2	10.000	
17 ESI_Positive_Sensitivity 3	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	QC	Gradient	1:A,2	10.000	
18 ESI_Positive_Sensitivity 4	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	QC	Gradient	1:A,2	10.000	
19 ESI_Positive_Sensitivity 5	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	QC	Gradient	1:A,2	10.000	
20 ESI_Positive_Sensitivity 6	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	QC	Gradient	1:A,2	10.000	
21							0.000	
22 ESI_Negative_Sensitivity 1	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	Standard	Gradient	1:A,2	10.000	System-level tests
23 ESI_Negative_Sensitivity 2	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	QC	Gradient	1:A,2	10.000	
24 ESI_Negative_Sensitivity 3	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	QC	Gradient	1:A,2	10.000	
25 ESI_Negative_Sensitivity 4	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	QC	Gradient	1:A,2	10.000	
26 ESI_Negative_Sensitivity 5	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	QC	Gradient	1:A,2	10.000	
27 ESI_Negative_Sensitivity 6	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	QC	Gradient	1:A,2	10.000	
28							0.000	
29 ESI_Positive_Sensitivity_Standalone_1	1 pg/ul Reserpine Fluidics Injections (standalone)	ESI_POS_Sensitivity_DDMYY	Sensitivity_Tests				0.000	Standalone Sensitivity tests
30							0.000	
31 ESI_Negative_Sensitivity_Standalone_1	0.5 pg/ul Chloramphenicol Fluidics Injections (standalone)	ESI_NEG_Sensitivity_DDMYY	Sensitivity_Tests				0.000	
32							0.000	Scanwave checks
33 Scanwave_Positive_1	100 fmol/ul Glu-Fibrinopeptide B Daughter Scan	Scanwave_POS_Daughter	Scanwave				0.000	
34 Scanwave_Positive_2	100 fmol/ul Glu-Fibrinopeptide B Scan/Wave Daughter Scan	Scanwave_POS_SWDaughter	Scanwave				0.000	
35							0.000	
36 Scanwave_Negative_1	100 fmol/ul Glu-Fibrinopeptide B Daughter Scan	Scanwave_NEG_Daughter	Scanwave				0.000	
37 Scanwave_Negative_2	100 fmol/ul Glu-Fibrinopeptide B Scan/Wave Daughter Scan	Scanwave_NEG_SWDaughter	Scanwave				0.000	
38							0.000	

Figure 28 - Installation sample list

Data presentation

CAUTION: Read this section carefully before progressing with the instrument setup and performance tests.

- For each acquisition made during installation, perform the following steps:
 - Print the experiment parameters or MS Tune page, as stated in the relevant section.
 - Label the printed copy with the related acquisition.
 - Where requested, print the calibration results.
- Ensure that the sample text on the Acquire window contains the name of the test currently being run (for example, positive ion sensitivity).
- Configure the chromatogram and spectrum headers to display the following information:
 - Top left-hand corner sample text
 - Center section instrument number
 - Top right-hand corner RawDataDate <space> <space> RawDataTime

Aligning the RF generators

1. Open the MassLynx MS Tune page.
2. Click **View > Advanced, View > System and View > System Diagnostics** to access the advanced MS Tune page tabs.

NOTE: This page is not for customer use and is password protected. The password is 1147.

3. Click the **Resolution** tab.
4. Click the **Mode Select** drop-down list and select **Align**.

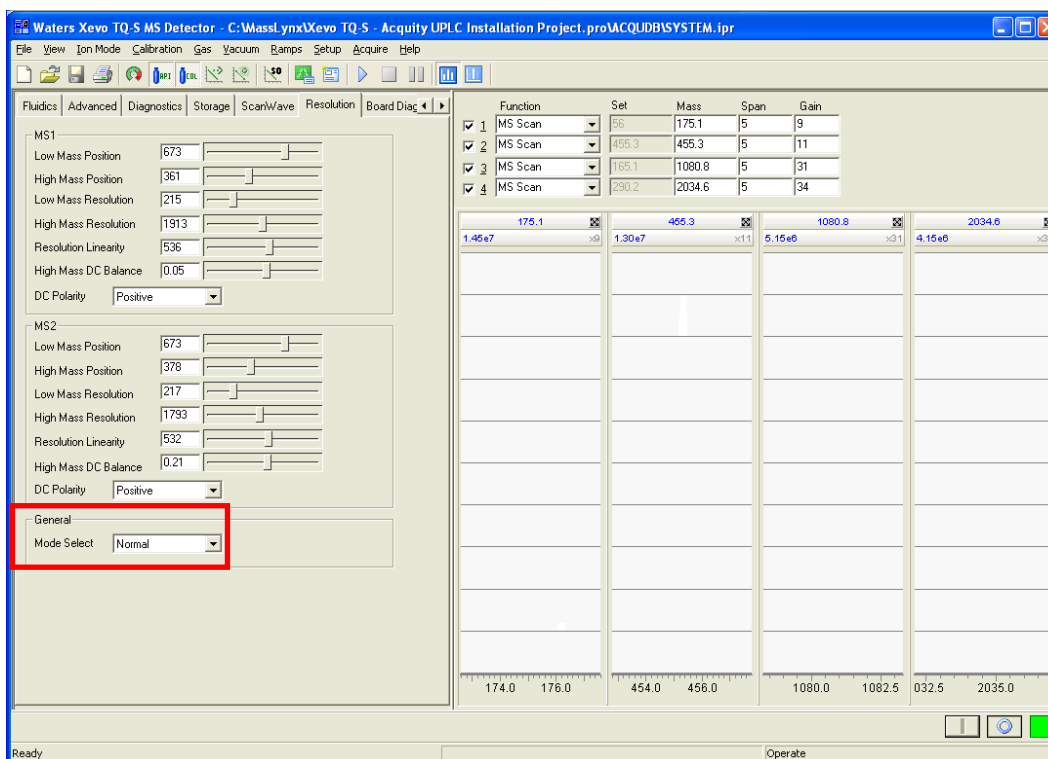


Figure 29 - Selecting Align mode

5. Click the **Quad Diagnostics** tab to access the Rec RF quadrupole readbacks.

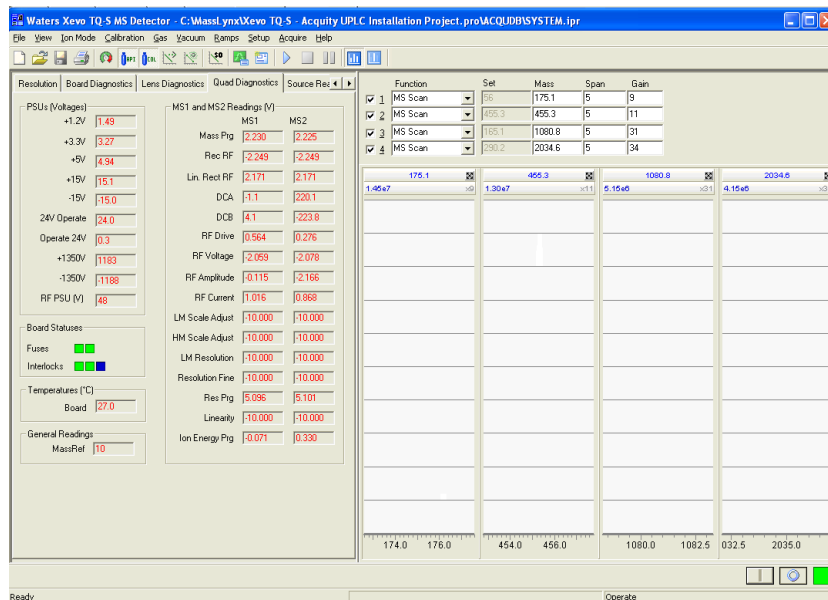


Figure 30 - Quad Diagnostics tab

6. Remove the instrument right-hand panel.

WARNING: Do not remove the covers from the RF generators – hazardous voltages will be exposed.

7. Locate the tuning control on the generators, see Figure 31.

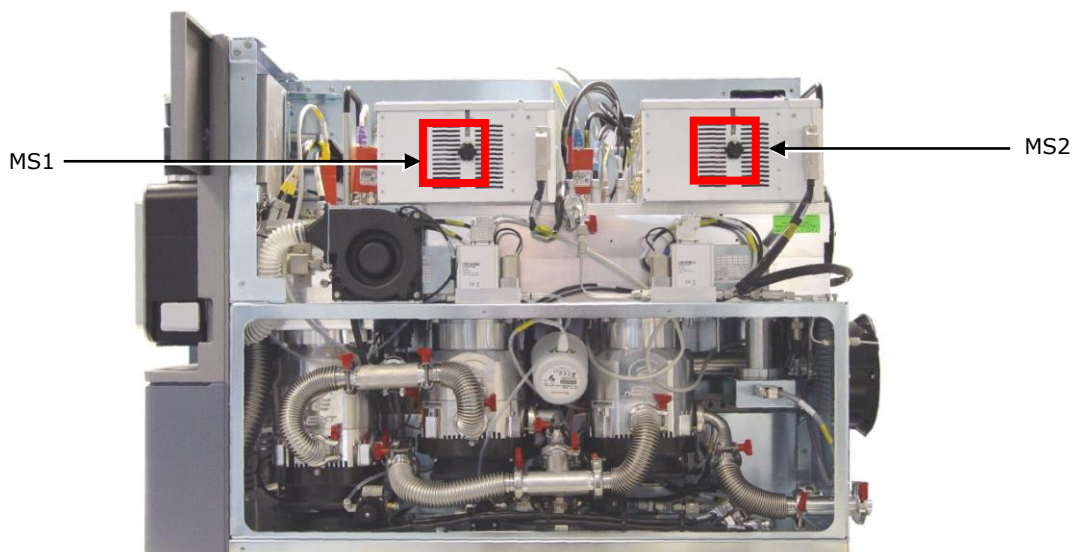


Figure 31 - Quadrupole RF generator tuning controls

8. Ensure the instrument is in Operate.

9. Using the tuning knob for the MS1 generator, tune to maximize the MS 1 Rec RF readback (the rectified RF voltage) to the voltage with the largest possible value.
10. Using the tuning knob for the MS2 generator, tune to maximize the MS 2 Rec RF readback to the voltage with the largest possible value.
11. When complete, click the **Resolution** tab and set the mode to **Normal**.
12. Open four MS Scan windows to display the masses 500, 1000, 1500, and 2039 Da with a span of 0.
13. Click the Quad Diagnostics tab.
14. Check the readback for the rectified RF and compare the values with the expected values in [Table 6](#) for each of the masses given.
15. Place the instrument in Standby.
16. When finished, lock the tuning knob in place using the locking screw, then refit the instrument side panel.

Table 6: Expected rectified RF (in Normal mode) for differing masses

Daughter Scan Mode Set Mass and Window Mass m/z with 0 span	MS1 Rec RF	MS2 Rec RF
500	~2.5	~2.5
1000	~4.9	~4.9
1500	~7.4	~7.4
2039	~10.0	~10.0

Initial instrument setup

For all of the tasks in the following section the instrument must be in Operate with the API gas on. The MS Tune page must be set to Positive Ion mode and be in MS mode unless otherwise stated.

NOTE: *The Advanced MS Tune page parameters and Interscan parameters must be set to automatic for installation and routine use.*

Mass scale and resolution setup

1. Load the **Instrument_Set_Up. Ipr** tune file. The initial source conditions are shown in [Table 7](#).

Table 7: Initial source conditions

Parameter	Setting	Comment
Capillary	3 kV	This value will be optimized later
Cone voltage	70 V	This value will be optimized later
Source Offset	60 V	Fixed
Desolvation temperature	200 °C	Fixed
Desolvation gas flow	500 L/h	Fixed
Cone gas flow	150 L/h	Fixed

2. Install a bottle of Setup Solution Xevo TQ-S on fluidics reservoir B.
3. Open the MS Tune page.
4. Click  to start the nitrogen flow, then click  to put the instrument in Operate mode.
5. Click Ion Mode > Electrospray +.
6. Click the **Fluidics** tab, then select **Reservoir B**.
7. Click . The purge function automatically starts after Reservoir B is selected.
8. Purge for a second time.
9. Select **Infusion** as the flow state, and set infusion flow rate to **10 µL/min**.
10. Ensure the isolation valve is open, then click .
11. Click **Calibration > Calibrate Instrument** in the Tune page menu bar. The Calibration dialog box appears.
12. Click **File > Open**, then select **Uncal.cal**.
13. Click **Open**. The Calibration window displays No Calibration for all calibration types.

NOTE: If the Calibration window does not display No Calibration for all calibration types, select **Calibrate** > **Default** to remove the calibration.

14. Click **File** > **Exit** to return to the Tune page.

15. Open the four masses shown in Figure 32 in the Mass Editor:

	Function	Set	Mass	Span	Gain
☒ 1	MS1 Scan	56	175.1	5	15
☒ 2	MS1 Scan	219	455.3	5	31
☒ 3	MS1 Scan	502	1080.8	5	35
☒ 4	MS1 Scan	614	2034.6	5	26

Figure 32 - Mass Editor

CAUTION: On the Xevo TQ-S, "MS Scan" refers to MS2 and "MS1 Scan" refers to MS1.

16. Ensure that the analyzer settings are correct for the MS1 scan function, see Figure 33.

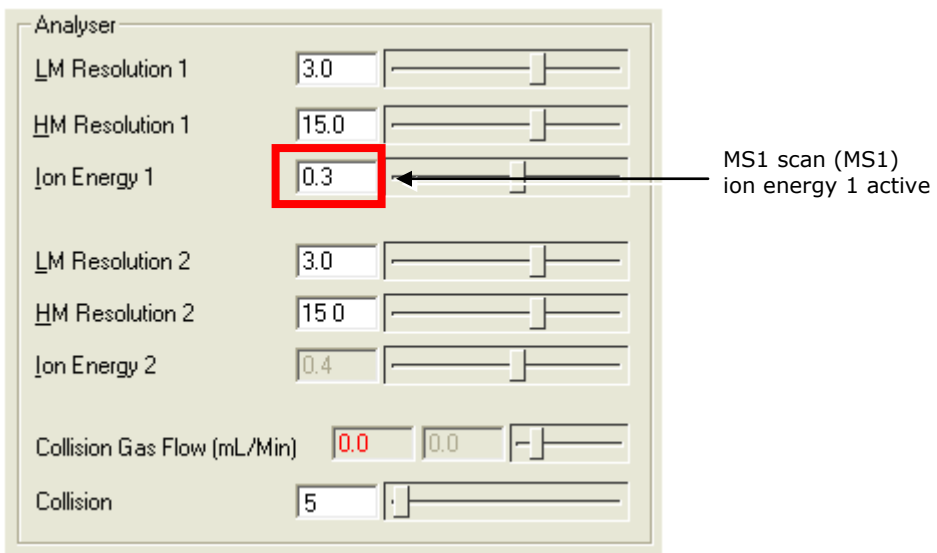


Figure 33 - Ion energy settings - MS1 scan

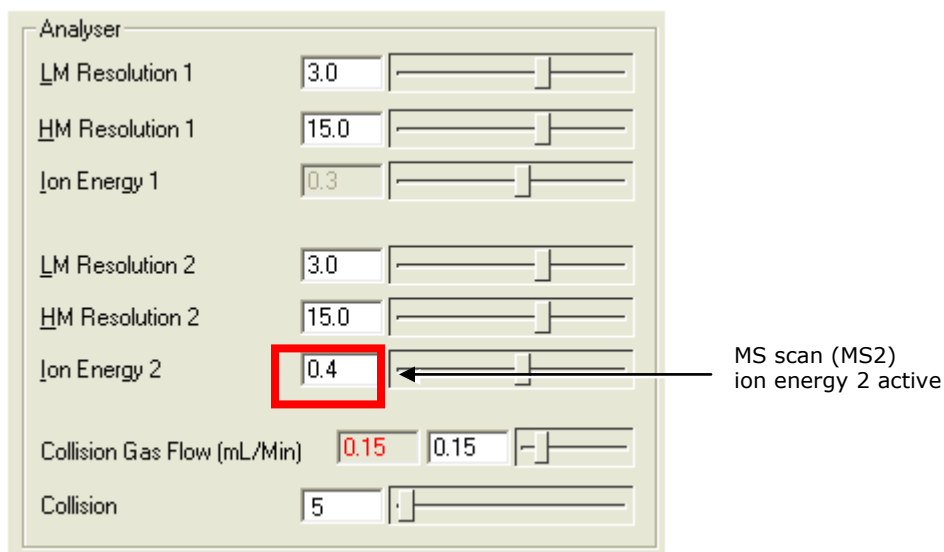


Figure 34 - Ion energy settings MS scan

NOTE: Using the correct value for ion energy is important to the performance of the quadrupole. If too low, then 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.

The following procedure ensures that the optimum balance between sensitivity and resolution is achieved:

17. Optimize the ion energy in MS1 Positive Ion mode:

- In Positive Ion mode, open a single MS1 Scan window to display mass 59 Da with a span of 3.
- Set the MS1 DC balance to **0** on the Resolution tab.
- Set the collision energy to **5** on the Source tab.
- Adjust the MS1 ion energy to maximize the intensity of the m/z 59 ion.
- Tune the ion energy so that the intensity of the m/z 59 ion drops to 5 to 10% of the maximum intensity obtained in step d.
- Add **0.5** to the current ion energy value. This is the optimal low mass ion energy for MS1.

18. Optimize the DC balance in MS1 mode:

- In Positive Ion mode, open a single MS1 Scan window to display mass 2034.6 Da with a span of 3.
- Adjust the DC balance to maximize the intensity of the 2034.6 Da signal.

NOTE: Do not adjust beyond ± 3 V.

- Adjust the DC balance so that intensity of the m/z 2034 ion drops to 5 to 10% of the maximum intensity.

- d. Adjust the DC balance value by ± 0.5 V so that the intensity of the m/z 2034 signal increases. This is the optimum DC balance.

19. Optimize the ion energy in MS2 Positive Ion mode:

- a. In Positive Ion mode, open a single MS Scan window to display mass 59 Da with a span of 3.
- b. Turn on the collision gas and set a flow of to give a collision cell pressure of **3.5E-3 mbar**.
- c. Set the MS2 DC balance to **0** on the Resolution tab.
- d. Set the collision energy to **5** on the Source tab.
- e. Optimize the MS2 ion energy to maximize the intensity of the m/z 59 ion.
- f. Tune the ion energy so that the intensity of the m/z 59 ion drops to 10% of the maximum intensity obtained in step e.
- g. Add **1.0** to the current ion energy value. This is the optimal low mass ion energy for MS2.

20. Optimize the DC balance in MS2 mode:

- a. In Positive Ion mode, open a single MS Scan window to display mass 2034.6 Da with a span of 3.
- b. Adjust the DC balance to maximize the intensity of the 2034.6 Da signal.

NOTE: Do not adjust beyond ± 3 V.

- c. Adjust the DC balance so that intensity of the m/z 2034 ion drops to 10 to 15% of the maximum intensity.
- d. Adjust the DC balance value by ± 1.0 V so that the intensity of the m/z signal increases. This is the optimum DC balance.

NOTE: See "[ScanWave optimization](#)" for details on optimizing ion energy in Negative Ion mode.

21. Save the Tune page as **Instrument_Setup.ipr**.

Acquiring MS1 data

1. Ensure that the four MS1 scan masses are displayed as shown in [Figure 32](#).
2. Turn off the collision gas.
3. Optimize the peak at 175.13 Da by tuning the probe position.
4. Optimize all four peak heights using the capillary and cone voltages.
5. Ensure the mass position is within 1.0 Da of the expected nominal mass and resolution for all four peaks is between 0.4 to 0.6 Da (FWHM).
6. If required, adjust the resolution, mass position, and linearity using the parameters on the Resolution tab of the MS Tune page.
7. Save the Tune page as **Instrument_Setup.ipr**.
8. Select the **Instrument_Setup.ipr** tune file for the MS1-01 sample in the sample list.
9. Highlight the **MS1_MasScaleResolution_1** sample (Mass Scale and Resolution Set Up MS1) in the sample list, then click **Start**.

The screenshot shows a dialog box titled "Function:1 MS1 Scan" with a red 'X' close button in the top right corner. The dialog is divided into several sections with blue headers:

- Mass (m/z)**: Contains "Start" (15) and "End" (2048) input fields.
- Method**: Contains "Ionization Mode" (ES+) and "Data" (MCA) dropdown menus.
- Time (Mins)**: Contains "Start" (0) and "End" (2.3) input fields.
- Scan Duration (secs)**: Contains "Scan Time" (10) input field.
- Cone Voltage**: Contains a checked "Use Tune Page" checkbox, "Cone Voltage (V)" (30) input field, an unchecked "Use Cone Voltage Ramp" checkbox, and a "CV Ramp..." button.
- APCl Probe**: Contains an unchecked "Use Tune Page Settings" checkbox and "Probe Temp" (20) input field.

At the bottom right are "OK" and "Cancel" buttons.

Figure 35 - Acquisition parameters

10. Click **Spectrum** on the main MassLynx page.
11. Click  or **File > Open** then select the appropriate filename and click **OK**.
12. Click  then click .
13. Click **Process > Smooth** and specify the parameters shown in [Figure 36](#), then click **OK**.

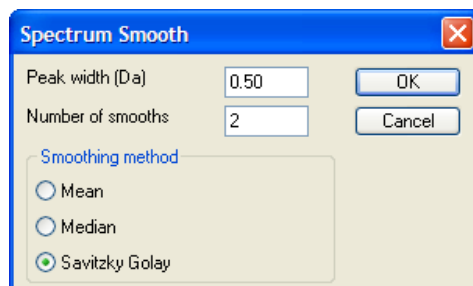


Figure 36 - Spectrum smooth parameters

14. Click **Display > View**, and specify the parameters shown in Figure 37, then click **OK**.

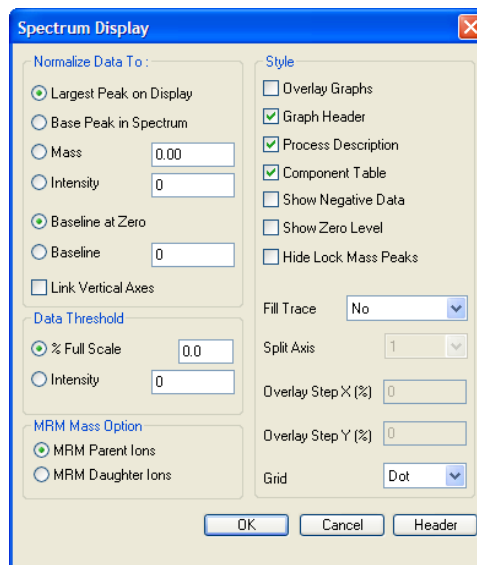


Figure 37 - Spectrum display parameters

15. Click **Display > Peak Annotation** and specify the parameters shown in Figure 38, then click **OK**.

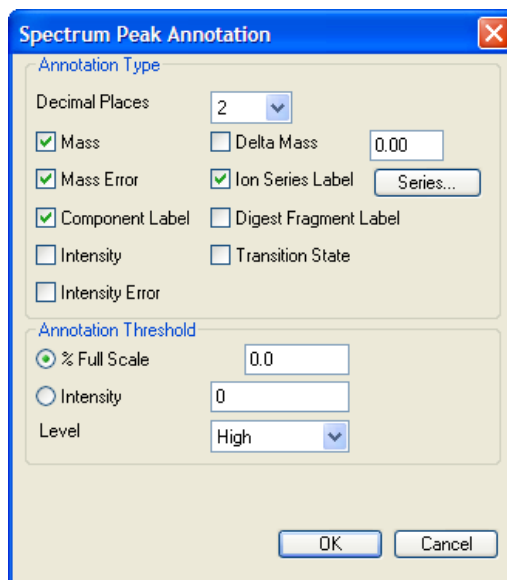


Figure 38 - Peak annotation parameters

16. Zoom in on the m/z 175.1 peak by left-clicking and dragging the Spectrum window.

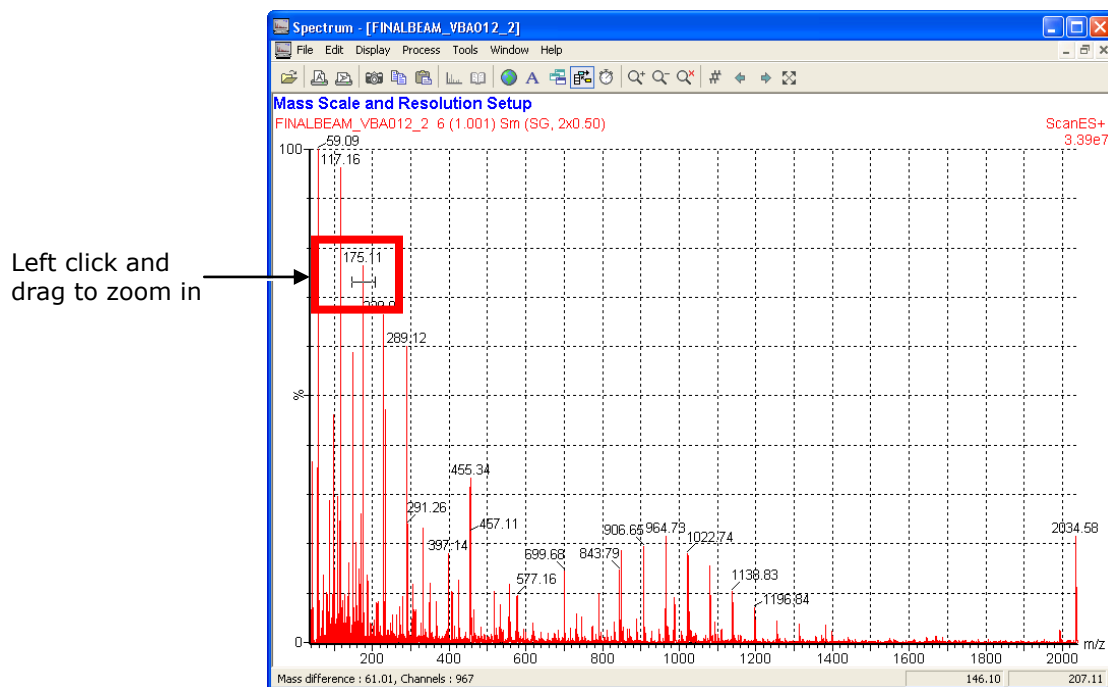
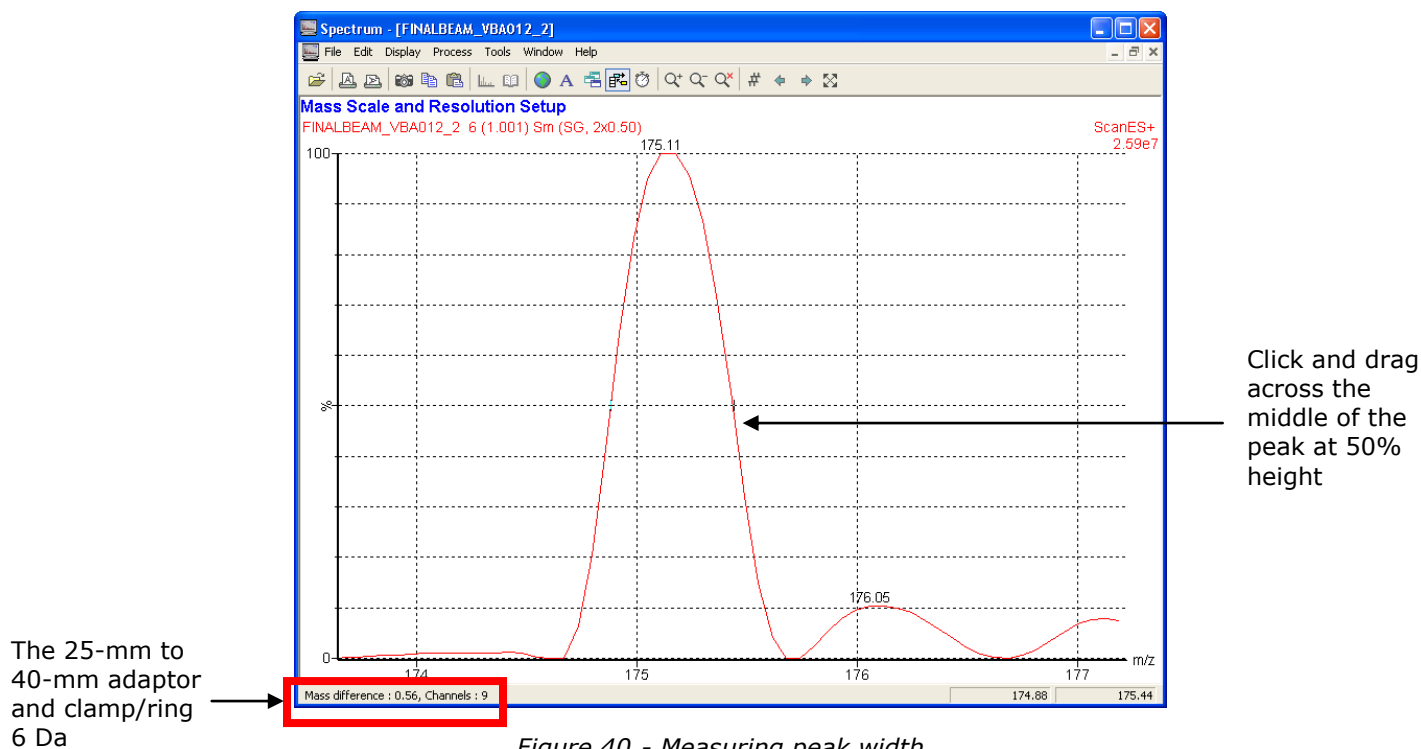


Figure 39 - Spectrum window

17. Measure the peak width at half-height.



18. Ensure the resolution and mass position are acceptable; the width at half-height of the peak must measure between 0.4 and 0.6 Da.
19. Ensure the resolution and mass position are acceptable for each of the other masses (m/z 455.3, 1080.8, 2034.6 and 59 Da).

CAUTION: *The variation between the resolution of the peaks at different masses must be kept at less than 0.1 Da. If the variation is too high, IntelliStart will not be able to set the resolution correctly.*

Ensure the masses are within 1.0 Da of their nominal mass positions.

20. If the resolution and mass position are not acceptable retune the resolution and mass position sliders on the Resolution tab on the Tune page.

NOTE: *The mass position and resolution values are set in the factory and stored on the flash memory card on the EPC. They require very little changing on installation.*

21. Print out the labeled spectrum when the mass scale and resolution is within specification.

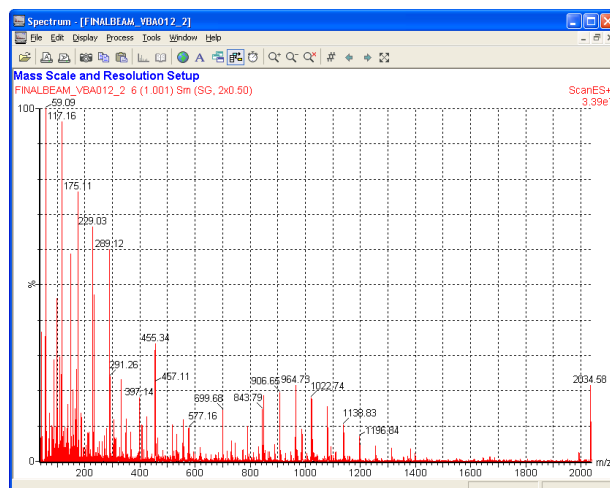


Figure 41 - A typical MS1 spectrum (setup solution)

22. Print out each individual peak (175.1, 455.3, 1080.8, and 2034.6).

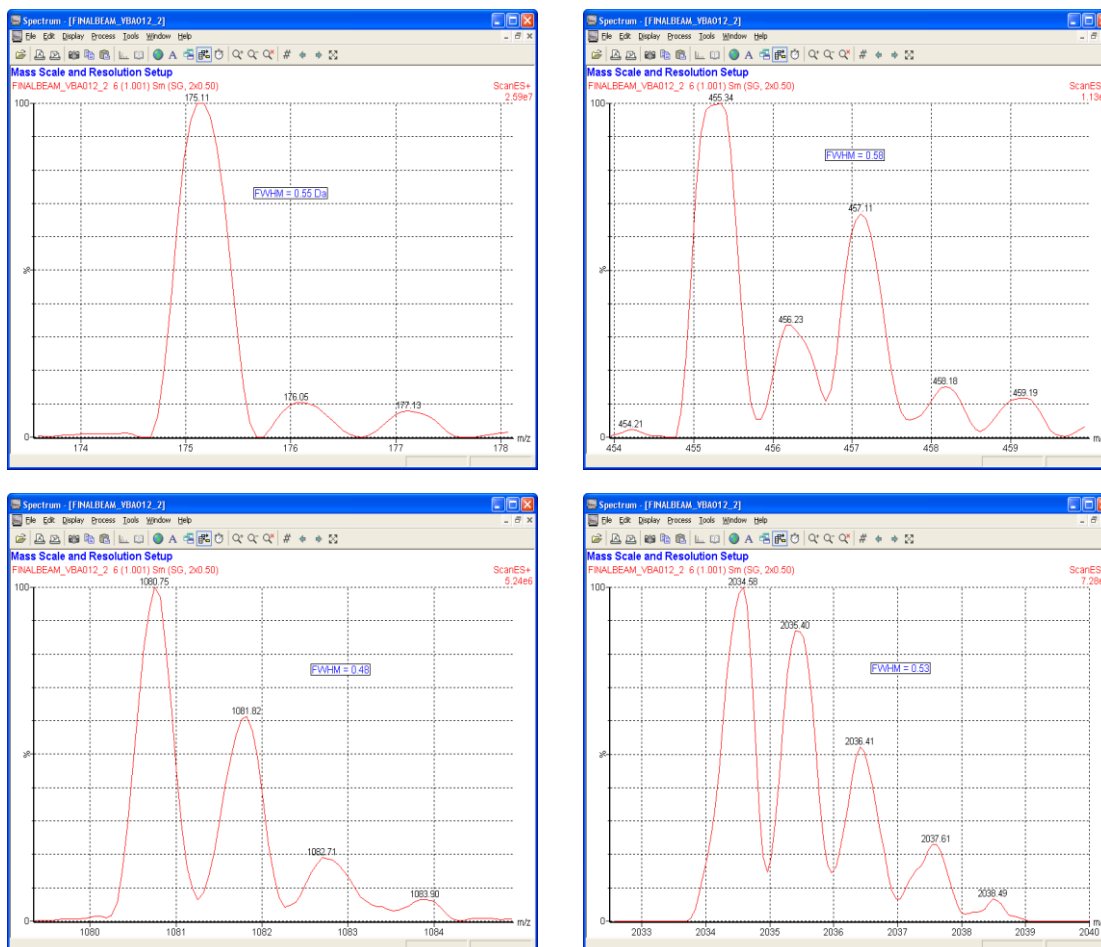


Figure 42 - Individual peaks (175.1, 455.3, 1080.8, and 2034.6)

Acquiring MS2 data

1. Turn on the collision gas and set the gas pressure to 3.5E-3 mbar.
2. Select MS mode for each of the four Tune page mass windows.
3. Display the four MS scan masses on the Tune page.
4. Ensure the mass position is within 1.0 Da of the expected nominal mass.
5. Ensure the resolution for all four peaks is between 0.4 to 0.6 Da (FWHM).
6. Check the resolution of the peak at 59 Da.
7. Click the **Resolution** tab of the MS Tune page.
8. Select the **Instrument_Setup.ipr** tune file for the MS2_MassScaleResolution_1 sample in the sample list.
9. Adjust the resolution, mass position, and linearity parameters, as required.
10. Click the the **MS2_MassScaleResolution_1** sample (Mass Scale and Resolution Set Up MS1) in the sample list, then click **Start**.
11. Repeat steps 10 to 22 of "Acquiring MS1 data" to process the acquired spectrum.

CAUTION: *On the Xevo TQ-S, "MS Scan" refers to MS2 and "MS1 Scan" refers to MS1.*

Backing up the flash memory settings

It is advisable to backup the information on the instrument flash memory to the host PC in case of accidental loss of settings.

1. Click the **Advanced** tab of the MS Tune page, and deselect **Manual Control**.
2. Place the instrument into Standby.
3. Exit MassLynx.
4. Browse to and double-click the C:\MassLynx\Vulcan\TargetRegistryEditor.exe file.
5. Enter **1147** as the password.

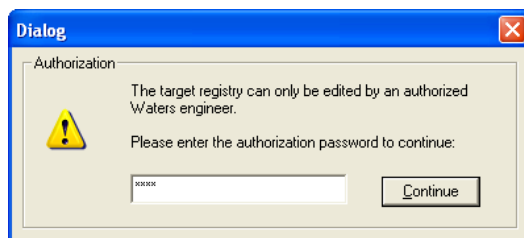


Figure 43 - Opening the Target Registry Editor

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

6. Right-click in the view and select **Export All**.
7. Export the settings to an appropriate folder on the host PC.
8. When complete, close the Target Registry Editor and re-open MassLynx.

Checking firmware revisions

Ensure that the PCB firmware is up to date. Current issue firmware can be found in the service note [Firmware: Xevo TQ-S](#).

ScanWave optimization

Load the factory ScanWave optimizations for Positive and Negative Ion modes from the DVD shipped with the instrument. The DVD is marked with the instrument serial number.

Positive enhancement check at 5000 Da/s

1. Load the saved positive optimization file, and click on **Setup > ScanWave > Optimisation > Optimisation Enabled** in the menu, to ensure that optimization is enabled.
2. Infuse 100 fmol/ μ L [Glu¹]-fibrinopeptide B at 10 μ L/min using the integrated fluidics.
3. Open one MS Tune page at 786 Da.
4. Adjust the source parameters so that the intensity of the precursor ion is <10% when the Tune page peak window gain is set to 1.
5. Leave the LM resolution on 3.0 and HM resolution on 15 and make a note of this position. Use this method in the "Daughters of" in the method editor, see [Figure 44](#).
6. Open the collision gas and set the collision gas pressure to **3E-3 mbar**.
7. Create two experimental methods, one with a single daughter scan and the other with a single ScanWave DS using the parameters in [Figure 44](#).

NOTE: *It is recommended that the collision cell pressure is set at 3E-3 mbar.*

The image shows two side-by-side software dialog boxes. The left dialog is titled "Function:1 Daughter Scan" and the right dialog is titled "Function:1 ScanWave DS". Both dialogs have a similar layout with tabs for "Mass (m/z)", "Time (Mins)", "Collision Energy", and "APol Probe".

Function:1 Daughter Scan parameters:

- Mass (m/z):** Daughters of: 785.84, Start: 100.00, End: 1100.00
- Time (Mins):** Start: 0, End: 2
- Collision Energy:** Use Tune Page: ☐, Collision Energy (V): 30, Use Collision Energy Ramp: ☐
- Cone Voltage:** Use Tune Page: ☒, Cone Voltage (V): 30, Use Cone Voltage Ramp: ☐
- APol Probe:** Use Tune Page Settings: ☐, Probe Temp: 600

Function:1 ScanWave DS parameters:

- Mass (m/z):** Daughters of: 785.84, Start: 100.00, End: 1100.00
- Time (Mins):** Start: 0, End: 2
- Scan Duration (secs):** Scan Speed: 5000 amu/sec, Scan Time: 0.2
- Collision Energy:** Use Tune Page: ☐, Collision Energy (V): 30, Use Collision Energy Ramp: ☐
- Cone Voltage:** Use Tune Page: ☒, Cone Voltage (V): 30, Use Cone Voltage Ramp: ☐
- APol Probe:** Use Tune Page Settings: ☐, Probe Temp: 600

Figure 44 - Daughter scan (left) and ScanWave DS (right) parameters

8. Create two runs on the sample list; one for the daughter scan experiment and one for the ScanWave DS experiment, acquire both runs.

NOTE: *Ensure that the sample text clearly indicates which experiment is being run.*

9. Open the resulting chromatograms on a single page and smooth both with the settings shown in [Figure 45](#).

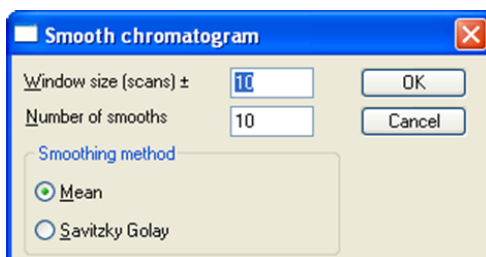


Figure 45 - Smoothing the chromatogram

10. Compare the smoothed chromatograms. Ensure the ratio of the maximum TIC values is $> \times 7$.

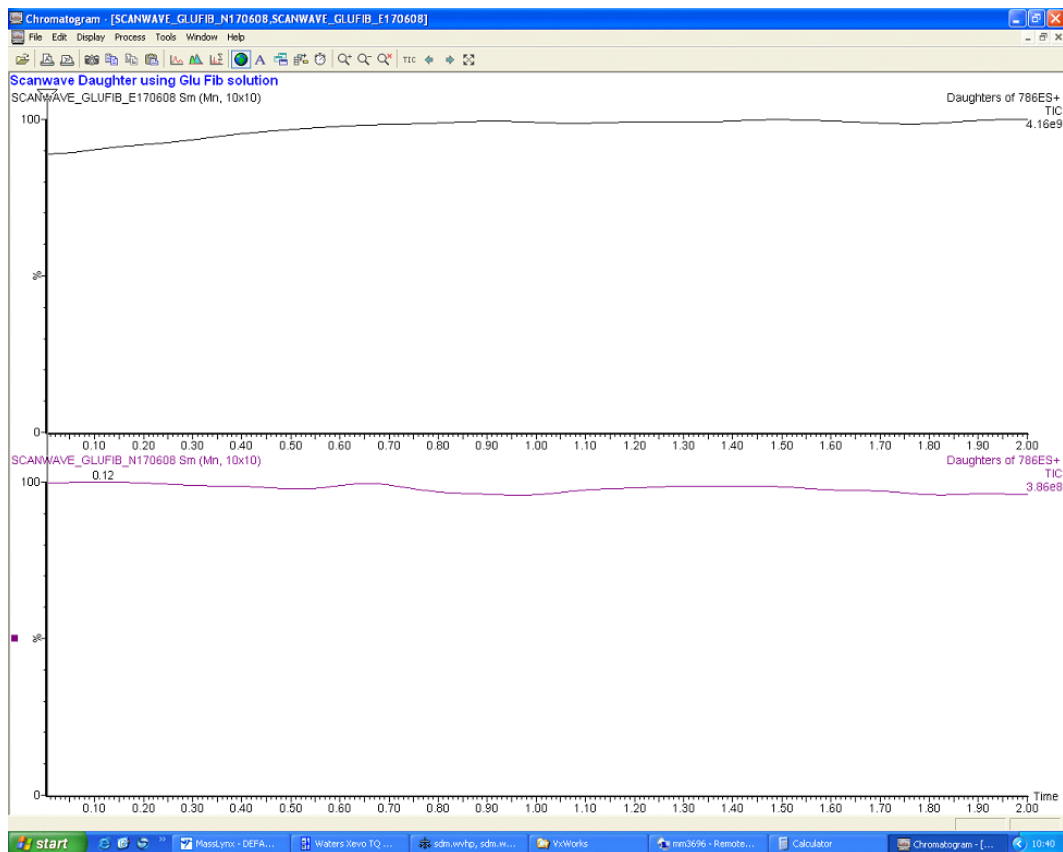


Figure 46 - Comparing the chromatograms

Negative enhancement check at 5000 Da/s

1. Load the saved negative optimization file, and click on **Setup > ScanWave > Optimisation > Optimisation Enabled** in the menu, to ensure that optimization is enabled.
2. Infuse 300 fmol/ μ L [Glu¹]-fibrinopeptide B at 10 μ L/min (20 μ L/min can be used if the signal intensity is very low) using the integrated fluidics.
3. Open one MS Tune page at 784 Da.
4. Adjust the source parameters so that the intensity of the precursor ion is $\sim 10\%$ when the Tune page peak window gain is set to 1.
5. Create two experimental methods, one with a single daughter scan and the other with a single ScanWave DS using the parameters shown in Figure 47.

NOTE: *It is recommended that the collision cell pressure is set at 3E-3 mbar.*

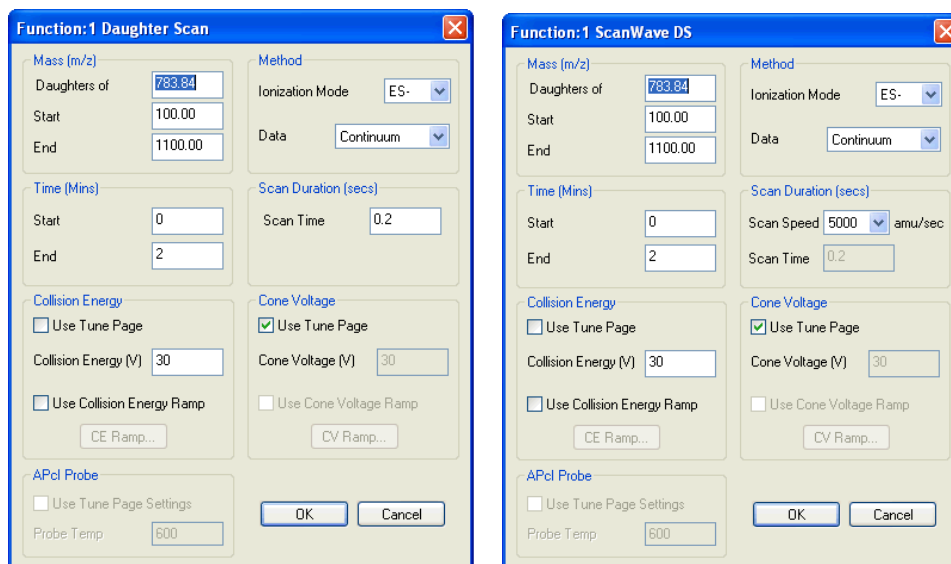


Figure 47 - Daughter scan (left) and ScanWave DS (right) parameters

6. Create two runs on the sample list, one for the daughter scan experiment and one for the ScanWave DS experiment, and acquire both runs.

NOTE: *Ensure that the sample text clearly indicates which experiment is being run.*

7. Open the resulting chromatograms on a single page and smooth both with the following settings.

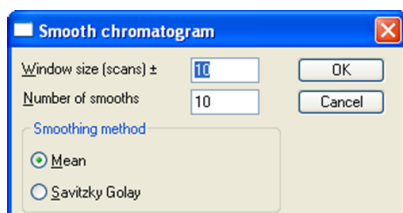


Figure 48 - Smoothing the chromatogram

8. Compare the smoothed chromatograms. Ensure the ratio of the maximum TIC values is $> \times 7$.

ScanWave specification failure

If the ScanWave specification fails, perform the following steps:

1. Open a single enhanced daughter window with the following parameters:
 - Set mass Value centred on the [Glu¹]-fibrinopeptide B peak (~786 Da)
 - Mass **600 Da**
 - Span **900**
 - Enhanced scope speed **1000 Da/s**
 - Interscan delay **0.02 s**
2. Tune the collision energy until the following conditions are met:
 - Highest massed peak used for the specification is present
 - Intensity of the lower massed daughter ions optimized
3. Modify the experiment files for both the enhanced and normal runs with this voltage value.
4. Set the flow rate to give a collision cell pressure of 3.0E-3 mbar.
5. Check the Penning gauge is 2.0E-5 mbar.

CAUTION: *If the pressure is too high, enhanced ScanWave may fail.*

6. Check the intensity of the MS1 (no collision gas) is 786 Da (positive) and 784 Da (negative).
 - a. Set the gain value to 1.
 - b. Tune the signal intensity to give a maximum of 10% of the window height (~1.80E7).
7. Tune the MS1 negative to maximize the intensity.
8. If the problem arises during the negative ScanWave, perform the following steps:
 - a. Check the infusion flow rate is 20 µL/min.
 - b. Tune until the enhanced TIC is >7E7 (when smoothed).

NOTE: *The fault may be caused by the amount of sample entering the instrument, rather than the ScanWave function.*

9. If automatic Advanced tuning is not being used for the differential aperture, entrance and exit lenses, ensure that the settings are the same for both daughter and enhanced daughter tuning.
10. Inspect the peaks ~0.5 Da FWHH on a combined spectra from the daughter scan and the enhanced daughter scan.
11. If the enhanced daughter scan peaks are reasonably intense but split, set the manual T-Wave pulse velocity to **142**.
12. Repeat the acquisition.
13. If the ScanWave optimization procedure continues to fail, perform the procedure in the Advanced Troubleshooting section of the [ScanWave Collision Cell Assembly Technical Reference Sheet](#) (715002816).

MS calibration using IntelliStart

Perform an instrument resolution setup and calibration for unit mass resolution mode in both positive and negative ion polarity:

1. Click the **MS Console** icon on the main MassLynx page.
2. Ensure a bottle calibration solution (phosphoric acid) is installed in fluidics reservoir B, and that the sample line has been purged twice.
3. Ensure a bottle IntelliStart resolution solution (sodium iodide/cesium iodide/methyl sulphate) is installed in fluidics reservoir C, and that the sample line has been purged twice.

CAUTION: *The IntelliStart resolution solution is supplied at high concentration and must be diluted before use, as described in [Preparing Test Samples / Xevo TQ-S \(715002796\)](#).*

4. Place the instrument in Operate and switch on the API gas.
5. Click the **IntelliStart** menu.
6. Specify the instrument resolution, ion polarity, and instrument calibration options, by clicking on the relevant check boxes as shown in [Figure 49](#).

NOTE: *Instrument Set-up options must be selected to access the Instrument Resolution and Instrument Calibration options.*

7. Click .

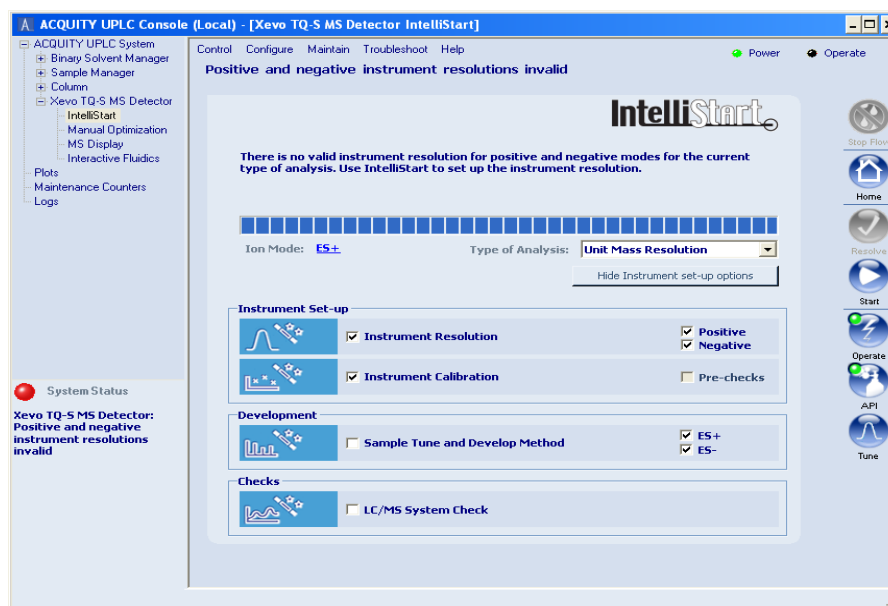
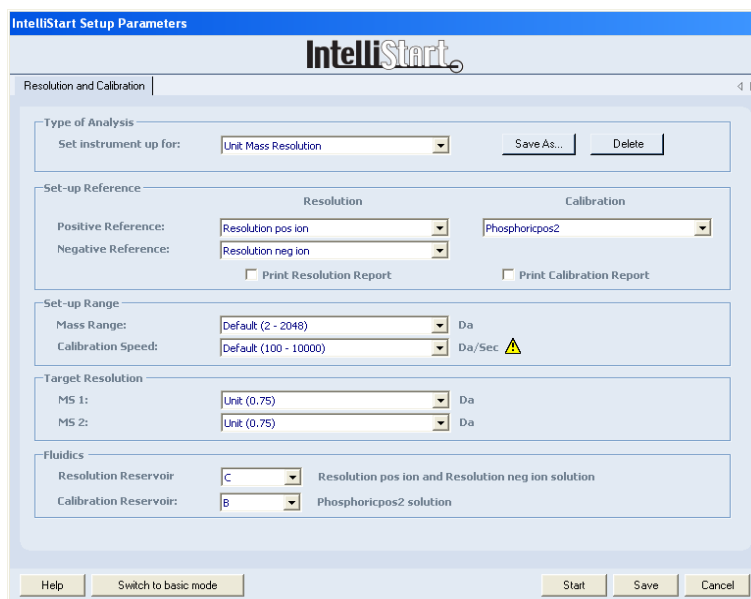


Figure 49 - Specifying IntelliStart options

8. Set up the following parameters in the Intellistart Setup Parameters dialog:
 - Set instrument up for **Unit Mass Resolution**
 - Resolution Reservoir **C**
 - Calibration Reservoir **B**



The image shows the 'IntelliStart Setup Parameters' dialog box. It has a title bar with the 'IntelliStart' logo. The 'Resolution and Calibration' tab is selected. The 'Type of Analysis' section has 'Unit Mass Resolution' selected in the dropdown, with 'Save As...' and 'Delete' buttons. The 'Set-up Reference' section has 'Resolution' and 'Calibration' sub-sections. Under 'Resolution', 'Positive Reference' is 'Resolution pos ion' and 'Negative Reference' is 'Resolution neg ion', with checkboxes for 'Print Resolution Report' and 'Print Calibration Report'. Under 'Calibration', 'Positive Reference' is 'Phosphoricpos2' and 'Negative Reference' is 'Phosphoricpos2', with a checkbox for 'Print Calibration Report'. The 'Set-up Range' section has 'Mass Range' set to 'Default (2 - 2048)' and 'Calibration Speed' set to 'Default (100 - 10000)'. The 'Target Resolution' section has 'MS 1' and 'MS 2' both set to 'Unit (0.75)'. The 'Fluidics' section has 'Resolution Reservoir' set to 'C' and 'Calibration Reservoir' set to 'B'. At the bottom are 'Help', 'Switch to basic mode', 'Start', 'Save', and 'Cancel' buttons.

Figure 50 - Specifying the IntelliStart parameters

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

9. Click **Start** to begin the IntelliStart optimization.
10. Ensure that the procedure is completed successfully (signified by cross check marks appearing in the Instrument Set-up icons) (Figure 51).

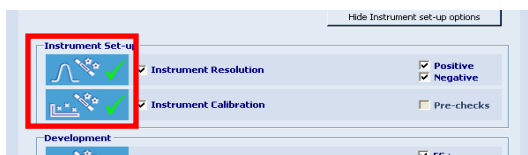


Figure 51 - Successful completion of Intellistart resolution and calibration.

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

After calibration, the cone may require cleaning. Rinse with water, and sonicate with 50:50 methanol/water. Methanol alone may not remove all the salts from the cone.

Performance tests

This section describes how to perform the performance tests during the installation of the Xevo TQ-S. The system-level tests monitor the performance of the entire Xevo TQ-S UPLC system (UPLC, Xevo TQ-S, column, sample and also solvents, for example).

The Xevo TQ-S standalone sensitivity tests monitor the performance of the Xevo TQ-S instrument alone. It is not necessary to complete the standalone sensitivity tests if the system-level tests have been passed.

If the system fails to meet the system-level specifications, the standalone sensitivity tests can be used to confirm the functionality of the Xevo TQ-S.

The results of these performance tests must be recorded in the installation checklist, and all data must be printed and correctly annotated.

CAUTION: *High background levels can be caused by contamination from LC systems. It may be necessary to flush the LC to pass the sensitivity specifications. For further information, refer to [Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems \(715001307\)](#), and [Cleaning the ACQUITY in LC/MS Systems \(715004064\)](#).*

The tests described are split into groups:

Xevo TQ-S ACQUITY, H-Class, or I-Class System and Xevo TQ-S (standalone) tests

- Resolution verification MS1
- Resolution verification MS2

Xevo TQ-S ACQUITY, H-Class, or I-Class System tests

- Gradient performance test
- ESI positive sensitivity and precision (also applies to UniSpray)
- ESI negative sensitivity and precision
- APCI sensitivity and precision (option)
- TUV and PDA precision tests (options)
- ELS and FLR tests (options)

Xevo TQ-S (standalone) tests

- ESI positive sensitivity (also applies to UniSpray)
- ESI negative sensitivity

Source options tests

- APCI sensitivity (system level)
- APCI sensitivity (standalone)
- APPI sensitivity
- NanoFlow sensitivity

NOTE: *A standalone Xevo TQ-S comprises any instrument installed with an LC system other than an ACQUITY UPLC.*

Xevo TQ-S ACQUITY, H-Class, or I-Class System and Xevo TQ-S (standalone) tests

Samples required

Table 8: Performance test sample

Test	Sample
High mass resolution verification	Setup Solution Xevo TQ-S

Resolution verification, positive ion, MS1

On infusion of Setup Solution Xevo TQ-S at 10 $\mu\text{L}/\text{min}$, the valley between the 2034.63 and 2035.63 Da peaks is <12% of the average height of the two peaks.

1. Attach a bottle containing Setup Solution Xevo TQ-S the reservoir B and purge twice.
2. Infuse Setup Solution Xevo TQ-S at 10 $\mu\text{L}/\text{min}$.
3. In the MS Tune page, open one MS1 scan mass window at m/z 2034.6 with a span of 5.
4. Set the initial source parameters as shown in [Figure 52](#).
5. Ensure the collision gas is turned off.
6. Tune the cone voltage to obtain maximum signal intensity.
7. Tune the MS1 resolution and ion energy values to obtain the best possible resolution.

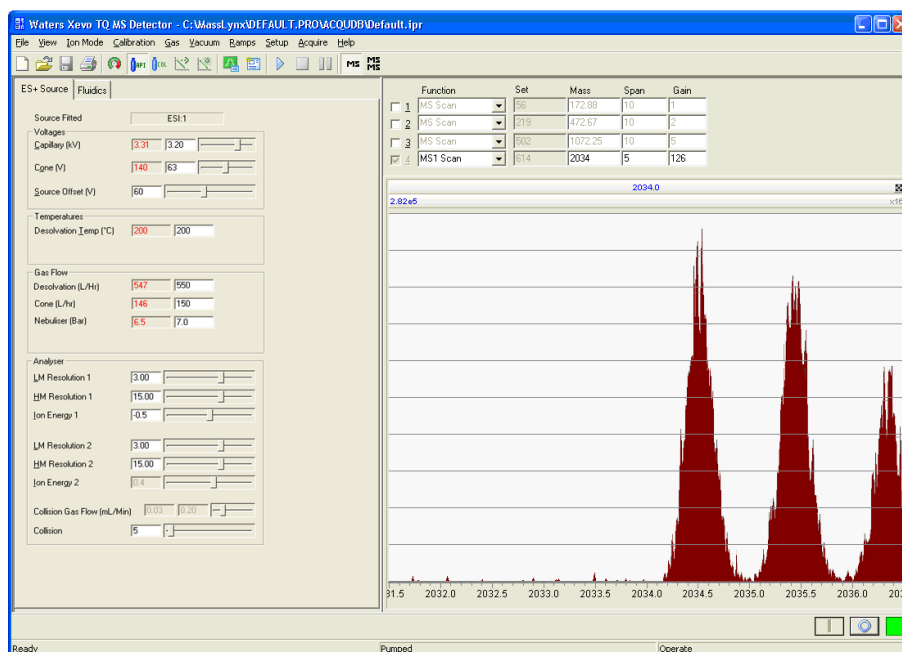


Figure 52 - Tuning for high mass resolution

8. Save the tune file as **MS1_HighMassVerification.ipr** and ensure that this Tune file has been selected in the sample list for the **MS1_HighMassVerification_1** sample.

9. Highlight the **MS1_HighMassVerification_1** sample (MS1 Mass Scale and Resolution Set Up) in the sample list, then click **Start**.

NOTE: The MS method parameters are shown in [Figure 53](#) for reference.

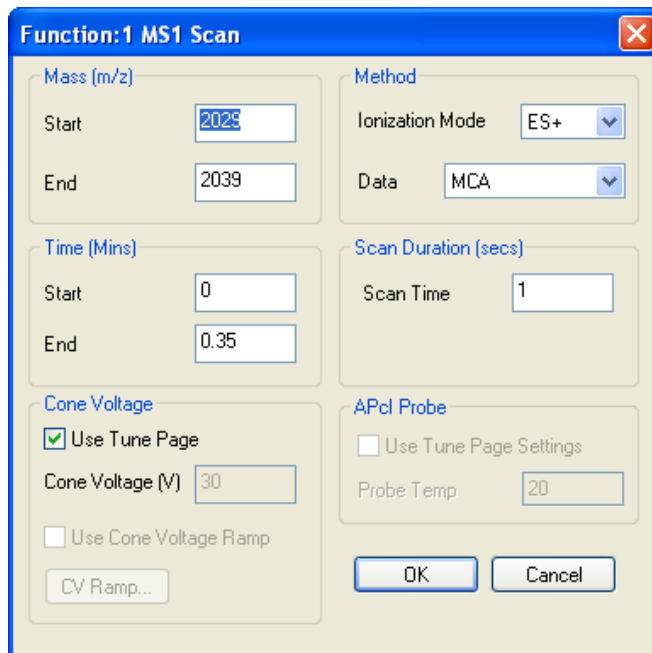


Figure 53 - MS method editor parameters

10. In the Spectrum viewer, click **Process > Smooth...** to smooth the spectrum with the settings shown in [Figure 54](#).

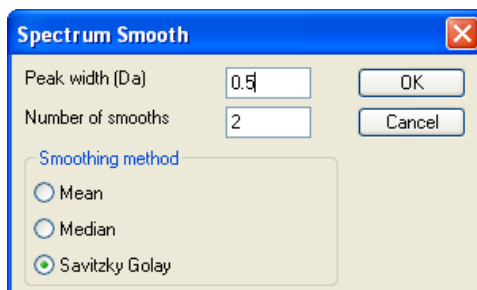


Figure 54 - Smoothing parameters

11. Measure the valley by clicking and dragging from the bottom of the valley to the average of the first two peaks.

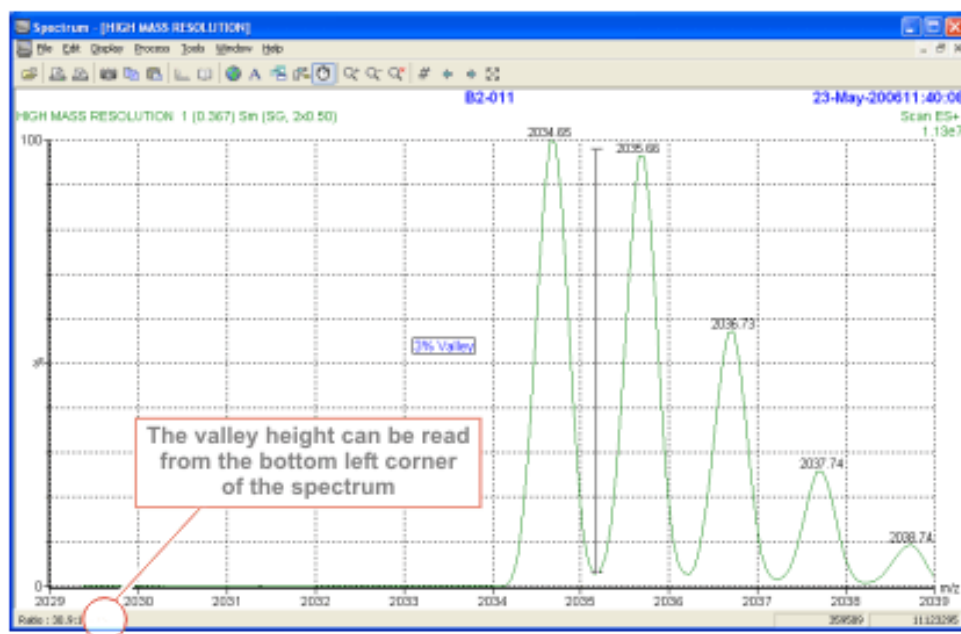


Figure 55 - Measuring the valley

12. Ensure the valley between m/z 2034.6 and 2035.6 is <12%.
13. Label the spectrum and print it.

Resolution verification, positive ion, MS2

On infusion of Setup Solution Xevo TQ-S at 10 $\mu\text{L}/\text{min}$, the valley between the 2034.63 and 2035.63 Da peaks is <12% of the average height of the two peaks.

1. Ensure the collision gas is turned on for MS acquisition.
2. Tune the source and source and analyser parameters to obtain best resolution on of the 2034.6 peak in MS mode.
3. Save the tune file as MS2_HighMassVerification.ipr.
4. Select this Tune file in the sample list for the MS_HMR_01 sample.
5. Click the **MS2_HighMassVerification_1** sample in the sample list, then click **Start**.
6. Process the spectrum in the same way as the MS1 spectrum.

CAUTION: On the Xevo TQ-S, "MS Scan" refers to MS2, "MS1 Scan" refers to MS1.

Xevo TQ-S ACQUITY, H-Class, or I-Class System tests

This section describes the tests required to verify the performance of the Xevo TQ-S ACQUITY UPLC System with all ACQUITY types. Specifically it describes tests of the Xevo TQ-S sensitivity and system reproducibility.

Samples required

NOTE: *If these system tests have been completed successfully it is not necessary to complete the Xevo TQ-S standalone sensitivity tests.*

Table 9: Performance test sample

Test	Sample
Gradient performance test	Six compound mix (700005472-1)
ESI positive sensitivity and precision	Six compound mix (700005472-1)
ESI negative sensitivity	Six compound mix (700005472-1)
UniSpray sensitivity and precision (option)	Six compound mix (700005472-1)
APCI sensitivity (option)	Six compound mix (700005472-1)
TUV precision (option)	10 ng/μL sulfadimethoxine (700002741)
PDA precision (option)	10 ng/μL sulfadimethoxine (700002741)
ELS precision (option)	0.16 mg/mL caffeine (700002848)
FLR precision (option)	5.0 pg/μL anthracene (700003694)

Mobile phase preparation

CAUTION: *All solvents, including additives, must be of the highest chemical purity; a minimum of LCMS grade is recommended.*

Choice of solvent, particularly water, is of primary importance in obtaining optimum performance. Failure to use high quality solvents will result in signal suppression, high background noise levels, and loss of sensitivity. An order of magnitude difference may result from water quality alone before other sources of system contamination/suppression are taken into account.

Deionized water from a well maintained in-house supply, for example Milli-Q or Elga, is the preferred option. Bottled water may be influenced by age and the material in which it is stored, as well as the original level of contaminants.

NOTE: See "[Appendix D: Solvent quality check \(LC/MS system test\)](#)" for a procedure to check solvent quality, specifically the sulfadimethoxine sodiation level.

CAUTION: ***Glassware must not be washed with detergents or washed with other general glassware. The glassware must be rinsed with the high purity solvents before use.***
Ensure that you have carried out the procedures required to prevent contamination, as detailed in [Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems \(715001307\)](#).

NOTE: Ammonia is supplied in the sample kit. If this is not available use aqueous ammonia with a specific gravity of 0.88 to 0.89 (~30% w/w ammonia).

Preparing the ACQUITY, H-Class, or I-Class System

NOTE: These tests require that the ACQUITY UPLC is fitted with a 10 μ L sample loop, 30 μ L PEEK needle, and 100 μ L metering syringe.

Table 10: ACQUITY lines

Solvent	ACQUITY	ACQUITY H-Class	ACQUITY I-Class (SM-FTN)	ACQUITY I-Class (SM-FL)
Water + 0.01% formic acid + 0.05% ammonia solution	N/A	N/A	SM-FTN: SM PURGE	SM-FL: SM PURGE
Add 100 μ L formic acid and 500 μ L ammonia solution to 1 L water	BSM: A1 and A2	QSM: A and C SM-FTN: SM PURGE and Seal Wash	BSM: A1 and A2	
100% acetonitrile + 0.01% formic acid Add 50 μ L formic acid to 500 mL of acetonitrile	BSM: B1 and B2 SM: Strong Wash	QSM: B and D SM-FTN: Strong needle Wash	BSM: B1 and B2 SM-FTN: SM Wash	BSM: B1 and B2 SM-FTN: Strong needle Wash
90:10 water/acetonitrile + 0.01% formic acid	BSM: Seal Wash SM: Weak Wash	N/A	BSM: Seal Wash	N/A

1. Place the lines in the relevant reservoir bottles as described in [Table 10](#).
2. Click **Control > Acquity UPLC System > System Start Up** to open the System Startup dialog box.
3. Prime each solvent line and wash line, using the settings shown in the appropriate table.

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

Preparing the diluent

1. Measure 500 mL of filtered LCMS-grade water, then transfer to a 500-mL reservoir bottle.

WARNING: Formic acid is hazardous. Take appropriate precautions and follow all Waters and customer health and safety procedures when using it.

2. Pipette 50 μ L of formic acid into the 500-mL reservoir bottle.
3. Pipette 250 μ L of ammonia solution into the 500-mL reservoir bottle

4. Cap the reservoir bottle and mix well.
5. Label the reservoir bottle as "Diluent".

Table 11: ACQUITY parameters table

Tabs	Parameters		
Prime solvents (BSM)	Prime A1 Prime A2 Prime B1 Prime B2 Prime seal wash		Checked
	Duration of prime		5 minutes
Prime solvents (SM)	Strong wash Weak wash Sample syringe		Checked
	Cycles		3
Optional:Characterize Volume (SM)	Characterize seal Characterize needle and loop volumes		Checked
	Configuration: Loop Needle		10.0 µL 30 µL
Equilibrate to method	QSM	Flow	0 mL/min
		Solvent A	100%
		Solvent B	0%
	CM	Column	Off
	SM	Sample	Off
	Other	Lamp On	Unchecked (Checked, only when PDA/TUV/FLR present)

Table 12: H-Class parameters table

Tabs	Parameters		
Prime solvents (QSM)	A B C D Seal wash		Checked
	Duration of prime		5 minutes
Prime solvents (SM)	Wash solvent		60 sec
	Purge solvent		20 cycles
Optional:Characterize Volume (SM)	Characterize seal		Unchecked
Equilibrate to method	QSM	Flow	0 mL/min
		Solvent A	100%
		Solvent B	0%
		Solvent C	0%
		Solvent D	0%
	CM	Column	Off
	SM	Sample	Off
	Other	Lamp On	Unchecked (Checked, only when PDA/TUV/FLR present)

Table 13: I-Class parameters table

Tabs	Parameters		
Prime solvents (BSM)	A1 B1 A2 B2 Seal wash		Checked
	Duration of prime		5 minutes
Prime solvents/SM	Wash solvent		60 sec
	Purge solvent		20 cycles
Optional:Characterize Volume (SM)	Characterize seal		Unchecked
Equilibrate to method	QSM	Flow	0 mL/min
		Solvent A	100%
		Solvent B	0%
	CM	Column	Off
	SM	Sample	Off
	Other	Lamp On	Unchecked (Checked, only when PDA/TUV/FLR present)

6. Place the ACQUITY UPLC outlet tubing into a suitable waste container.
7. Flush the system to waste using a 50:50 mixture of the mobile phase at 2 mL/min for 10 minutes.
8. Attach the inlet of the ACQUITY UPLC BEH C₁₈ (2.1 x 50-mm, 1.7- μ m) column (186002350) and place it in the column heater.
9. Connect the outlet of the coulmn to the port marked LC on the Xevo TQ-S diverter valve.
10. Place a 2-mL vial containing 1 mL of blank (diluent) into position 1:1 of the sample manager.
11. Place a 2-mL vial containing 1 mL of gradient, sensitivity and precision test sample into position 1:2 of the sample manager.
12. From the Inlet Method editor, click **Acquity sampler > Plate Generator** to open the rack editor.
13. Continue to press the **Previous Rack** icon until **ANSI-48Vial2mLHolder** appears in the Rack Name field.
14. Enter the parameters shown in [Figure 56](#).

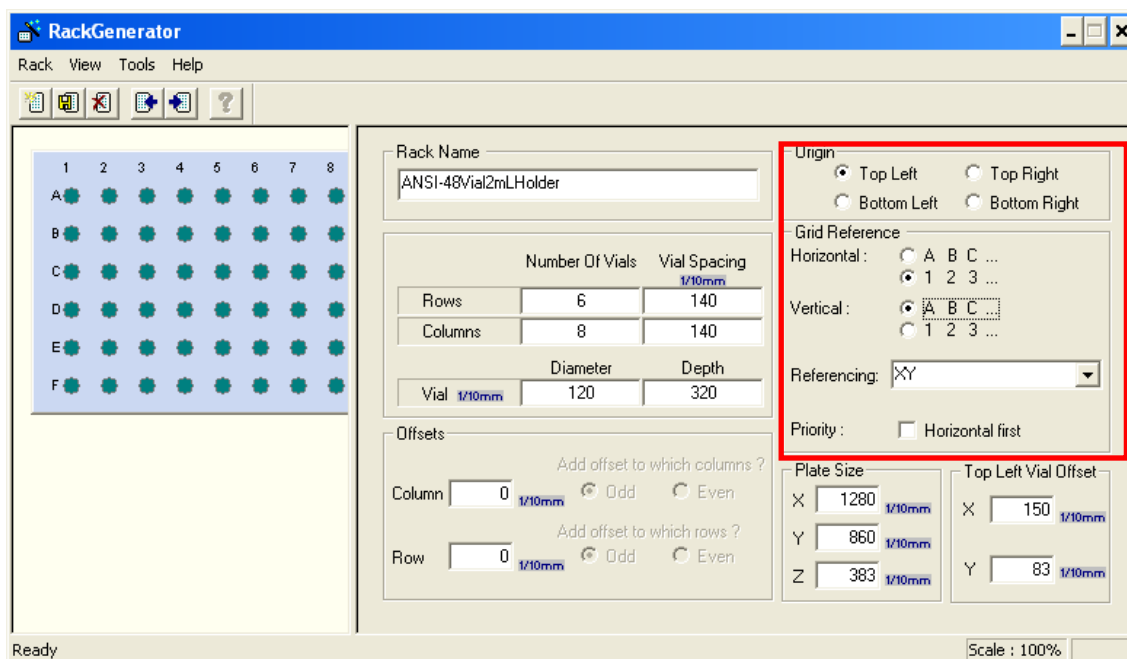


Figure 56 - Rack generator settings for ANSI-48Vial2mLHolder

- Click **Rack > Save Current Rack**, which saves the vial location format.

Creating the ACQUITY inlet method

The inlet method for the Xevo TQ-S ACQUITY system tests is included in the installation project with the file name "Gradient". The settings are shown in the following tables.

Table 14: ACQUITY BSM inlet method parameters

Parameter	Value
Run time	2.50 min
Pressure limit low	0 PSI
Pressure limit high	15000 PSI
Seal Wash	1 min
Solvent A1	Water (+0.01% formic acid +0.05% ammonia)
Solvent B1	Acetonitrile (+0.01% formic acid)

Table 15: ACQUITY BSM inlet gradient

Step	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

Table 16: ACQUITY SM parameters

Parameter	Value
Run time	2.50 min
Sample loop option	Full loop
Weak wash solvent	800 µL water: acetonitrile 90/10 (+0.01% formic acid)
Strong wash solvent	200 µL acetonitrile (+0.01% formic acid)
Loop offline	Disable
Load ahead	Disable
Column temperature	40.0 °C
Sample temperature	15.0 °C

Table 17: ACQUITY SM advanced parameters

Parameter	Setting
Full loop overfill factor	De-selected
Syringe draw rate	De-selected
Needle placement (from bottom)	De-selected
Air gaps	De-selected

Creating the H-Class inlet method

The inlet method for the Xevo TQ-S H-Class system tests is included in the installation project with the file name "Gradient". The settings are shown in the following tables.

Table 18: H-Class QSM inlet method parameters

Parameter	Value
Run time	2.50 min
Pressure limit low	0 PSI
Pressure limit high	15000 PSI
Seal Wash	1 min
Solvent A	Water (+0.01% formic acid+0.05% ammonia)
Solvent B	Acetonitrile (+0.01% formic acid)
Solvent C	Water (+0.01% formic acid+0.05% ammonia)
Solvent D	Acetonitrile (+0.01% formic acid)

Table 19: H-Class QSM inlet gradient

Step	Time (min)	Flow (mL/min)	% A	% B	% C	% D	Curve
1	Initial	0.800	90.0	10.0	0.0	0.0	Initial
2	0.20	0.800	90.0	10.0	0.0	0.0	6
3	1.50	0.800	30.0	70.0	0.0	0.0	6
4	1.80	0.800	30.0	70.0	0.0	0.0	6
5	2.00	0.800	90.0	10.0	0.0	0.0	6
6	2.50	0.800	90.0	10.0	0.0	0.0	6

Table 20: H-Class FTN parameters

Parameter	Value
Wash solvent	Water (+0.01% formic acid+0.05% ammonia)
Pre-inject	0 sec
Post-inject	6 sec
Purge solvent	Acetonitrile (+0.01% formic acid)
Load ahead	De-selected
Loop offline	De-selected
Column temperature	40.0 °C
Sample temperature	15.0 °C

Table 21: H-Class FTN advanced parameters

Parameter	Setting
Syringe draw rate	De-selected
Needle placement (from bottom)	De-selected
Air gaps	De-selected
No injection	De-selected

Creating the I-Class inlet method

The inlet method for the Xevo TQ-S I-Class system tests is included in the installation project with the file name "Gradient". The settings are shown in the following tables.

Table 22: I-Class BSM inlet method parameters

Parameter	Value
Run time	2.50 min
Pressure limit low	0 PSI
Pressure limit high	15000 PSI
Seal Wash	1 min
Gradient start	At injection
Solvent A1	Water (+0.01% formic acid +0.05% ammonia)
Solvent B1	Acetonitrile (+0.01% formic acid)

Table 23: I-Class BSM inlet gradient

Step	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

Table 24: I-Class FTN parameters

Parameter	Value
Wash solvent	Acetonitrile (+0.01% formic acid)
Pre-inject	0 sec
Post-inject	6 sec
Purge solvent	Water (+0.01% formic acid+0.05% ammonia)
Load ahead	De-selected
Loop offline	De-selected
Column temperature	40.0 °C
Sample temperature	15.0 °C

Table 25: I-Class FTN advanced parameters

Parameter	Setting
Syringe draw rate	De-selected
Needle placement (from bottom)	De-selected
Air gaps	De-selected
No injection	De-selected

Preparing sample for gradient, sensitivity and precision tests

To prepare the test sample, use Xevo TQ-S stock solution (700005472-1) from the Waters Xevo TQ-S Sample Kit (700005472).

CAUTION: Do not use the stock solution if it has exceeded its expiry date.

Table 26: Xevo TQ-S stock solution components

Component	Neutral monoisotopic mass
Acetaminofen 2 ng/μL	151.07
Caffeine 2 ng/μL	194.09
Sulphadimethoxine 1 ng/μL	310.08
Verapamil 500 pg/μL	454.29
Chloramphenicol 500 pg/μL	322.00
17-α-hydroxyprogesterone 50 ng/μL	330.23

Preparing the tuning solution

1. Add 1 mL of stock solution to a 100-mL volumetric flask.
2. Make up to 100 mL using diluent.
3. Stopper and invert the flask several times to fully mix.
4. Label the flask as "Tuning Solution".

Preparing sample for gradient, sensitivity, and precision tests

1. Add 100 μL of tuning solution to a 2-mL vial.
2. Add 900 μL of diluent to the vial.
3. Cap and shake the vial to fully mix.
4. Label the vial as "Stock 1".
5. Add 100 μL of Stock 1 to a 2-mL vial.
6. Add 900 μL of diluent to the vial.
7. Cap and shake the vial to fully mix.
8. Label the vial as "Gradient, Sensitivity/Precision solution".

The target solution contains the component concentrations shown in [Table 27](#).

Table 27: System test sample components

Component	Concentration (pg/μL)	Test
Acetaminofen	0.2	Gradient
Caffeine	0.2	Gradient
Sulphadimethoxine	0.1	Gradient/precision/ESI/UniSpray positive sensitivity
Verapamil	0.05	Gradient
Chloramphenicol	0.05	ESI negative sensitivity
17-α-hydroxyprogesterone	5	Precision/APCI positive sensitivity

Tuning the Xevo TQ-S and running system tests

Before tuning the Xevo TQ-S and running system tests perform the following steps:

1. On the MS Tune page, click **Setup > Inter-scan Setup**.
2. Set the Interscan parameters to automatic.
3. Click the **Advanced Tune Parameters** tab and ensure the **Enable Manual Controls** check box is deselected.

Gradient performance MS method

The MS method for the gradient performance test is included in the installation project with the file name **Gradient**. The gradient performance test settings are shown in [Figure 57](#).

Function:1 MRM

Method

Ionization Mode: ES+

Span: 0.2

Use Tune Cone Voltage: ☐

Use Tune Collision Energy: ☐

Retention Window (Mins)

Start: 0

End: 2.5

APCI Probe

Use Tune Page Settings: ☐

Probe Temp: 20

PIC Scan

Use Default PIC Scan function: ☒

PIC Scan: ScanWave DS

Use Default Threshold: ☒

Activation Threshold: 20

Minimum Threshold: 500000

Use Default Collision Energy: ☒

Collision Energy: 20

Channels

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	Acetaminophen	152.0700	110.0600	☐	0.025	40	16	☐	
2	Caffeine	195.0900	138.0400	☐	0.025	40	16	☐	
3	Sulfadimethoxine	311.0800	156.0100	☐	0.025	40	20	☐	
4	Verapamil	455.2900	165.0900	☐	0.025	40	30	☐	

Add Delete Clear All Undo Redo Fill Down

OK Cancel

Figure 57 - Gradient performance test MS method

Creating an ESI sensitivity and precision MS method using IntelliStart

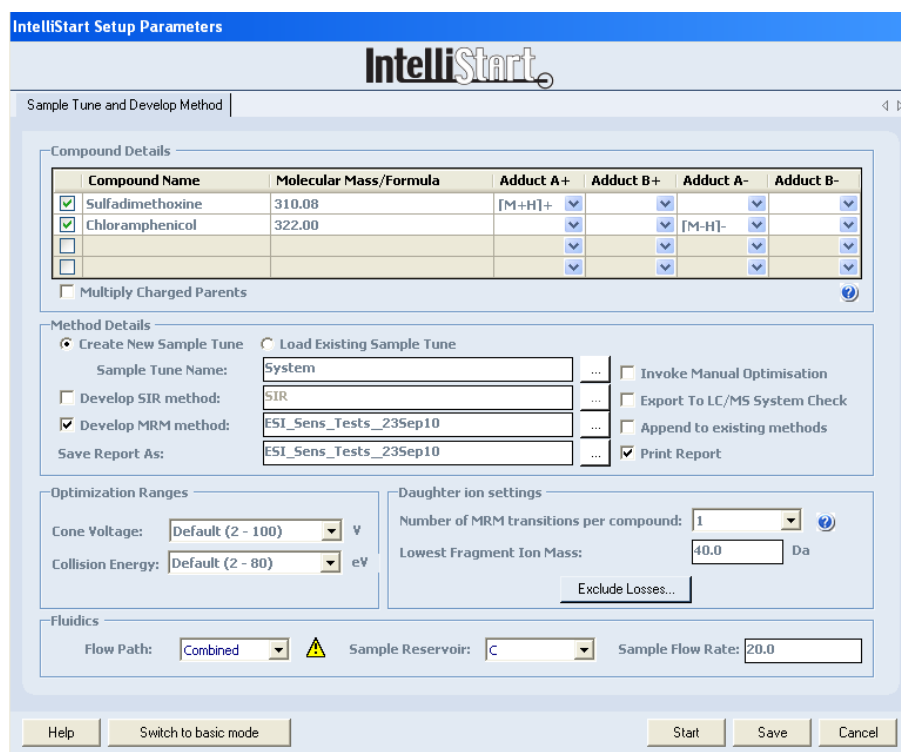
NOTE: Once created, this method can also be used for the UniSpray system sensitivity test.

1. Select the Xevo TQ-S window in the MS Console.
2. Place the instrument in Operate, with the API and collision gas flows turned on.
3. Set the ACQUITY UPLC to deliver 50:50 water/acetonitrile from A1:B1 at 0.8 mL/min.
4. Perform the following steps to set the initial ion probe position:
 - a. Set the initial horizontal position of the probe by setting the vernier screw on the top of the source housing (X) to 6 on the vernier scale.
 - b. Set the initial vertical position of the probe by setting the collar on the top of the probe (Z) to 7 on the vernier scale.
 - c. Turn the adjuster knob at the top of the probe to set the capillary position so that approximately 0.5 mm is visible at the probe tip.

5. Install a 30-mL bottle containing 10 mL of "Tuning Solution diluted" on fluidics reservoir C.
6. Click Interactive Fluidics > Enable Control.
7. Ensure reservoir **C, combined flow**, is selected and the purge procedure has completed.
8. Repeat the purge procedure.
9. Click **IntelliStart** and set the type of analysis to **Unit Mass Resolution**.

CAUTION: *The instrument resolution and calibration procedures must have been performed during the initial instrument setup section. If not, perform these procedures using IntelliStart, ensuring that both pass successfully.*

10. Select **Sample Tune and Develop Method**, ensuring that both **ES+** and **ES-** are selected.
11. Click .
12. Click **Switch to Advanced Mode** and set the parameters and file names as shown in Figure 58.



IntelliStart Setup Parameters

Sample Tune and Develop Method

Compound Details

Compound Name	Molecular Mass/Formula	Adduct A+	Adduct B+	Adduct A-	Adduct B-
☒ Sulfadimethoxine	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: System

☐ Develop SIR method: SIR

☒ Develop MRM method: ESI_Sens_Tests_23Sep10

Save Report As: ESI_Sens_Tests_23Sep10

☐ Invoke Manual Optimisation

☐ Export To LC/MS System Check

☐ Append to existing methods

☒ Print Report

Optimization Ranges

Cone Voltage: Default (2 - 100) V

Collision Energy: Default (2 - 80) eV

Daughter ion settings

Number of MRM transitions per compound: 1

Lowest Fragment Ion Mass: 40.0 Da

Fluidics

Flow Path: Combined

Sample Reservoir: C

Sample Flow Rate: 20.0

Buttons: Help, Switch to basic mode, Start, Save, Cancel

Figure 58 - IntelliStart method development ESI system tests

CAUTION: *The molecular mass values entered must be the neutral monoisotopic masses, not [M+H]⁺ m/z values.*

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

NOTE: A green check mark appears on the icon when the procedure has completed successfully.

IntelliStart automatically determines an MRM transition for sulfadimethoxine and chloramphenicol and optimizes the MS/MS parameters to create an MS method file.

14. From the MassLynx sample list page, open the MS method editor and browse to the **ESI_SENS_DDMMYY.exp** file created by IntelliStart.

NOTE: *IntelliStart may save the MS method outside of the current project, for example in the default, System QC or any other project that has been previously accessed through IntelliStart.*

15. Ensure the method contains two channels:

- Sulfadimethoxine in ESI positive (nominal mass 311 > 156)
- Chloramphenicol in ESI negative (nominal mass 321 > 152)

16. Set the retention end time to **2.5 minutes**.

17. Set the dwell times to **0.025 s**.

18. Save the method using the same file name.

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

NOTE: *The negative ion channel will be deleted shortly.*

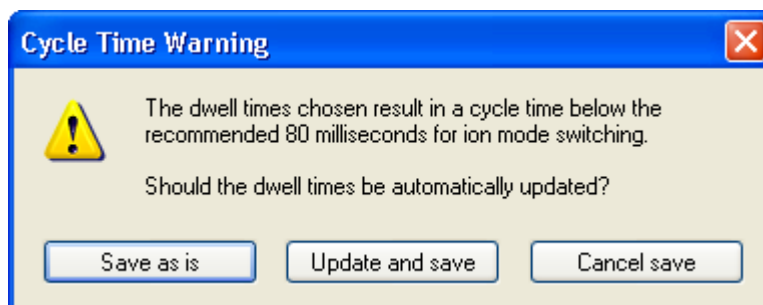


Figure 59 - Cycle time warning

Creating an ESI positive sensitivity and precision MS method

1. From the sample list page, open the MS method editor, and browse to the ESI_SENS_DDMmMY.exp file created by IntelliStart.
2. Delete the negative ion chloramphenicol channel and save the method as **ESI_POS_System_DDMmMY.exp**.

Function:1 MRM

Method

Ionization Mode: ES+

Span: 0

Use Tune Cone Voltage: ☐

Use Tune Collision Energy: ☐

Retention Window (Mins)

Start: 0

End: 2.5

APd Probe

Use Tune Page Settings: ☐

Probe Temp: 20

PIC Scan

Use Default PIC Scan function: ☒

PIC Scan: ScanWave DS

Use Default Threshold: ☒

Activation Threshold: 20

Minimum Threshold: 500000

Use Default Collision Energy: ☒

Collision Energy: 20

Channels

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	sulfadimethoxine	311.1757	156.0416	☐	0.025	38	18	☐	IntelliStart Generated

Add Delete Clear All Undo Redo Fill Down

OK Cancel

Figure 60 - Example MS method created for system precision/ESI positive sensitivity

Optimizing the capillary/impactor voltage and probe position

NOTE: The optimum probe positions are different for ESI positive, ESI negative, UniSpray positive, and APCI positive. The probe position must be optimized immediately before running the sensitivity acquisitions and must be re-optimized for each sensitivity specification.

1. From the MS console, click Xevo TQ-S Detector > Manual Optimization.
2. Open the **System.ipr** tune file.
3. Monitor the **311 > 156 MRM** transition and ensure that the cone voltage and collision energy values match those in the MS method created by IntelliStart.
4. Set the desolvation gas flow to **1000 L/Hr**, and the desolvation temperature to **600 °C**.
5. Select vial **C**, set the flow rate to **20 µL/min** combined flow and start the syringe pump.
6. Optimize the capillary (or impactor) voltage by entering the required value, and check for the intensity rising or falling, for an increasing or decreasing signal respectively.

NOTE: Right-click on the signal plot and click **Plot properties** to set the time window. Set the time window to 1 min to make optimization easier.

7. Optimize the probe position for maximum intensity.
8. Optimize the capillary length for maximum intensity.
9. Perform the following steps to minimize any thermal ionization:
 - a. Set the capillary voltage to zero.
 - b. Any residual signal observed from thermal ionization must be $<10\%$ of the optimized signal intensity. If not, move the ESI probe position away from the sample cone to achieve this.
 - c. If adjustment was required, re-optimize the capillary voltage in the new probe position.
10. Stop the syringe pump.
11. Click **Save** in the Manual Optimization IntelliStart window and save the tune file as **SYSTEM.ipr**.

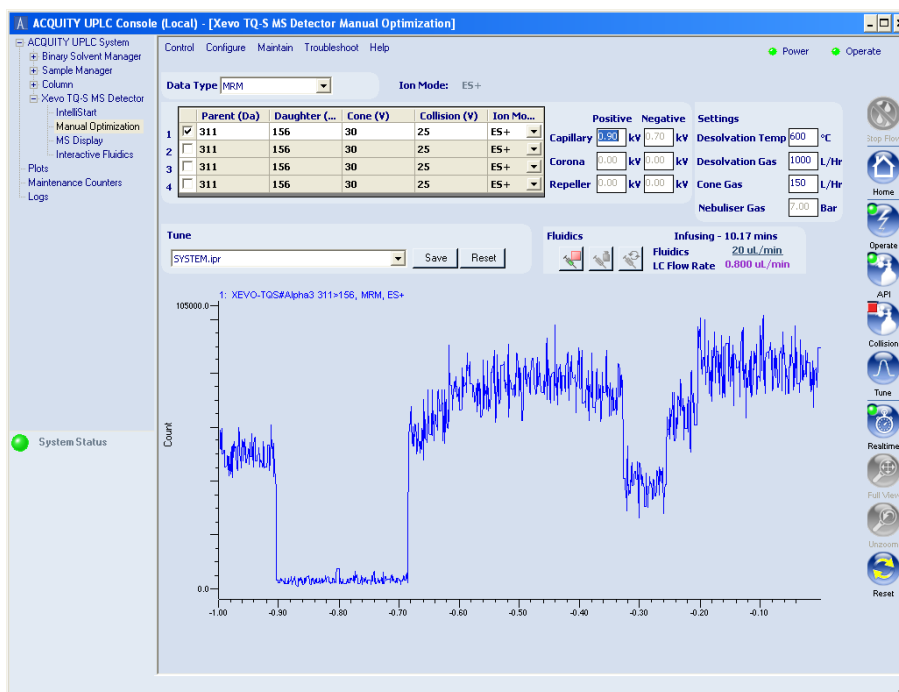


Figure 61 - Manual optimization of the capillary voltage and probe position

Creating an ESI negative sensitivity and precision MS method

1. From the MassLynx sample list page, open the MS method editor and browse to the **ESI_SENS_DDMMYY.exp** file created by IntelliStart.
2. Delete the positive ion sulfadimethoxine channel and save the method as **ESI_NEG_System_DDMMYY**.

Function:1 MRM

Method

Ionization Mode: ES-

Span: 0

Use Tune Cone Voltage: ☐

Use Tune Collision Energy: ☐

Retention Window (Mins)

Start: 0

End: 2.5

APol Probe

Use Tune Page Settings: ☐

Probe Temp: 20

PIC Scan

Use Default PIC Scan function: ☒

PIC Scan: ScanWave DS

Use Default Threshold: ☒

Activation Threshold: 20

Minimum Threshold: 500000

Use Default Collision Energy: ☒

Collision Energy: 20

Channels

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	Chloramphenicol	321.0319	151.9596	☐	0.025	28	16	☐	IntelliStart Generated

Add Delete Clear All Undo Redo Fill Down

OK Cancel

Figure 62 - Example MS method created for system precision/ESI negative sensitivity

Creating a sample list

1. Open the sample list as **WAAXXX_DDMMYY.spl** (where ddmmmyy = date, and XXX is the serial number of the instrument).
2. From the MS inlet editor, open the **Gradient** method.
3. Click  to load the initial conditions to the ACQUITY UPLC system (90:10 A1:B1 at 0.8 mL/min).
4. Equilibrate the column until the BSM system pressure is stable.

NOTE: Expect a Delta PSI value of less than 50 psi once the system has equilibrated.

	File Name	File Text	MS File	MS Tune File	Sample Type	Inlet File	Bottle	Inject Volume	Conc A	Inlet Presur
1	MS1_MassScaleResolution_1	MS1 Mass scale and resolution setup	MS1_MassScaleResolution	Instrument_Setup				0.000		
2								0.000		
3	MS2_MassScaleResolution_1	MS2 Mass scale and resolution setup	MS2_MassScaleResolution	Instrument_Setup				0.000		
4								0.000		
5	MS1_HighMassVerification_1	MS1 High mass resolution verification	MS1_HighMassVerification	MS1_HMVerification				0.000		
6								0.000		
7	MS2_HighMassVerification_1	MS2 High mass resolution verification	MS2_HighMassVerification	MS2_HMVerification				0.000		
8								0.000		
9	Column_Conditioning 1	Blank	Gradient	SYSTEM	Blank	Gradient 1	1	10.000		Forward Gradient 8min equil
10	Column_Conditioning 2	Blank	Gradient	SYSTEM	Blank	Gradient 1	1	10.000		
11	Gradient 1	Gradient	Gradient	SYSTEM	Standard	Gradient 1	2	10.000		
12	Gradient 2	Gradient	Gradient	SYSTEM	Standard	Gradient 1	2	10.000		
13	Gradient 3	Gradient	Gradient	SYSTEM	Standard	Gradient 1	2	10.000		
14								0.000		
15	ESI_Positive_Sensitivity 1	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
16	ESI_Positive_Sensitivity 2	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
17	ESI_Positive_Sensitivity 3	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
18	ESI_Positive_Sensitivity 4	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
19	ESI_Positive_Sensitivity 5	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
20	ESI_Positive_Sensitivity 6	Sulfadimethoxine 1 pg on column	ESI_POS_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
21								0.000		
22	ESI_Negative_Sensitivity 1	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
23	ESI_Negative_Sensitivity 2	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
24	ESI_Negative_Sensitivity 3	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
25	ESI_Negative_Sensitivity 4	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
26	ESI_Negative_Sensitivity 5	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
27	ESI_Negative_Sensitivity 6	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMMYY	SYSTEM	Standard	Gradient 1	2	10.000		
28								0.000		
29	ESI_Positive_Sensitivity_Standalone_1	1 pg/ul Reserpine Fluidics Injections (standalone)	ESI_POS_Sensitivity_DDMMYY	Sensitivity_Tests				0.000		
30								0.000		
31	ESI_Negative_Sensitivity_Standalone_1	0.5 pg/ul Chloramphenicol Fluidics Injections (standalone)	ESI_NEG_Sensitivity_DDMMYY	Sensitivity_Tests				0.000		
32								0.000		
33	Scanwave_Positive_1	100 fmol/ul Glu-Fibrinopeptide B Daughter Scan	Scanwave_POS_Daughter	Scanwave				0.000		
34	Scanwave_Positive_2	100 fmol/ul Glu-Fibrinopeptide B ScanWave Daughter Scan	Scanwave_POS_SWDaughter	Scanwave				0.000		
35								0.000		
36	Scanwave_Negative_1	100 fmol/ul Glu-Fibrinopeptide B Daughter Scan	Scanwave_NEG_Daughter	Scanwave				0.000		
37	Scanwave_Negative_2	100 fmol/ul Glu-Fibrinopeptide B ScanWave Daughter Scan	Scanwave_NEG_SWDaughter	Scanwave				0.000		
38								0.000		

Figure 63 - WAAXXX_DDMMYY.spl sample list

Gradient performance test

NOTE: Specification - the standard deviation of the retention times for each sample peak must be ≤ 0.047 minutes.

Use the optimum probe position determined for the positive ion sensitivity specification for the gradient performance test.

1. Highlight the gradient test injections samples (rows 9 to 13) then click **Run > Start**.
2. Click **Acquire Sample Data only**.
3. Click **OK** to perform the analysis.
4. Perform the following procedure to calculate the standard deviation of the peak retention times for all four components over the three replicate injections:
 - a. Click the **TargetLynx** tab at the side of the sample list.
 - b. Highlight the three gradient sample lines in the sample list.
 - c. Click **Process samples** from the side menu and select **Gradient_RT method**.

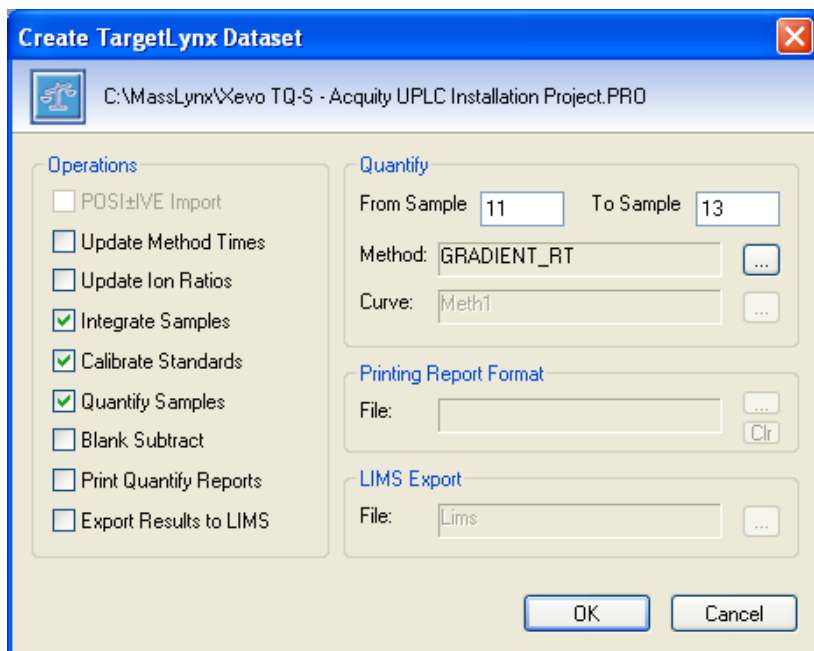


Figure 64 - Gradient_RT method dialog

- d. TargetLynx will smooth and determine the retention times of the sample peaks.
- e. Print the TargetLynx report. The report contains the retention times of all the peaks for each of the compounds.
- f. Verify that the compounds have eluted from the ACQUITY UPLC system in the following order:
 - Acetaminophen
 - Sulfadimethoxine
 - Caffeine
 - Verapamil

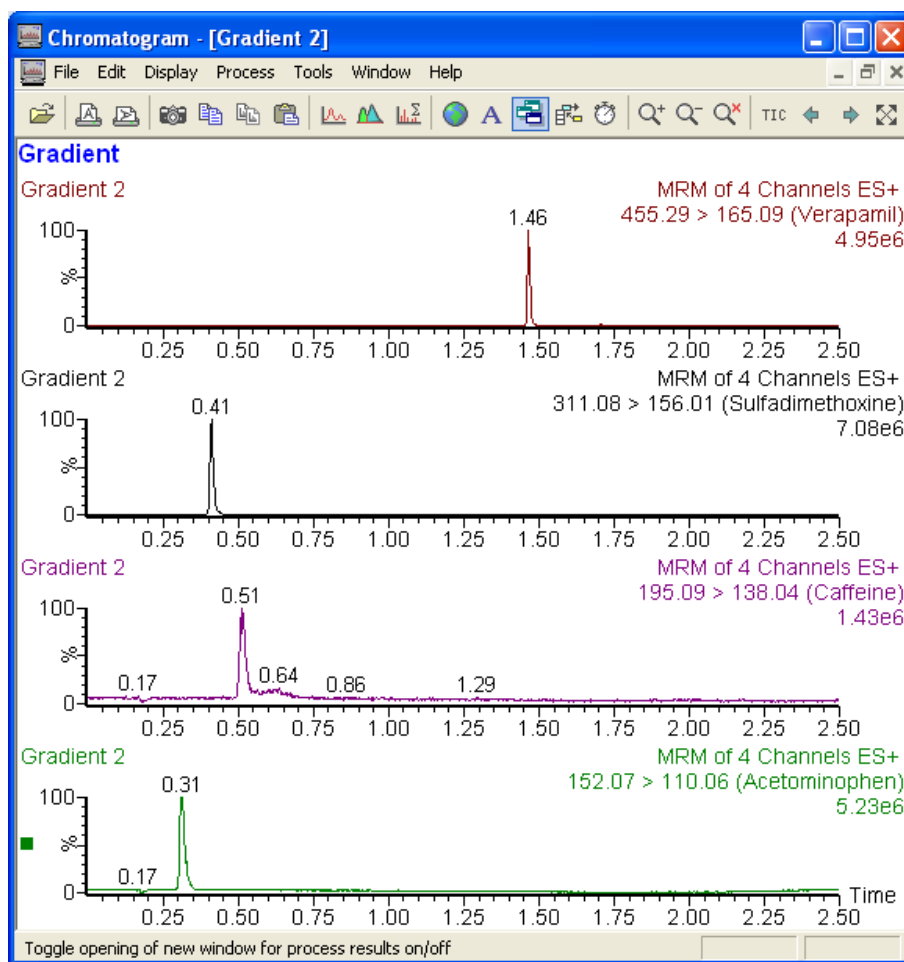


Figure 65 - Example gradient performance chromatogram

NOTE: Peak heights are for indication only.

- g. Use the Windows scientific calculator to calculate standard deviation of the retention times for each compound.

NOTE: Refer to "[Appendix B: Using the Windows statistical calculator](#)" for instructions on how to use the windows scientific calculator.

- h. Record the standard deviation values of the retention times for each of the compound sets in the [Xevo TQ-S ACQUITY UPLC System Installation Checklist](#) (715002460).

NOTE: The peak retention times over each component over three replicate injections have a standard deviation of ≤ 0.047 minutes

ESI positive ion sensitivity and precision

NOTE: Follow the same procedure for the UniSpray system sensitivity and precision test, adding a set of six UniSpray sample injections to the sample list.

For a 10 µL loop injection of 0.1 pg/µL sulfadimethoxine, ensure the following conditions are met:

- The signal-to-noise ratio of the sample peaks (RMS, ignoring zeroes and with no extra processing) is >3000:1
 - The average peak area for the six replicate injections is ≥60,000
 - The peak areas over six replicate injections have a %RSD ≤3.0%
 - The peak retention times over six replicate injections have a standard deviation of ≤0.047 minutes
1. From the MS Tune page, open Instrument Threshold Settings and enable SIR/MRM Chromatogram spike removal.
 2. From the MS inlet editor, open the **Gradient** method.
 3. Click  to load the initial conditions to the ACQUITY UPLC system (90:10 A1:B1 at 0.8 mL/min).
 4. Equilibrate the column until the BSM system pressure is stable.
 5. In the sample list, select the seven ESI_Positive_Sensitivity samples (lines 15 to 21 in the sample list).
 6. Click **Run > Start**.
 7. When the run has finished inspect the chromatogram and determine the retention time of the sulfadimethoxine peak and the noise range that will be used for calculation the signal to noise.

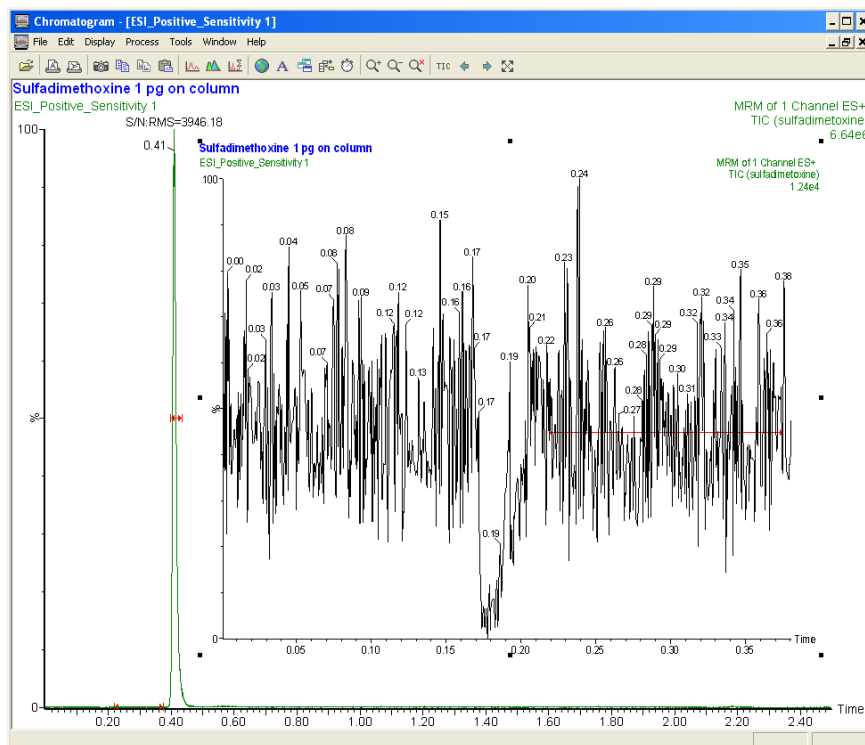


Figure 66 - Example of positive ion sensitivity test injection chromatogram

8. Choose a noise range covering 0.15 min from before the peak.

NOTE: Use noise that is flat, and free from any large noise spikes.

9. Open the TargetLynx method, and enter the noise window start and noise window end parameters using the times determined in step 8.

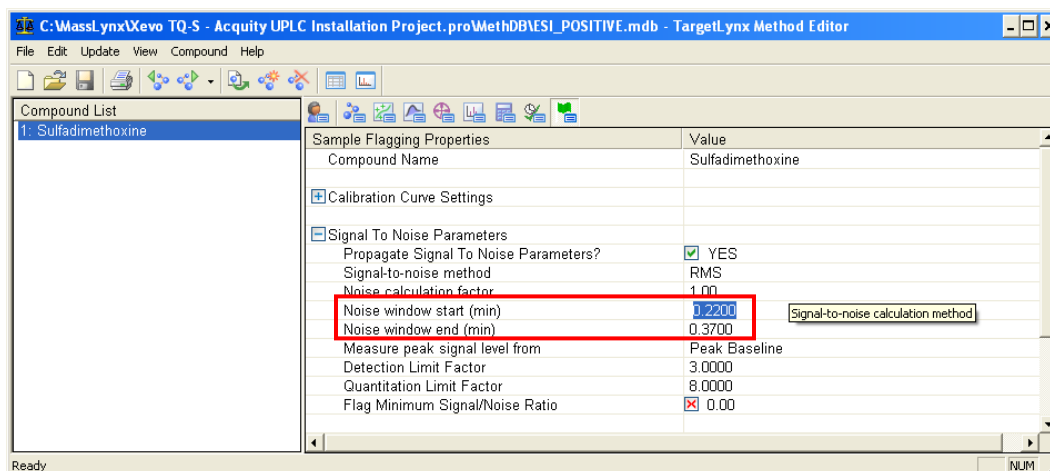


Figure 67 - Method editor noise parameters

10. With the ESI_Pos TargetLynx method open, right-click and drag across the sulfadimethoxine peak in the chromatogram.

NOTE: The predicated retention time parameter is automatically entered.

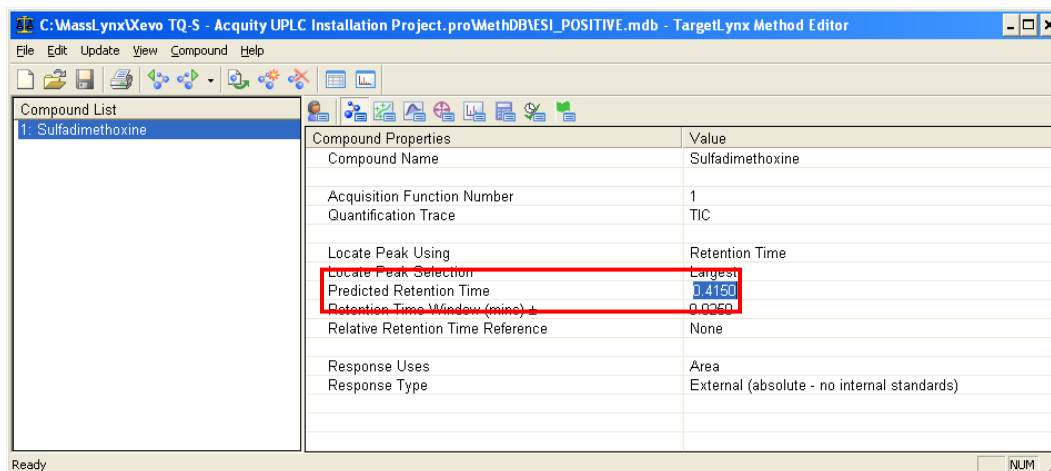


Figure 68 - Method editor retention parameters

11. Save the TargetLynx method.
12. Highlight the six samples in the sample list and process using the TargetLynx method ESI_Positive.

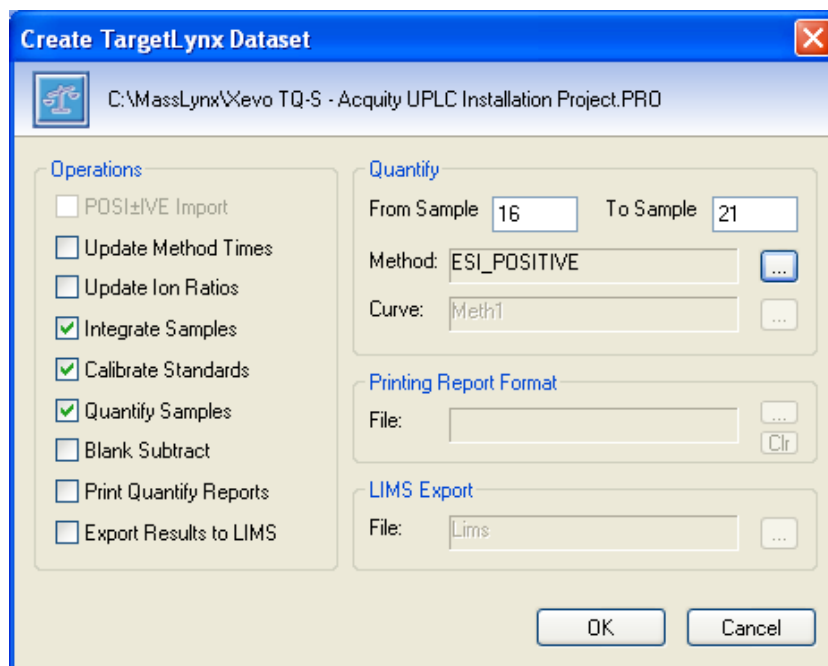


Figure 69 - Processing the ESI positive chromatograms to determine signal-to-noise

13. Open the TargetLynx report and click **File > Apply Layout**.
14. Select the **Sensitivity.qlt** file from the methDB folder located inside the installation project.

15. The resulting TargetLynx report contains the signal-to-noise measurements for the six chromatograms.
16. Save the report with an appropriate name.
17. Print the report.

NOTE: The report displayed on-screen will differ from the hardcopy report (illustrated below).

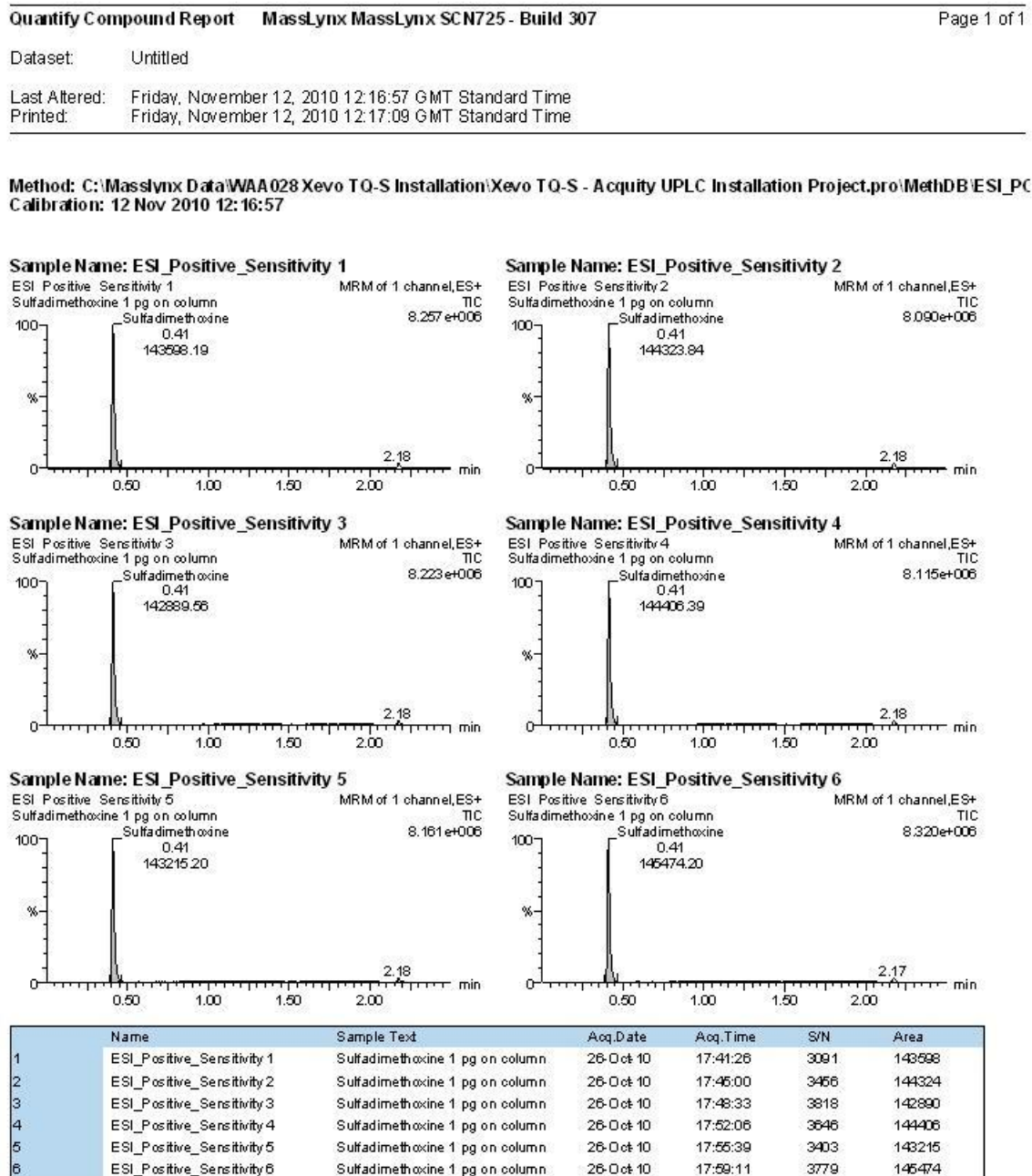


Figure 70 - Hardcopy TargetLynx report showing calculated signal-to-noise values

18. Calculate the average signal-to-noise for the six injections.
19. Record the result in the [Xevo TQ-S ACQUITY UPLC System Installation Checklist](#) (715002460).

NOTE: *The expected average signal-to-noise ratio is >3000:1.*

The expected peak area is >60,000.

20. Process the all six injections using the TargetLynx method **ESI_Positive_Area**.
21. Apply the report Layout **Reproducibility**.
22. The TargetLynx report shows the areas for each of the smoothed peaks, calculate the %RSD of the injections areas.
23. Calculate the peak areas over six replicate injections.

NOTE: *The expected %RSD is ≤3.0%.*

24. Calculate the peak retention times over six replicate injections.

NOTE: *The expected standard deviation is ≤0.047 minutes.*

Poor solvents can cause the baseline of the chromatogram to be very uneven, this makes it difficult for TargetLynx to integrate the peaks well. It is acceptable to manually modify peak integration if automatic integration has given poor results.

Optimize the capillary voltage and probe position ESI negative

NOTE: The optimum capillary and probe positions are different for ESI positive, ESI negative and APCI positive. Optimize the probe position immediately before running the sensitivity acquisitions, and re-optimize it for each sensitivity specification.

1. Click **Manual Optimization** in the MS console.
2. Open the Tune file **System.exp**.
3. Set the **321 > 152 MRM** transition to be monitored.
4. Set the desolvation gas flow to **1000 L/Hr**, and the desolvation temperature to **500 °C**.
5. Type in the required value to optimize the capillary voltage, and check for the baseline rising or falling, for an increasing or decreasing signal respectively.
6. Optimize the probe position for maximum intensity.
7. Optimize the capillary length for maximum intensity
8. Perform the following steps to minimize any thermal ionization:
 - a. Set the capillary voltage to zero.
 - b. Ensure any residual signal observed from thermal ionization is <10% of the optimized signal intensity.
 - c. To reduce the residual signal, move the ESI probe position away from the sample cone.
 - d. If adjustment was required, re-optimize the capillary voltage in the new probe position.
9. Click **Save** in the Manual Optimization IntelliStart window and save the tune file as **System.exp.ipr**.

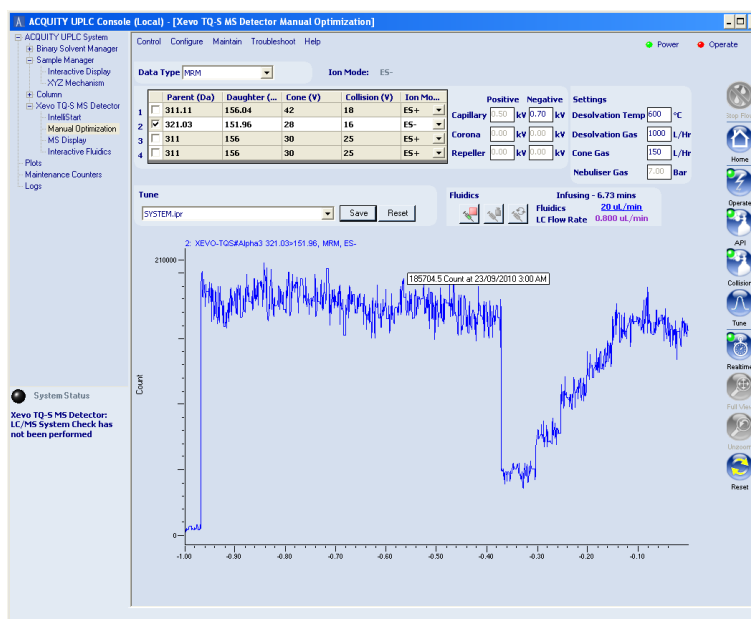


Figure 71 - Manual optimization of the capillary voltage and probe position

ESI negative ion sensitivity and precision

For a 10 µL loop injection of 0.05 pg/µL chloramphenicol, ensure the following conditions are met:

- The signal-to-noise ratio of the sample peaks (RMS, ignoring zeroes and with no extra processing) is >400:1
 - The average peak area for the six replicate injections is ≥1000
1. In the sample list select the six ESI_Negative_Sensitivity samples (lines 22 to 27 in the sample list).
 2. Click **Run > Start**.
 3. Process the acquired data in the same way the positive ion data were processed using TargetLynx. Use the TargetLynx Methods and layouts shown below:

Table 28: TargetLynx processing methods

Specification	Method	Layout
Signal-to-noise	ESI_NEGATIVE.mdb	SENSITIVITY.qlt
Reproducibility	ESI_NEGATIVE_AREA.mdb	REPRODUCIBILITY.qlt

NOTE: The noise range and retention time parameters must be defined in the TargetLynx methods.

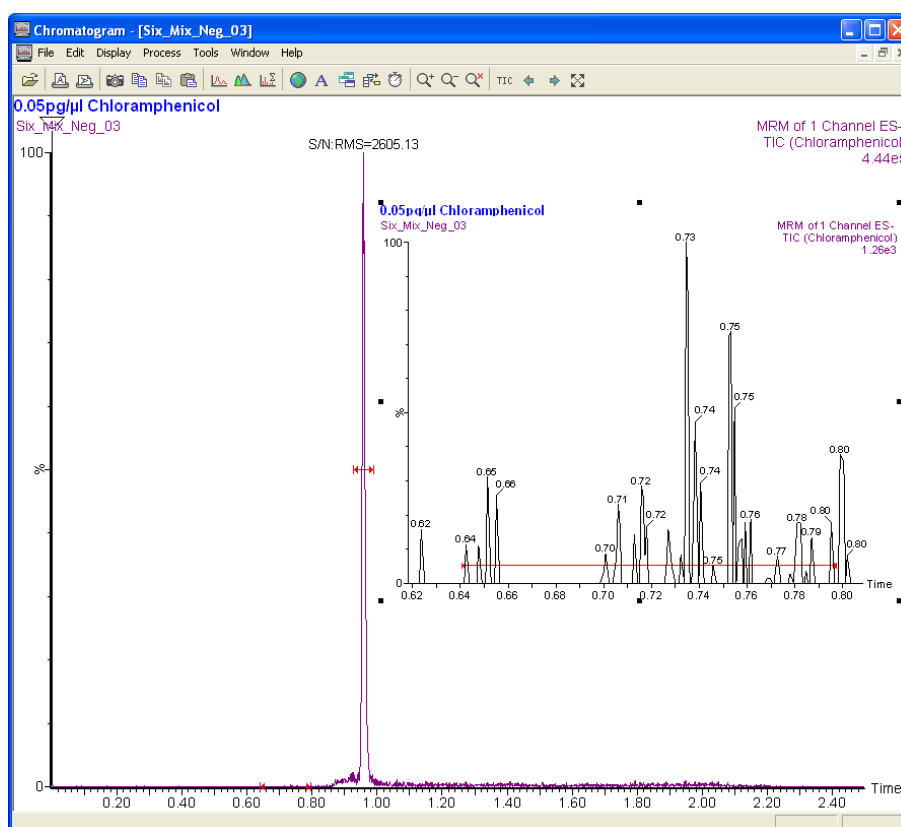


Figure 72 - Example negative ion chromatogram

4. Calculate the average signal-to-noise for the six injections.
5. Record the result in the [Xevo TQ-S ACQUITY UPLC System Installation Checklist](#) (715002460).

NOTE: *The expected average signal-to-noise ratio is >400:1.*

6. Process the all six injections using the TargetLynx method **ESI_Negative_Area**.
7. Apply the report Layout **Reproducibility**.

Creating an APCI positive sensitivity and precision MS method using IntelliStart

CAUTION: Perform this procedure after completing the ESI specifications.

1. From the MS Tune page, open Instrument Threshold Settings and enable SIR/MRM Chromatogram spike removal.
2. Place the instrument in Standby, with the API gas switched off.
3. Remove the ESI probe and fit the APCI probe and corona pin.
4. When prompted, select the **APCI Source** check box, and click **OK**.

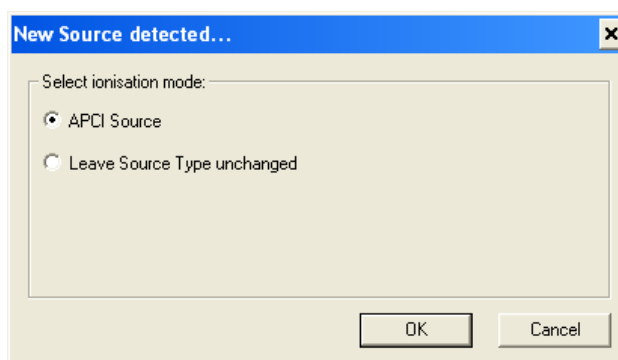


Figure 73 - New source dialog

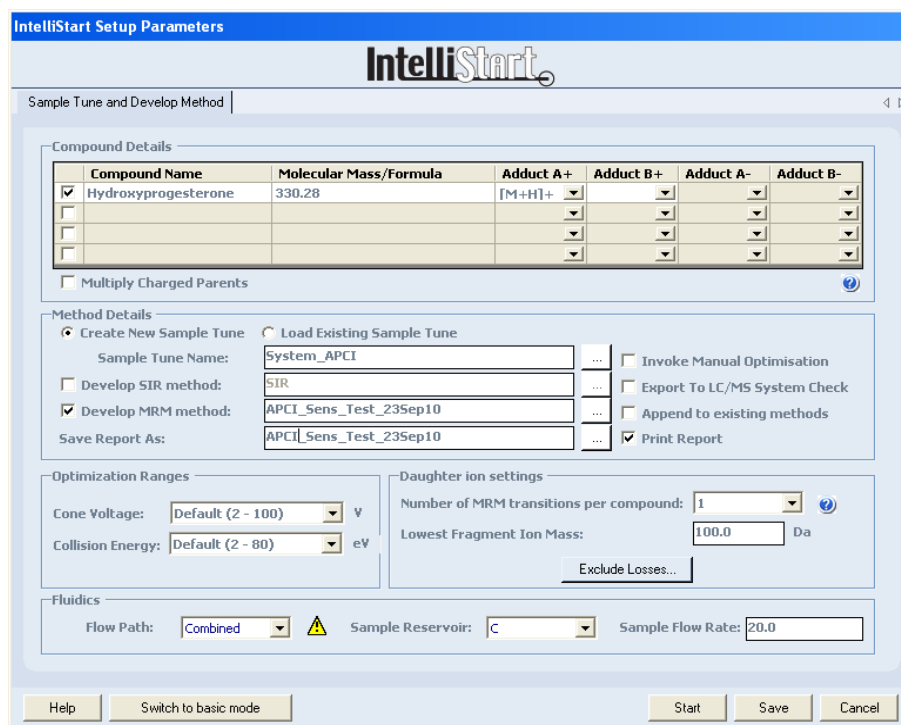
5. Click the **Ion Mode APcI+** tab on the Tune page.
6. Select the **Xevo TQ-S** window in the MS Console.
7. Place the instrument in Operate, with the API and collision gas flows turned on.
8. Set the ACQUITY UPLC to deliver 50:50 water/acetonitrile from A1:B1 at 0.8 mL/min.
9. Perform the following steps to set the initial ion probe position:
 - a. Set the initial horizontal position of the probe by setting the vernier screw on the top of the source housing (X) to 5 on the vernier scale.
 - b. Set the initial vertical position of the probe by setting the collar on the top of the probe (Z) to 7 on the vernier scale.
10. Click Interactive Fluidics > Enable Control.
11. Ensure parameters are set as follows:
 - Reservoir **C**
 - Flow **Combined**
12. Ensure the purge procedure is completed.
13. Click **IntelliStart** and set the type of analysis to **Unit Mass Resolution**.

CAUTION: The instrument resolution and calibration procedures must have been performed during the initial instrument setup section. If not, perform these procedures using IntelliStart, ensuring that both pass successfully.

14. Click **Sample Tune and Develop Method** and ensure that **APCI+** is selected.

15. Click .

16. Click **Switch to Advanced Mode** and set the parameters as shown in [Figure 74](#).



The image shows the 'IntelliStart Setup Parameters' dialog box. It has a title bar with the 'IntelliStart' logo. Below the title bar is a tab labeled 'Sample Tune and Develop Method'. The main area is divided into several sections:

- Compound Details:** A table with columns: Compound Name, Molecular Mass/Formula, Adduct A+, Adduct B+, Adduct A-, and Adduct B-. The first row is checked and contains 'Hydroxyprogesterone', '330.28', and '[M+H]+' for Adduct A+. There are three empty rows below it. A checkbox 'Multiply Charged Parents' is at the bottom left of this section.
- Method Details:**
 - Radio buttons: 'Create New Sample Tune' (selected) and 'Load Existing Sample Tune'.
 - Text fields: 'Sample Tune Name: System_APCI', 'Develop SIR method: SIR', 'Develop MRM method: APCI_Sens_Test_23Sep10', and 'Save Report As: APCI_Sens_Test_23Sep10'.
 - Checkboxes: 'Invoke Manual Optimisation', 'Export To LC/MS System Check', 'Append to existing methods', and 'Print Report' (checked).
- Optimization Ranges:**
 - 'Cone Voltage: Default (2 - 100) V' (dropdown)
 - 'Collision Energy: Default (2 - 80) eV' (dropdown)
- Daughter ion settings:**
 - 'Number of MRM transitions per compound: 1' (dropdown)
 - 'Lowest Fragment Ion Mass: 100.0 Da' (text field)
 - 'Exclude Losses...' button
- Fluidics:**
 - 'Flow Path: Combined' (dropdown)
 - 'Sample Reservoir: C' (dropdown)
 - 'Sample Flow Rate: 20.0' (text field)

At the bottom are buttons: 'Help', 'Switch to basic mode', 'Start', 'Save', and 'Cancel'.

Figure 74 - IntelliStart method development for APCI tests

CAUTION: The lowest fragment mass must be set to 100.0 Da to ensure that the correct fragment is selected by IntelliStart

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

18. Open the MS Method Editor and browse to the **APCI_Sens_Test_DDMMY.exp** file created by IntelliStart.

19. Set the **331.23 > 109.7** transition for 17- α -hydroxyprogesterone in the ESI positive.
20. Set the analysis end time to **2.5 minutes**.
21. Set the dwell times to **0.025 s**.
22. Save the method file.

Function: 1 MRM

Method

Ionization Mode: API+
Span: 0

Use Tune Cone Voltage: ☐
Use Tune Collision Energy: ☐

Retention Window (Mins)
Start: 0
End: 2.5

APCI Probe
☒ Use Tune Page Settings
Probe Temp: 20

PIC Scan
☒ Use Default PIC Scan function
PIC Scan: ScanWave DS
☒ Use Default Threshold
Activation Threshold: 20
Minimum Threshold: 500000
☒ Use Default Collision Energy
Collision Energy: 20

Channels

	Compound Name	Parent (m/z)	Daughter (m/z)	Acq	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	Hydroxyprogester	331.2481	109.1370	☐	0.025	35	28	☐	IntelliStart Generated

IntelliStart Generate

Add Delete Clear All Undo Redo Fill Down

OK Cancel

Figure 75 - Example APCI positive sensitivity method

Optimizing the corona current, cone gas, and probe position APCI positive

NOTE: The optimum capillary and probe positions are different for ESI positive, ESI negative, and APCI positive. The probe position must be optimized immediately before running the sensitivity acquisitions, and must be re-optimized for each sensitivity specification.

1. From the MS console, click Xevo TQ-S Detector > Manual Optimization.
2. Open the APCI_Sens_Test_DDMMYY.exp tune file.
3. Monitor the 331.23 > 109.7 MRM transition.
4. Set the probe temperature to **600 °C** and the desolvation gas flow to **1000 L/Hr**.
5. Optimize the corona current by entering the required value, and check for the baseline rising or falling, for an increasing or decreasing signal respectively.
6. Optimize the nebulizer pressure for maximum intensity.

NOTE: The typical nebulizer pressure optimum is approximately 4 bar.

7. Optimize the probe position for maximum intensity.
8. Optimize the capillary length for maximum intensity.
9. Optimize the capillary length for maximum intensity.
10. Optimize the cone gas for maximum intensity.
11. Click **Save** in the Manual Optimization IntelliStart window and save the tune file as **System_APCI.ipr**.

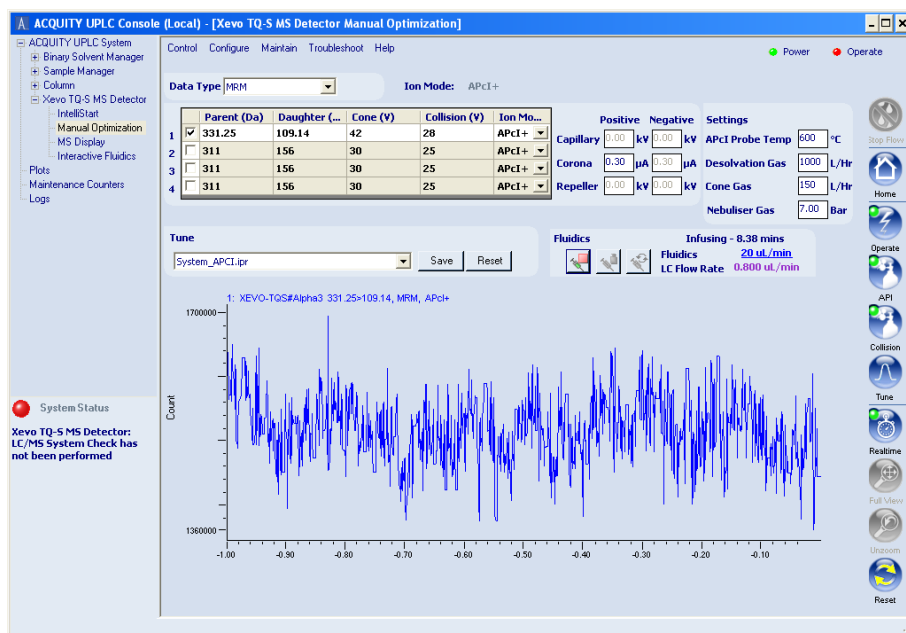


Figure 76 - Manual optimization of the corona current, nebulizer pressure, and probe position

APCI positive ion sensitivity and precision

NOTE: *Specification - 1250:1 RMS and peak area $\geq 10,000$ for a 10 μL injection of 5 pg/ μL 17- α -hydroxyprogesterone.*

For a 10 μL loop injection of 5 pg/ μL 17- α -hydroxyprogesterone, ensure the following conditions are met:

- The signal-to-noise ratio of the sample peaks (RMS, ignoring zeroes and with no extra processing) is $>1250:1$
 - The average peak area for the six replicate injections is $\geq 10,000$
 - The peak areas over six replicate injections have a %RSD $\leq 3.0\%$
 - The peak retention times over six replicate injections have a standard deviation of ≤ 0.047 minutes
1. Open the **Source_Options_WAAXXX_DDMMYY.spl** sample list and save with the instrument serial number and date amended.
 2. Select the six APCI_Positive_Sensitivity samples (lines 1 to 6 in the sample list).
 3. Click **Run > Start**.
 4. Process the acquired data in the same way the positive ion data were processed using TargetLynx. Use the TargetLynx Methods and layouts shown below:

Table 29: TargetLynx processing methods

Specification	Method	Layout
Signal-to-noise	APCI_POSITIVE.mdb	SENSITIVITY.qlt
Reproducibility	APCI_POSITIVE_AREA.mdb	REPRODUCIBILITY.qlt

NOTE: *Remember to define the noise range and retention time parameters in the TargetLynx methods.*

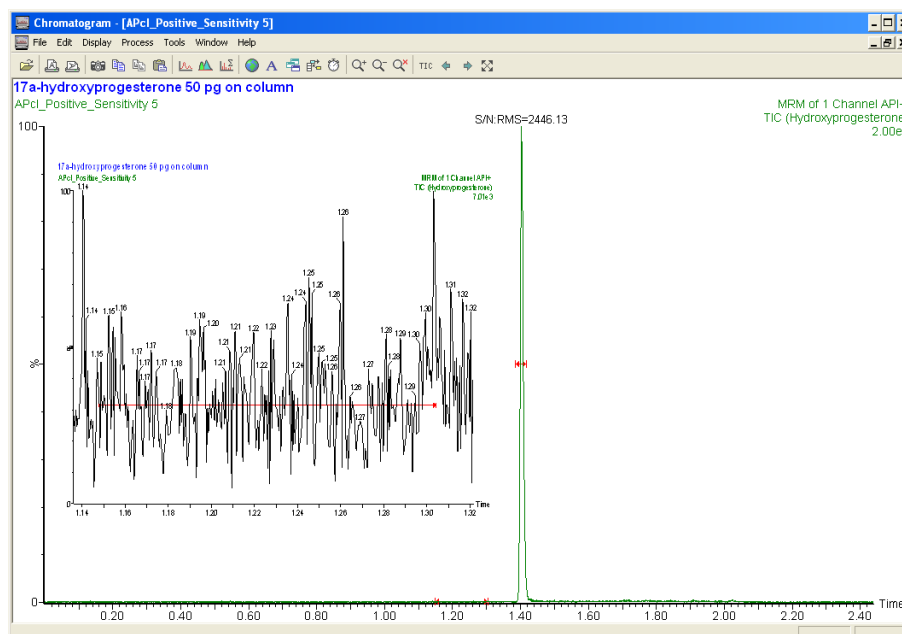


Figure 77 - Example APCI Chromatogram

5. Calculate the average signal-to-noise for the six injections.
6. Record the result in the [Xevo TQ-S ACQUITY UPLC System Installation Checklist](#) (715002460).

NOTE: The expected average signal-to-noise ratio is >1250:1.

The expected peak area is >10,000.

7. Process the all six injections using the TargetLynx method **ESI_Positive_Area**.
8. Apply the report Layout **Reproducibility**.
9. The TargetLynx report shows the areas for each of the smoothed peaks, calculate the %RSD of the injections areas.
10. Calculate the peak areas over six replicate injections.

NOTE: The expected %RSD is $\leq 3.0\%$.

11. Calculate the peak retention times over six replicate injections.

NOTE: The expected standard deviation is ≤ 0.047 minutes.

TUV or PDA detector precision tests (options)

Table 30: Performance test sample

Component	Concentration
Sulfadimethoxine	10 ng/μL

Sample preparation

To prepare the test samples, use Solution 1 (1 mg/mL sulfadimethoxine in methanol) from the Waters ACQUITY UPLC MS Startup Solution Kit (700002741).

1. Add 100 μL of solution 1 to a 10-mL volumetric flask and dilute to 10 mL with diluent.

NOTE: Use the diluent prepared for the sensitivity and precision tests (10:90 acetonitrile/water +0.01% formic acid)

2. Stopper and invert the flask several times to fully mix.
3. Label the flask as "10 ng/μL TUV/PDA test solution".

Setting up the TUV or PDA detector

NOTE: Ensure that the **UV_DETECTORS_WAAXXX_DDMMYY.SPL** sample list is loaded

Spectrum Chromatogram Map Edit ▾ Samples ▾

	File Name	File Text	MS File	MS Tune File	Sample Type	Inlet File	Bottle	Inject Volume
1	PDA_Blank_01	Blank injection	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:1	10.000
2	PDA_Blank_02	Blank injection	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:1	10.000
3	Sulfadimethoxine_PDA_Test_Injec...	PDA Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
4	Sulfadimethoxine_PDA_Test_Injec...	PDA Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
5	Sulfadimethoxine_PDA_Test_Injec...	PDA Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
6	Sulfadimethoxine_PDA_Test_Injec...	PDA Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
7	Sulfadimethoxine_PDA_Test_Injec...	PDA Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
8	Sulfadimethoxine_PDA_Test_Injec...	PDA Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
9								0.000
10	TUV_Blank_01	Blank injection	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:1	10.000
11	TUV_Blank_02	Blank injection	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:1	10.000
12	Sulfadimethoxine_TUV_Test_Injec...	TUV Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
13	Sulfadimethoxine_TUV_Test_Injec...	TUV Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
14	Sulfadimethoxine_TUV_Test_Injec...	TUV Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
15	Sulfadimethoxine_TUV_Test_Injec...	TUV Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
16	Sulfadimethoxine_TUV_Test_Injec...	TUV Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000
17	Sulfadimethoxine_TUV_Test_Injec...	TUV Precision Test	Optical Detector (Divert to waste)	Optical_Detector		Xevo-UPLC-PDA Specifi...	1:3	10.000

Figure 78 - UV_DETECTORS_WAAXXX_DDMMYY.SPL sample list

4. On the MS Tune page, open the **Optical_Detector.ipr** Tune page.

CAUTION: The MS method provided in the project diverts the sample to waste to prevent contamination of the mass spectrometer.

- Set up TUV or PDA detector methods for the UV detector precision tests using the following settings:

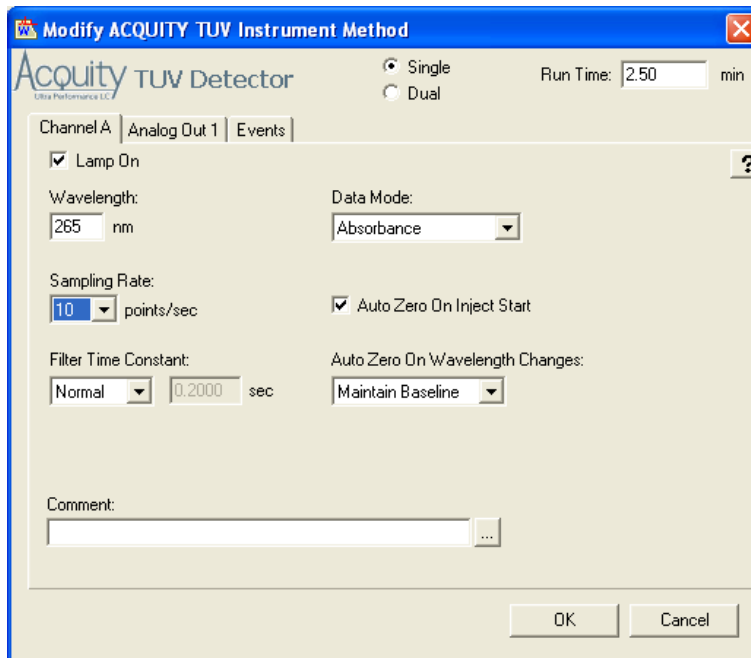


Figure 79 - TUV detector settings

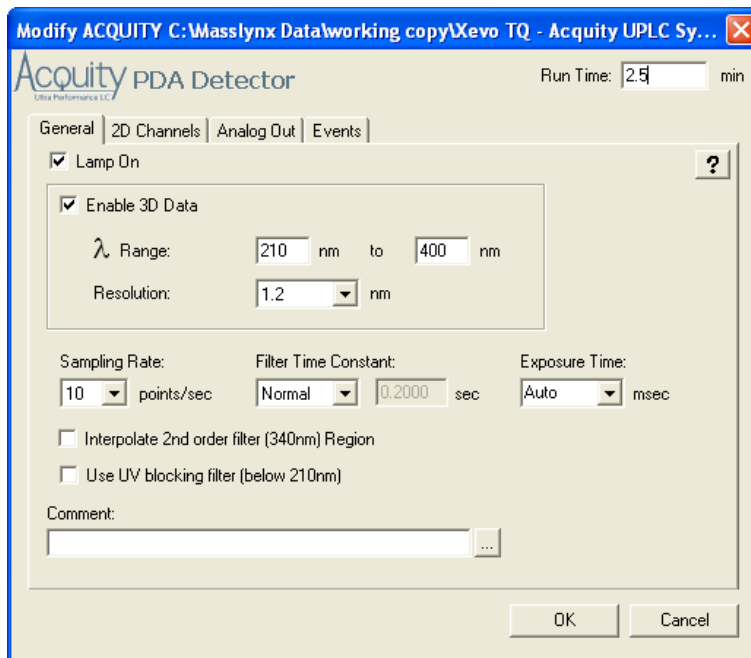


Figure 80 - PDA detector settings

CAUTION: For the PDA detector, extract the wavelength at 265 nm. If an old style PDA is used, set the filter response to zero.

6. Place a 2-mL vial containing 1 mL of gradient, sensitivity and precision test sample into position 1:B,2 of the sample manager.
7. Place a 2-mL vial containing 1 mL of diluent into position 1:B,1 of the sample manager.
8. Click the TUV or PDA tests in the sample list (as applicable).
9. Click **Start** to begin the acquisitions.
10. Process the acquired data in the same way the positive ion data were processed using TargetLynx. Use the TargetLynx Methods and layouts shown below:

Table 31: TargetLynx processing methods

Specification	Method	Layout
Reproducibility	UV_AREA.mdb	REPRODUCIBILITY.qlt

11. Use the Windows scientific calculator to calculate standard deviation of the retention times, peak heights and peak areas for each compound.

NOTE: Refer to "[Appendix B: Using the Windows statistical calculator](#)" for instructions.

12. The %RSD for peak areas must be $\leq 0.5\%$.
13. The %RSD for peak heights must be $\leq 0.9\%$.
14. Record the standard deviation in the [Xevo TQ-S ACQUITY UPLC System Installation Checklist](#) (715002460).

ELS or FLR detector precision tests (options)

1. For the ELS or FLR detectors, perform the system precision tests as detailed in [ACQUITY UPLC System Performance Tests](#) (715001134).

Calculate the %RSD for the peak areas and heights using the following steps:

1. Process the results as described in the [ACQUITY UPLC System Performance Tests](#) (715001134).
 - The %RSD for peak areas must be $\leq 5\%$ (ELSD) or $\leq 3\%$ (FLR).
 - The %RSD for peak heights must be $\leq 5\%$ (ELSD) or $\leq 4.5\%$ (FLR).
2. Record the %RSD for peak areas and heights in the [Xevo TQ-S ACQUITY UPLC System Installation Checklist](#) (715002460).

Xevo TQ-S (standalone) tests

1. Ensure the sample list **WAAXXX_DDMMY.Y** (saved earlier) is open.
2. Load the installation sample list (in the format XEVO_TQS_UPLC.fmt) from the **C:\MassLynx\Xevo TQ-S - Acquity UPLC Installation Project.pro\SampleDB** directory.

	File Name	File Text	MS File	MS Tune File	Sample Type	Inlet File	Bottle	Inject Volume
1	MS1_MassScaleResolution_1	MS1 Mass scale and resolution setup	MS1_MassScaleResolution	Instrument_Setup				0.000
2								0.000
3	MS2_MassScaleResolution_1	MS2 Mass scale and resolution setup	MS2_MassScaleResolution	Instrument_Setup				0.000
4								0.000
5	MS1_HighMassVerification_1	MS1 High mass resolution verification	MS1_HighMassVerification	MS1_HMVerification				0.000
6								0.000
7	MS2_HighMassVerification_1	MS2 High mass resolution verification	MS2_HighMassVerification	MS2_HMVerification				0.000
8								0.000
9	Column_Conditioning 1	Blank	Gradient	SYSTEM	Blank	Gradient	1:A,1	10.000
10	Column_Conditioning 2	Blank	Gradient	SYSTEM	Blank	Gradient	1:A,1	10.000
11	Gradient 1	Gradient	Gradient	SYSTEM	Standard	Gradient	1:A,2	10.000
12	Gradient 2	Gradient	Gradient	SYSTEM	Standard	Gradient	1:A,2	10.000
13	Gradient 3	Gradient	Gradient	SYSTEM	Standard	Gradient	1:A,2	10.000
14					Standard			0.000
15	ESI_Positive_Sensitivity 1	Sulfamethoxazole 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	Standard	Gradient	1:A,2	10.000
16	ESI_Positive_Sensitivity 2	Sulfamethoxazole 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	Standard	Gradient	1:A,2	10.000
17	ESI_Positive_Sensitivity 3	Sulfamethoxazole 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	Standard	Gradient	1:A,2	10.000
18	ESI_Positive_Sensitivity 4	Sulfamethoxazole 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	Standard	Gradient	1:A,2	10.000
19	ESI_Positive_Sensitivity 5	Sulfamethoxazole 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	Standard	Gradient	1:A,2	10.000
20	ESI_Positive_Sensitivity 6	Sulfamethoxazole 1 pg on column	ESI_POS_SYSTEM_DDMYY	SYSTEM	Standard	Gradient	1:A,2	10.000
21								0.000
22	ESI_Negative_Sensitivity 1	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	Analyte	Gradient	1:A,2	10.000
23	ESI_Negative_Sensitivity 2	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	Analyte	Gradient	1:A,2	10.000
24	ESI_Negative_Sensitivity 3	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	Analyte	Gradient	1:A,2	10.000
25	ESI_Negative_Sensitivity 4	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	Analyte	Gradient	1:A,2	10.000
26	ESI_Negative_Sensitivity 5	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	Analyte	Gradient	1:A,2	10.000
27	ESI_Negative_Sensitivity 6	Chloramphenicol 0.5 pg on column	ESI_NEG_SYSTEM_DDMYY	SYSTEM	Analyte	Gradient	1:A,2	10.000
28								0.000
29	ESI_Positive_Sensitivity_Standalone_1	1 pg/ul Reserpine Fluidics Injections (standalone)	ESI_POS_Sensitivity_DDMYY	Sensitivity_Tests				0.000
30								0.000
31	ESI_Negative_Sensitivity_Standalone_1	0.5 pg/ul Chloramphenicol Fluidics Injections (standalone)	ESI_NEG_Sensitivity_DDMYY	Sensitivity_Tests				0.000
32								0.000
33	Scanwave_Positive_1	100 fmol/ul Glu-Fibrinopeptide B Daughter Scan	Scanwave_POS_Daughter	Scanwave				0.000
34	Scanwave_Positive_2	100 fmol/ul Glu-Fibrinopeptide B Scan/Wave Daughter Scan	Scanwave_POS_SWDaughter	Scanwave				0.000
35								0.000
36	Scanwave_Negative_1	100 fmol/ul Glu-Fibrinopeptide B Daughter Scan	Scanwave_NEG_Daughter	Scanwave				0.000
37	Scanwave_Negative_2	100 fmol/ul Glu-Fibrinopeptide B Scan/Wave Daughter Scan	Scanwave_NEG_SWDaughter	Scanwave				0.000

Figure 81 - Installation sample list (standalone sensitivity lines highlighted)

ESI positive ion sensitivity (and UniSpray option)

NOTE: Follow the same procedure for the UniSpray test, except for step 23.

Make five sample infusions of reserpine (1 pg/μL in 70:30 acetonitrile/water) and three 5-second blank infusions (70:30 acetonitrile/water) into a flow of 200 μL/min 70:30 acetonitrile/water, using the system fluidics.

Ensure the following conditions are met:

- The signal-to-noise ratio of the sample peaks (RMS, ignoring zeroes and with no extra processing) is >15,000:1
- The %RSD for the five sample peak heights is <10%
- The blank intensity is <10% of the average sample intensity

1. Click  to select **MS mode** on the MS Tune page.
2. Ensure the collision gas is off.
3. Click **View > System** on the MS Tune page to display the advanced MS Tune page controls.

4. Set the scope display to **MS1** scan with a span of **5** and mass of **609 Da**.
5. Set the initial source conditions as shown in [Table 32](#).

Table 32: Initial source conditions

Parameter	Setting	Comment
Impactor/Capillary	3 kV	This value will be optimized later
Cone voltage	60 V	This value will be optimized later
Source Offset	60 V	Fixed
Desolvation temperature	500 °C	Fixed
Desolvation gas flow	1000 L/h	Fixed
Cone gas flow	150 L/h	Fixed

6. Ensure the instrument fluidics are plumbed as shown in [Figure 22](#).
7. Set the LC system to deliver mobile phase (70:30 acetonitrile/water) at 200 µL/min.
8. Install a bottle of tuning solution (reserpine 5 pg/µL in 70:30 acetonitrile/water) in reservoir C.
9. Purge the fluidics.
10. On the MS Tune page, click the **Fluidics** tab.
11. Enter the parameters listed in [Table 33](#), and start infusion.

Table 33: Fluidics parameters for tuning

Parameter	Setting
Infusion flow rate	20 µL/min
Reservoir	C
Flow state	Combined

12. Ensure that the m/z 609 reserpine ion is visible on the spectrum display.
13. Optimize the source conditions to maximize the intensity of the m/z 609.3 parent ion.
14. Optimize the analyzer settings to achieve a resolution of 0.7 ± 0.1 Da on the m/z 609.3 parent ion.

NOTE: Note the exact mass position of the peak center. If the optimization of the mass resolution alters the peak position, accurately note the new m/z value for use in the MS method.

15. Select **MS/MS mode** on the MS Tune page, and click .
16. Switch on the collision gas and set an initial flow rate of **0.15 mL/min**.

17. Adjust the collision gas flow rate to obtain a collision cell pressure of $\sim 3.5\text{E-}3$ mbar.
18. Set the collision energy to a starting value of **38 V**.
19. Set the scope display to **Daughter Scan** with a span of **3 Da** around **195 Da**.
20. Optimize the source conditions to maximize the intensity of the m/z 195 daughter ion.
21. Optimize the MS/MS conditions for the $609 > 195$ transition.
22. Tune the analyzer settings to achieve a resolution of 0.7 ± 0.1 Da FWHM on the m/z 195 fragment.

NOTE: *If the optimization of the mass resolution alters the peak position, accurately note the new m/z value for use in the MS method.*

23. Minimize any thermal ionization:

NOTE: *This step and its sub-steps are not needed for UniSpray.*

- a. Set the capillary voltage to zero.
 - b. Thermal ionization must be $<10\%$ of the optimized signal intensity.
 - c. To reduce the thermal ionization, move the ESI probe position away from the sample cone.
 - d. If adjustment was required, re-optimize the capillary voltage in the new probe position.
24. Select the tune file in the sample list.
 25. Stop the fluidic infusion flow.
 26. Create an MRM MS method using the experiment setup parameters shown in [Figure 82](#) and [Figure 83](#).

NOTE: *An MS method file, ESI_POS_SYSTEM_DDMMYY, is provided in the installation project. If this file is used, the mass positions in the method must be adjusted to match those noted during the optimization steps, and the method re-saved.*

The method provided in the project contains a method events table which performs the sample injections automatically. There is no need to use the sample inject button  if this method is used.

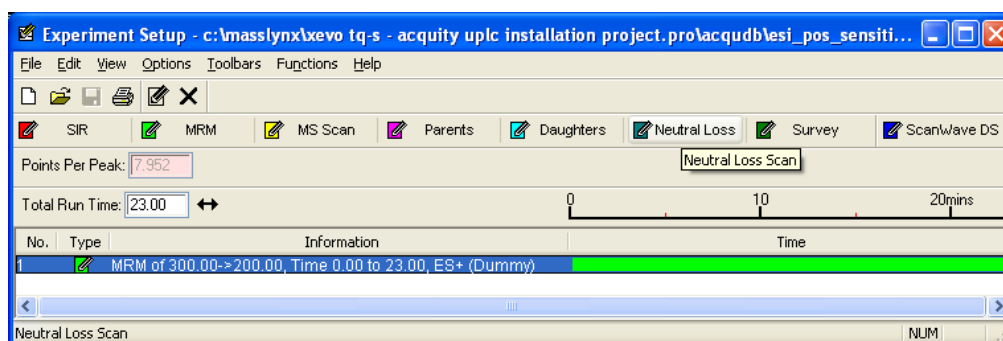


Figure 82 - Method editor

Figure 83 - MS method setup for positive ion

CAUTION: *The mass positions must be completed with those observed during tuning.*

For the parent and daughter m/z values, use the positions noted during the optimization steps.

27. Save the MS experiment using the format **POS_Sensitivity_DDMMYY** (where ddmmmyy = date)
28. Install a bottle of specification solution (reserpine 1 pg/ μ L in 70:30 acetonitrile/water) in the instrument reservoir B.
29. Install a bottle of blank solution (70:30 acetonitrile/water) in the instrument reservoir A.
30. On the MS Tune page, click the **Fluidics** tab.
31. Enter the parameters listed in [Table 34](#).

NOTE: *If necessary, reconfigure the inlet system to allow sample list acquisitions. However, ensure the LC flow rate is on.*

Table 34: Fluidics parameters for specification

Parameter	Setting
Infusion flow rate	200 μ L/min
Flow state	LC
Fill volume	250 μ L

32. Set the reservoir selection to **A**.
33. Purge the fluidics.

34. Start the **ESI_Positive_Sensitivity_Standalone_1** acquisition from the sample list (line 29).
35. Display the MRM transition TIC in the Chromatogram browser.
36. Click **Process > Signal To Noise...** in the Chromatogram window.
37. Click **Ignore Zeros > No Extra Processing > RMS**.
38. To measure the signal, right-click and drag across the first infusion peak.
39. To measure the noise, right-click and drag across a 30-second section of noise, 1 minute after the infusion peak.
40. Click **OK** to calculate the signal-to-noise ratio.
41. Repeat steps 38 to 40 for each sample peak.
42. Calculate the average signal-to-noise ratio.

NOTE: *The expected average signal-to-noise ratio is >15,000:1.*

43. Calculate the %RSD for the five sample peak heights.

NOTE: *The expected %RSD is <10%. This is the %RSD of S/N values. Refer to "[Appendix B: Using the Windows statistical calculator](#)" for instructions for calculating the average, standard deviation, and %RSD values.*

44. Calculate the average signal intensity for the five sample peaks.
45. Calculate the average signal intensity for the three blanks.

NOTE: *The expected average signal intensity of the blanks must be <10% of the average signal intensity of the five sample peaks.*

46. Print the result showing the average signal-to-noise ratio and %RSD.
47. Print the result showing the blank signal intensity.
48. Print the experimental record.

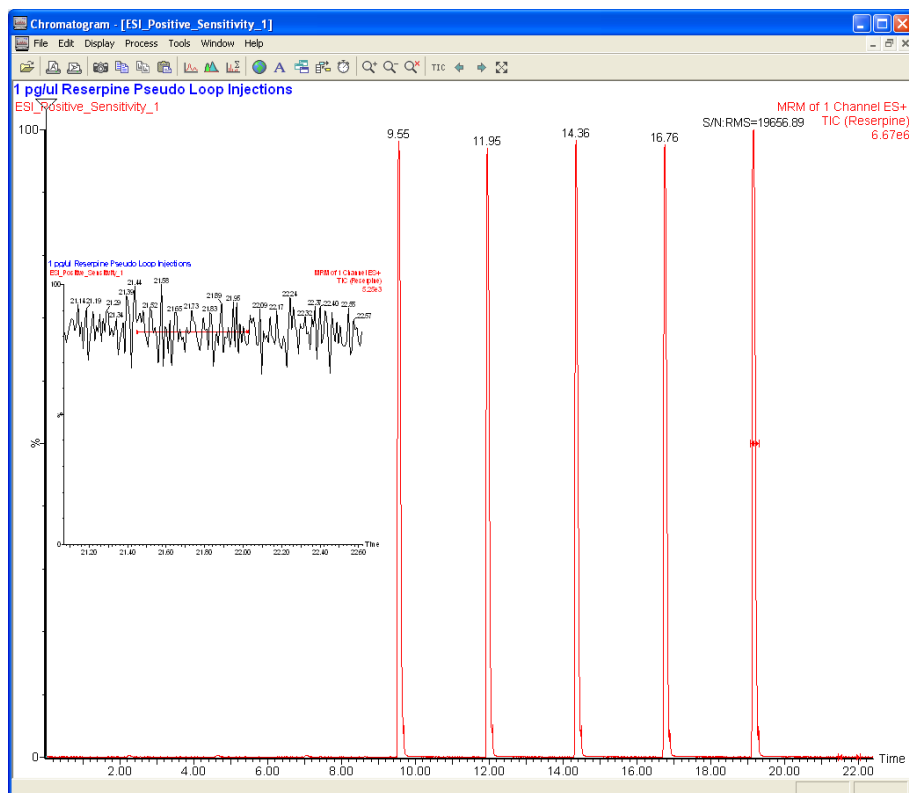


Figure 84 - Typical standalone ESI positive sensitivity chromatogram

ESI negative ion sensitivity

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 a flow of 200 μ L/min 70:30 acetonitrile/water, using the system fluidics.

Ensure the following conditions are met:

- The signal-to-noise ratio of the sample peaks (RMS, ignoring zeroes and with no extra processing) is >3000:1
 - The %RSD for the five sample peak heights is <10%
 - The blank intensity is <10% of the average sample intensity
1. Perform the "ESI positive ion sensitivity" procedure using the following parameters:
 - Create a new MS method using the m/z 321 to 152 transition in ES- mode
 - Set an initial collision energy of **25 V**
 - Use a tuning solution of 5 pg/ μ L chloramphenicol in 70:30 acetonitrile/water

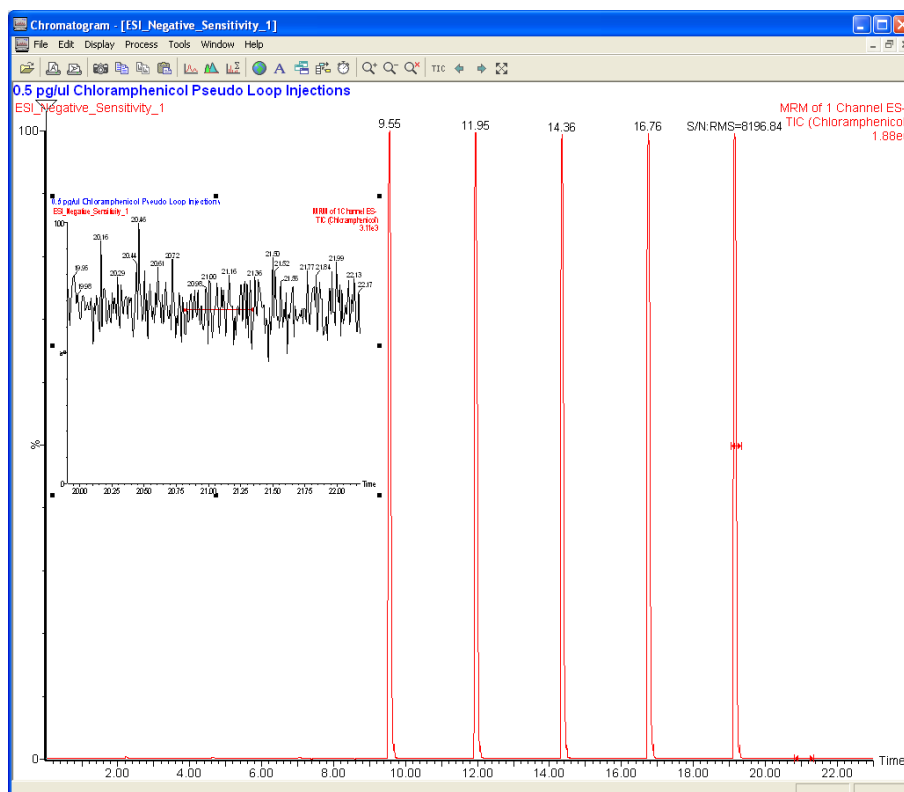


Figure 85 - Typical ESI negative sensitivity chromatogram

Source options tests

The specifications described in this section apply to source options which do not ship with the core instrument.

1. Open the sample list SOURCE_OPTIONS_WAAXXX_DDMMYY.SPL.spl.
2. Save and rename the sample list where:
 - XXX is the serial number of the instrument
 - ABC are the engineer's initials
 - DDMMYY is the date
3. Load the installation sample list format **Installation_Specifications.fmt** from the C:\MassLynx\ Xevo TQ-S - Acquity UPLC System Specifications Project.PRO directory.

APGC source (option)

For installation of the APCI source, refer to the following documents:

- [Waters APCI Installation Manual](#) (715002452)
- [Installing the APCI Source Upgrade Kit](#) (715002881)

Positive ion, APCI (option, standalone Xevo TQ-S only)

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

Ensure the following conditions are met:

- The signal-to-noise ratio of the sample peaks (RMS, ignoring zeroes and with no extra processing) is >2500:1
 - The %RSD for the five sample peak heights is <10%
 - The blank intensity is <10% of the average sample intensity
1. Install the APCI probe and corona pin.
 2. On the MS Tune page, click **Ion Mode > APCI Positive**.
 3. Set the initial source conditions as shown in [Table 36](#).
 4. Perform the "[ESI positive ion sensitivity](#)" procedure using the following parameters:
 - Create a new MS method using the m/z 331.2 to 109.1 transition in APCI+ mode
 - Set dwell to 0.1 seconds
 - Use a tuning solution of 500 pg/ μ L 17- α -hydroxyprogesterone in 70:30 acetonitrile/water

Table 35: Fluidics parameters for specification

Parameter	Setting
Infusion flow rate	400 μ L/min
Flow state	LC
Fill volume	250 μ L

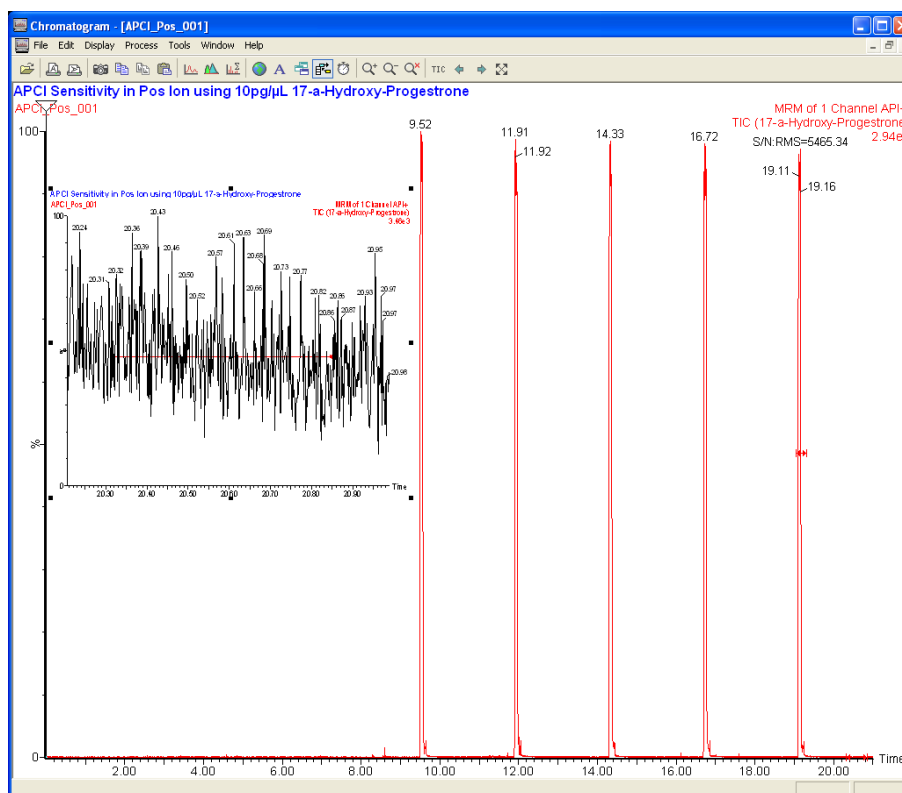


Figure 86 - Typical APCI positive ion sensitivity chromatogram

Positive ion, APPI (option)

Make five sample infusions of 100 pg/ μ L naphthalene (100 pg/ μ L in 100% methanol) and three blank infusions (100% methanol) into a flow of 0.2 mL/min 100% methanol, using the system fluidics.

Ensure the following conditions are met:

- The signal-to-noise ratio of the sample peaks (RMS, ignoring zeroes and with no extra processing) is $\geq 240:1$.
 - The %RSD for the five sample peaks is $\leq 10\%$.
 - The blank intensity is $\leq 10\%$ of the average sample intensity.
1. Install the APPI source, APCI probe, and corona pin.
 2. On the MS Tune page, click **Ion Mode > APPI Positive**.
 3. Set the initial source conditions and fluidics parameters as shown in [Table 36](#) and [Table 37](#).
 4. Perform the "[ESI positive ion sensitivity](#)" procedure, but with the following parameters:
 - Create a new MS method using the m/z 128 to 102 transition in APPI+ mode
 - Set dwell to 0.5 seconds
 - Use a tuning solution of 1000 pg/ μ L naphthalene in 100% methanol

Table 36: Initial source conditions for APPI

Parameter	Setting	Comment
Repeller	1 kV	Optimize
Cone voltage	60 V	Optimize
Source Offset	60 V	Fixed
Desolvation temperature	300 °C	Fixed
Desolvation gas flow	1000 L/h	Fixed
Cone gas flow	150 L/h	Fixed

Table 37: Fluidics parameters for specification

Parameter	Setting
Infusion flow rate	200 μ L/min
Flow state	LC
Fill volume	250 μ L

Positive ion, NanoFlow ESI (option)

NOTE: *This source option specification requires an external syringe pump capable of delivering a stable flow of 400 nL/min.*

Make a direct infusion of 100 fmol/ μ L [Glu¹]-fibrinopeptide B (in 50:50 acetonitrile/water +0.1% formic acid) at 400 nL/min using an external syringe pump. MS2 full scan spectra are acquired, 700 to 900 Da at 8 sec/scan.

NOTE: *The signal-to-noise ratio (peak-to-peak with no extra processing) of the 785.8 peak (three scans combined) region must be $\geq 1E7$.*

1. Install the NanoFlow source enclosure.
2. Install the Universal NanoFlow sprayer.
3. Install the camera drivers:
 - a. Exit MassLynx.
 - b. Click **Start > Control Panel > Add Hardware**.
 - c. Follow the wizard instructions to add the drivers to Windows XP.
 - d. When prompted, browse to the driver directory on the MassLynx DVD, for example, **D:\Redist\NanoFlow Camera Driver**.
 - e. Click **OK** and ensure the installation completes.
 - f. Open MassLynx.
4. Connect the camera cable from the Video output on the rear of the instrument to the host PC through the USB converter box.
5. Open the MS Tune page.
6. Click **Ion Mode > NanoFlow +**.
7. Infuse 100 fmol/ μ L [Glu¹]-fibrinopeptide B (in 50:50 acetonitrile/water +0.1% formic acid) at 400 nL/min using an external syringe pump.
8. Click  to select MS mode on the MS Tune page.
9. Switch on the collision gas and set an initial flow rate of **0.15 mL/min**.
10. Adjust the collision gas flow rate to obtain a collision cell pressure of $\sim 3.5E-03$ mbar.
11. Set the scope display to **MS Scan** with a mass of **785.8 Da** and a span of **5**.
12. Set the initial source conditions as shown in [Table 38](#).

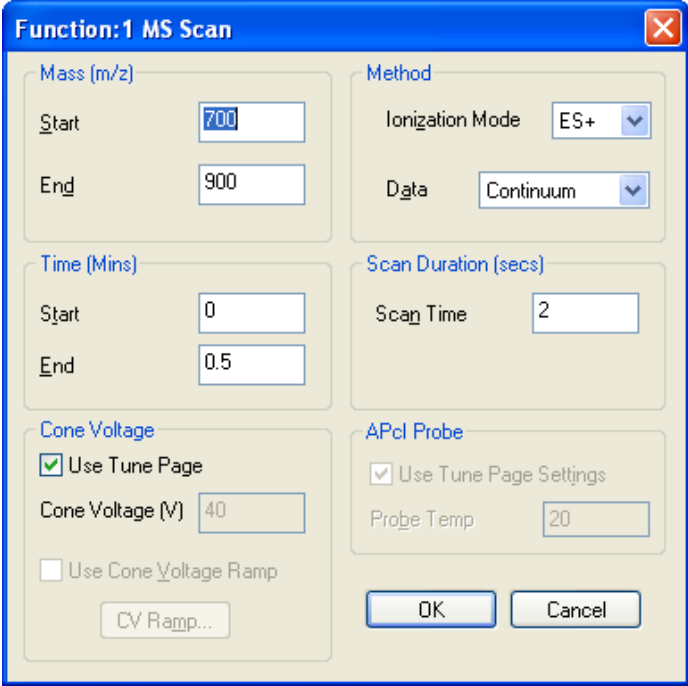
Table 38: Initial source conditions for NanoFlow ESI

Parameter	Setting	Comment
Capillary	3.0 kV	Optimize
Cone voltage	45 V	Optimize
Source Offset	60 V	Recommended
Purge gas flow	15 L/h	Recommended
Cone gas flow	0 L/h	Recommended
Nanoflow (bar)	0.1 L/h	Optimize

13. Optimize the source conditions and/or the sprayer position to maximize the intensity of the multiply charged distribution.
14. Save the tune file as NANOFLOW_POS_OPTION_DDMMYY.
15. Select the tune file in the sample list.

NOTE: Monitor the sprayer tip using the camera display to help optimize the position. Click  to open the camera display.

16. Highlight **NANOFLOW_POS_01** in the sample list, then click **Start**.


Figure 87 - MS method editor for NanoFlow

17. Click **Chromatogram** on the main MassLynx page.
18. Click  or **File > Open** and select the appropriate filename, then click **OK**.

19. Click  then click **Process > Combine Spectra**.

20. Specify three scans to combine, as shown in Figure 88, then click **OK**.

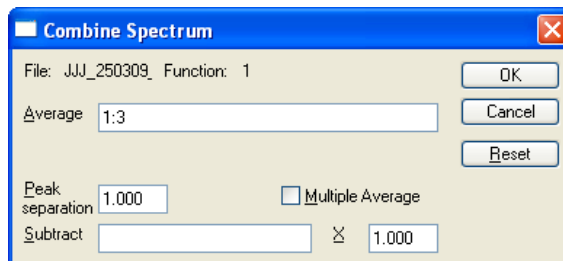


Figure 88 - Combine Spectra window

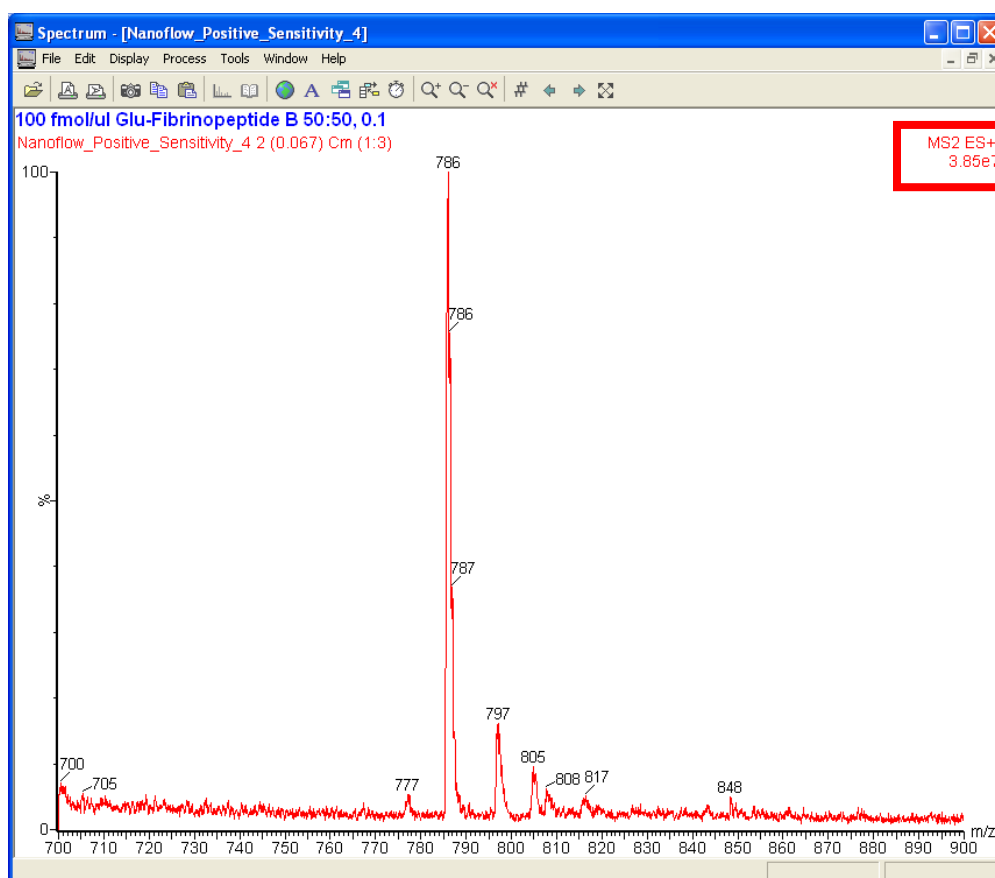


Figure 89 - Typical NanoFlow ESI spectrum

21. Print the result showing the signal intensity.

22. Print the experimental record.

Positive ion, TRIZAIC (option)

CAUTION: To avoid communication problems, ensure that the source PCB firmware is at revision P or later.

The TRIZAIC source installation tests are performed using a nanoACQUITY UPLC system.

Ensure that the rear panel PCB on the nanoACQUITY UPLC Sample Manager has been updated to either 210000325 or 210000418.

Initial setup

1. Remove the LockSpray baffle on the TRIZAIC source when used on tandem quadrupole instruments:
 - a. Loosen the grub screw on the baffle.
 - b. Withdraw the baffle from the motor shaft.

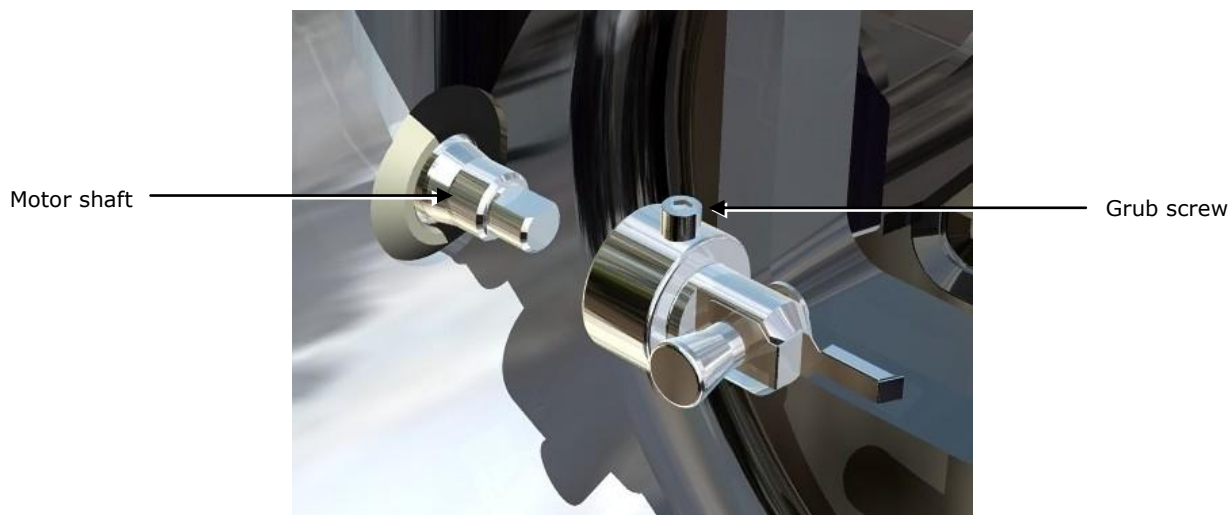


Figure 90 - Baffle location and fixings

2. Attach the TRIZAIC source to the mass spectrometer.

NOTE: For the correct procedure on how to achieve this, refer to the TRIZAIC UPLC System Guide (Documentation CD 715002131).

3. Close MassLynx and shut down the PC.
4. Shut down the nanoACQUITY.

CAUTION: Disconnecting the TRIZAIC power cord when the sample manager is powered on will cause damage to the equipment.

5. Connect the TRIZAIC power cord to the QSPI connection of the nanoACQUITY UPLC Sample Manager.
6. Switch the nanoACQUITY on.

7. Wait for the Sample Manager and Binary Sample Manager to complete their boot-up sequence.
8. Switch the PC on, and launch MassLynx.
9. Ensure the nanoTile appears on the Inlet Method SM tab, and the console tree.
10. Connect the power to the source.
11. Click the **TRIZAIC** source type.
12. Place the instrument in Positive Ion mode and set the initial source parameters shown in [Table 39](#).

Table 39: Typical Tune page parameters (TRIZAIC configuration)

Parameter	Value
Capillary voltage	3.0 kV
Sampling cone	30 V
Source Offset	70 V
Source temperature	100 °C
Cone gas	50 L/Hr
Nanoflow gas	0.30 Bar
Purge gas	Off

CAUTION: *Do not apply voltage to the electrospray emitter without supplying flow to the tile. This will raise the optimal electrospray potential and limit the lifetime of the emitter.*

Do not touch the electrospray emitter as it may bend.

The infusion tile must only be used to calibrate the instrument, and during the set pressure diagnostic test.

13. Connect the infusion line to the port 2 of the diverter valve for infusion.
14. Close the TRIZAIC clamp without the tile present.

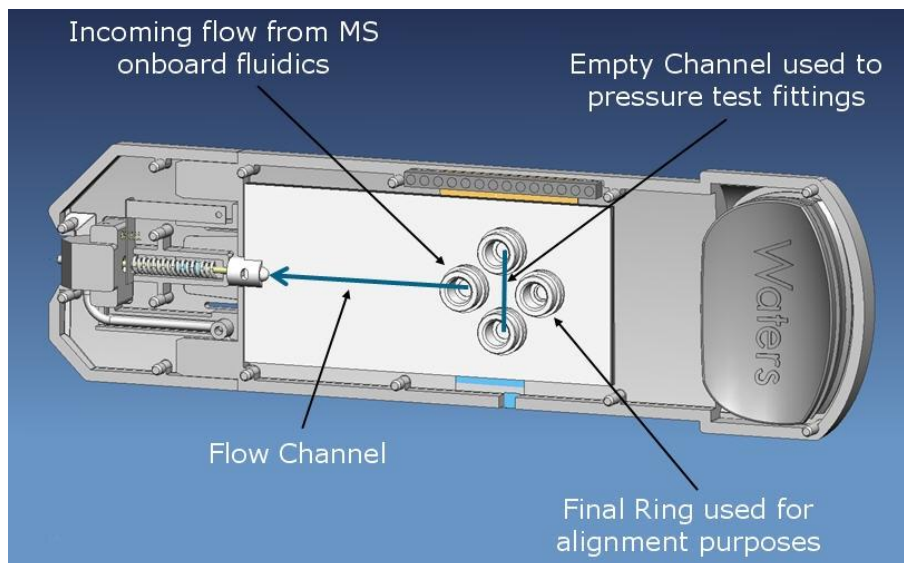


Figure 91 - Calibration tile flow path

15. Make up a solution of 100 fmol/ μ L [Glu1]-fibrinopeptide B in 0.1% formic acid in 70:30 water/acetonitrile.
16. Set a flow rate of 30 μ L/min to infuse the solution.
17. Wait for liquid to emerge in the fitting closest to the source.
18. Stop the flow.
19. Open the clamp and place the infusion tile in clamp, as described above.
20. Set the flow to the infusion tile to 1 μ L/min.
21. When droplets form, turn the capillary voltage on.
22. Adjust the following parameters for an optimal signal (Figure 92):
 - Stage position
 - Voltage
 - Cone gas
 - NanoFlow gas

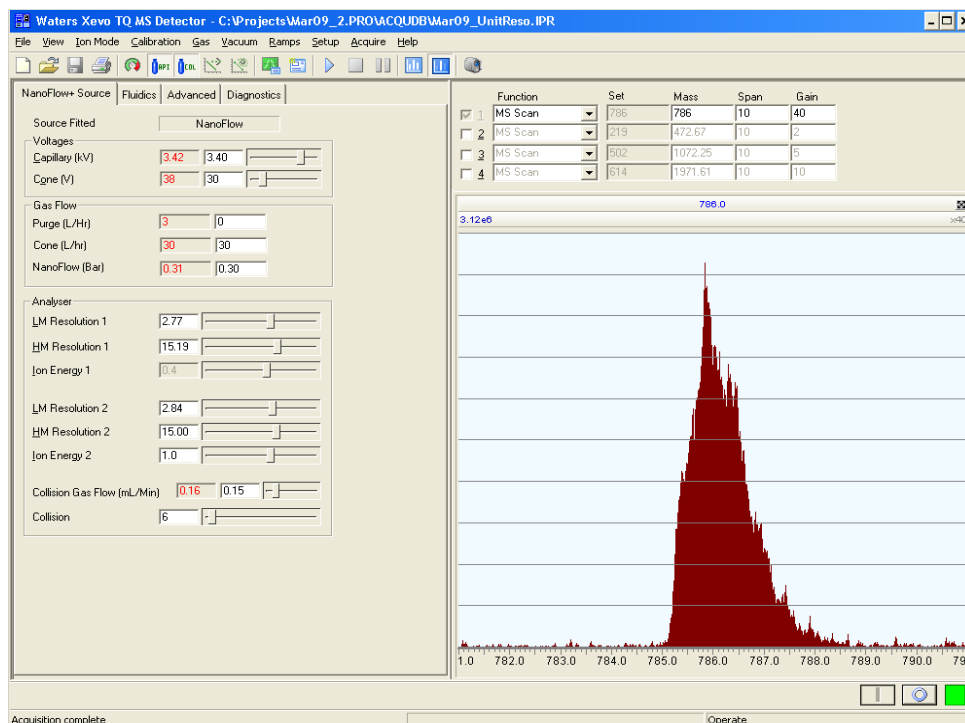


Figure 92 - Example Tune page (Xevo TQ MS)

NOTE: Expect the signal to be similar for Xevo TQ MS, and order of magnitude larger for Xevo TQ-S.

23. Once the stage has been optimized for maximum sensitivity, perform the following steps:
 - a. Turn off the capillary voltage.
 - b. Mark the position of the emitter with the crosshair.
 - c. Stop the [Glu¹]-fibrinopeptide B infusing.
24. Save the Tune page as **TRIZAIC_Tune_ddmmmyy.ipr** (where ddmmmyy = date).
25. Replace the infusion tile with the nanoTile.

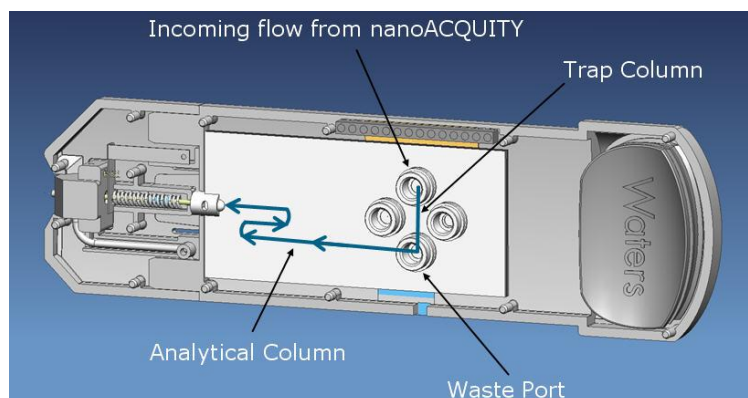


Figure 93 - nanoTile flow path

nanoACQUITY UPLC setup

1. Make the connections between the nanoACQUITY and the nanoTile (Figure 94).

NOTE: Refer to document [nanoACQUITY UPLC 10k PSI Plumbing](#) (715001069) and also the [nanoACQUITY UPLC System with 2D Operator's Guide](#) (71500097502) if required.

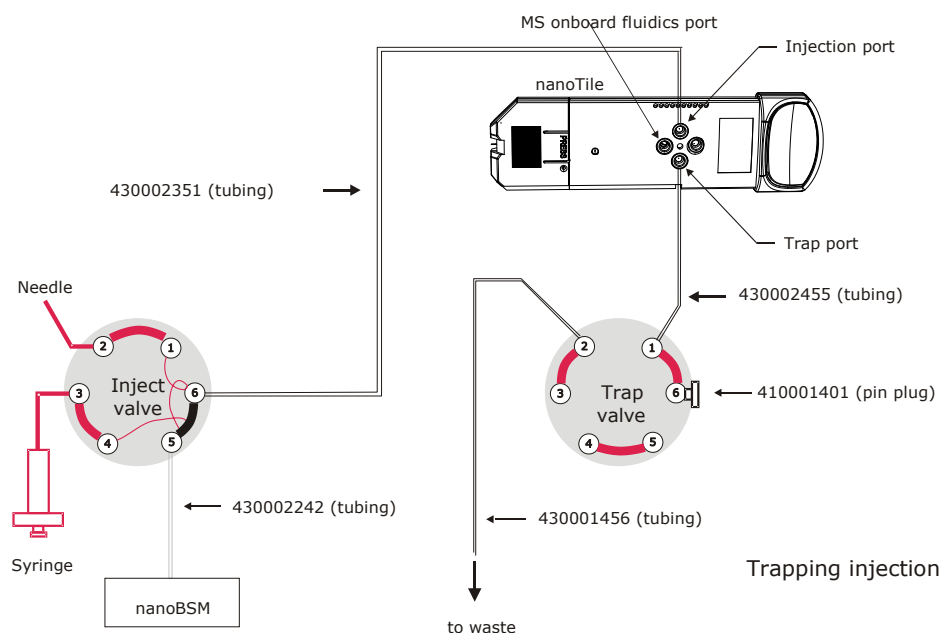


Figure 94 - Diagram of connections (TRIZAIC)

2. Place solvent line A into a reservoir bottle containing water plus 0.1% formic acid.
3. Place solvent line B into a reservoir bottle containing acetonitrile plus 0.1% formic acid.
4. Set the flow rate to **8 $\mu\text{L}/\text{min}$** in Trapping mode at **85% B** for five minutes.

CAUTION: During this stage ensure that the column temperature remains switched off.

Ensure the voltage is off during trapping, and on during analytical.

5. Stop the flow and switch to Analytical mode.
6. Set the flow rate to **0.45 $\mu\text{L}/\text{min}$** at **85% B** and allow the column to flush for five minutes.
7. Stop the flow and switch to Trapping mode.
8. Set the flow rate to **8 $\mu\text{L}/\text{min}$** at **50% B** and leave to flush for five minutes.
9. Stop the flow and switch to Analytical mode.
10. Set the flow rate to **0.45 $\mu\text{L}/\text{min}$** at **50% B** and leave to flush for five minutes.

11. Stop the flow and switch to Trapping mode.
12. Set the flow rate to **8 µL/min** at **3% B** and leave to flush for five minutes.
13. Stop the flow and switch to Analytical mode.
14. Set the flow rate to **0.45 µL/min** at **3% B** and leave to flush for five minutes.
15. Record the backpressure achieved at 3% B.

Sample preparation

Perform the following procedure using the vial of Waters MassPREP Digestion Standard Mixture 1 (186002865).

Table 40: Waters MassPREP Digestion Standard Mixture 1 components

Component	Quantity
Enolase (P00924)	50 pmol
ADH (P00330)	50 pmol
BSA (P02769)	50 pmol
Phos B (P00489)	50 pmol

1. Reconstitute the vial in 100 µL of 85/15 H₂O/CAN to make a 500 fmol/µL stock solution of each protein.
2. Dilute above 20 times to make a 25 fmol/µL stock solution of each protein.
3. Dilute above 10 times to make a 2.5 fmol/µL working solution of each protein.

nanoACQUITY UPLC method

1. Access the inlet method editor, and click  , to open the binary solvent manager method editor.
2. Create an inlet method using the parameters shown in [Table 41](#).

Table 41: Binary solvent manager gradient values

	Time (mins)	Flow (µL/min)	%A	%B	Curve
1	Initial	0.45	97.0	3.0	Initial
2	30.00	0.45	60	40	6
3	35.00	0.45	15.0	85.0	6
4	40.00	0.45	15.0	85.0	6
5	45.00	0.45	97.0	3.0	6

3. Click  to customize a mobile phase name.

4. Click the **Trapping** tab and set the parameters shown in [Table 42](#).

Table 42: Binary solvent manager trapping conditions

Parameter	Value
Run time	70 min
Flow rate	8 µL/min
%A1	99%
%B1	1%
Pressure limit (low)	0 psi
Pressure limit (high)	5000 psi
Sample loading time	2 min

5. Click the **Autosampler** icon to open the sample manager method editor.
 6. Create an autosampler method using the parameters in [Table 43](#).

Table 43: Autosampler method parameters

Parameter	Value
Run time	60 min
Sample loop option	2 µL Partial Loop
Loop offline	Disable
No injection	Leave unchecked
Wash solvents (weak)	100% Water + 0.1% Formic Acid
Wash solvent volume (weak)	1000 µL
Wash solvents (strong)	100% Acetonitrile + 0.1% Formic Acid
Wash solvent volume (strong)	200 µL
nanoTile Temperature	35 °C
Sample temperature	8 °C

7. In the Autosampler window, click **Advanced**.
 8. Clear the check boxes for the advanced features.

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

9. In the inlet editor, click **File > Save As**, and save the inlet method as **TRIZAIC_TQMS_ddmmyy**.

10. Click  to load the inlet method to the nanoACQUITY UPLC system.

MS method

Each function contains transitions from one precursor mass to two fragment ions. The appearance of a peak in both mass channels indicates the presence of the peptide.

1. Enter the masses, cone voltages, and collision energies into the MS method (Figure 95).

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	PHO T88 - UNIDENTIFIED	527.2900	581.2700	☒	0.094	22	20	☐	
2	PHO T88 - UNIDENTIFIED	527.2900	841.4200	☒	0.094	22	20	☐	

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	BSA T10 - UNIDENTIFIED	582.3200	595.3100	☒	0.094	24	20	☐	
2	BSA T10 - UNIDENTIFIED	582.3200	951.4700	☒	0.094	24	20	☐	

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	ADH T17 - UNIDENTIFIED	626.3400	752.3700	☒	0.094	22	22	☐	
2	ADH T17 - UNIDENTIFIED	626.3400	851.4400	☒	0.094	22	22	☐	

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	ENL T43 - UNIDENTIFIED	644.9000	834.4000	☒	0.094	30	24	☐	
2	ENL T43 - UNIDENTIFIED	644.9000	947.5000	☒	0.094	30	24	☐	

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	BSA T59 - UNIDENTIFIED	653.3600	712.4400	☒	0.094	30	26	☐	
2	BSA T59 - UNIDENTIFIED	653.3600	1055.5600	☒	0.094	30	26	☐	

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	ENL T4 - UNIDENTIFIED	708.9000	623.3000	☒	0.094	30	20	☐	
2	ENL T4 - UNIDENTIFIED	708.9000	948.5000	☒	0.094	30	24	☐	

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	PHO T33 - UNIDENTIFIED	721.8500	1067.4700	☒	0.094	30	24	☐	
2	PHO T33 - UNIDENTIFIED	721.8500	1230.5400	☒	0.094	30	24	☐	

	Compound Name	Parent (m/z)	Daughter (m/z)	Au	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
1	ADH T19 - UNIDENTIFIED	814.5000	456.2500	☒	0.094	50	36	☐	
2	ADH T19 - UNIDENTIFIED	814.5000	487.3300	☒	0.094	50	36	☐	

Figure 95 - MS method daughter transition masses

2. In the MS method, set the parameters shown in [Table 44](#).

Table 44: MS method parameters

Parameter	Value
Ionization Mode	ES+
Span	0
Use Tune Cone Voltage	Unchecked
Use Tune Collision Energy	Unchecked
Start	0
End	60

3. In the method options, set the parameters shown in [Figure 96](#).

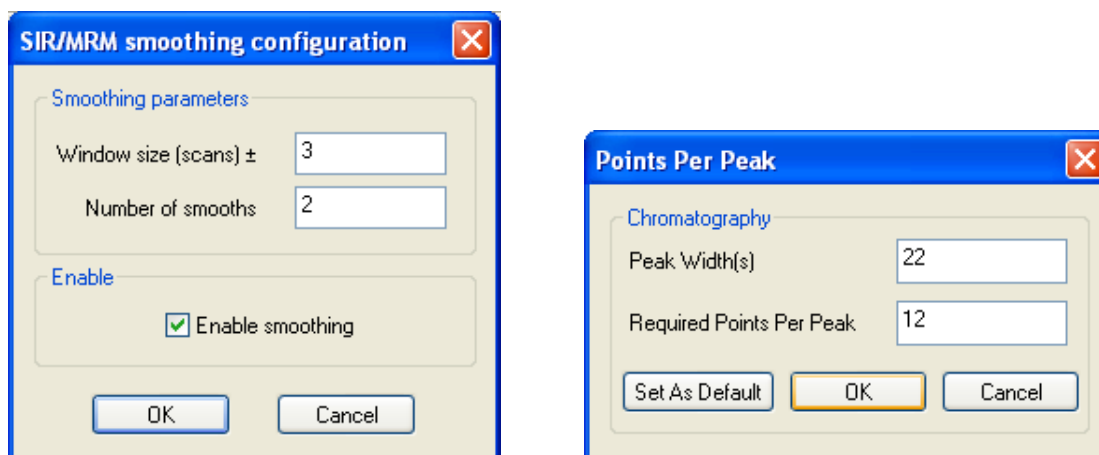


Figure 96 - MS method option settings

4. In the MS method editor, click **File > Save As**, and save the MS method as **TRIZAIC_TQMS_ddmmyy**.

Acquiring data

1. Insert the vial of 2.5 fmol/ μ L Mix 1 working solution in position 1:A,1 (1:1) on tray 1 in the sample manager.
2. Insert the vial of water with 0.1 formic acid (the same as mobile phase A1) in position 1:B,1 (1:2) on tray 1 in the sample manager
3. Create a sample list with one blank and five sample injections and set the injection volumes to 2 μ L.
4. Select all six lines in the sample list and click the **Play** icon.

Performance tests

The peak retention times for each component over three replicate injections must have a standard deviation of ≤ 0.25 min.

1. Record the results in the [Xevo TQ-S ACQUITY UPLC System Installation Checklist](#) (715002460).

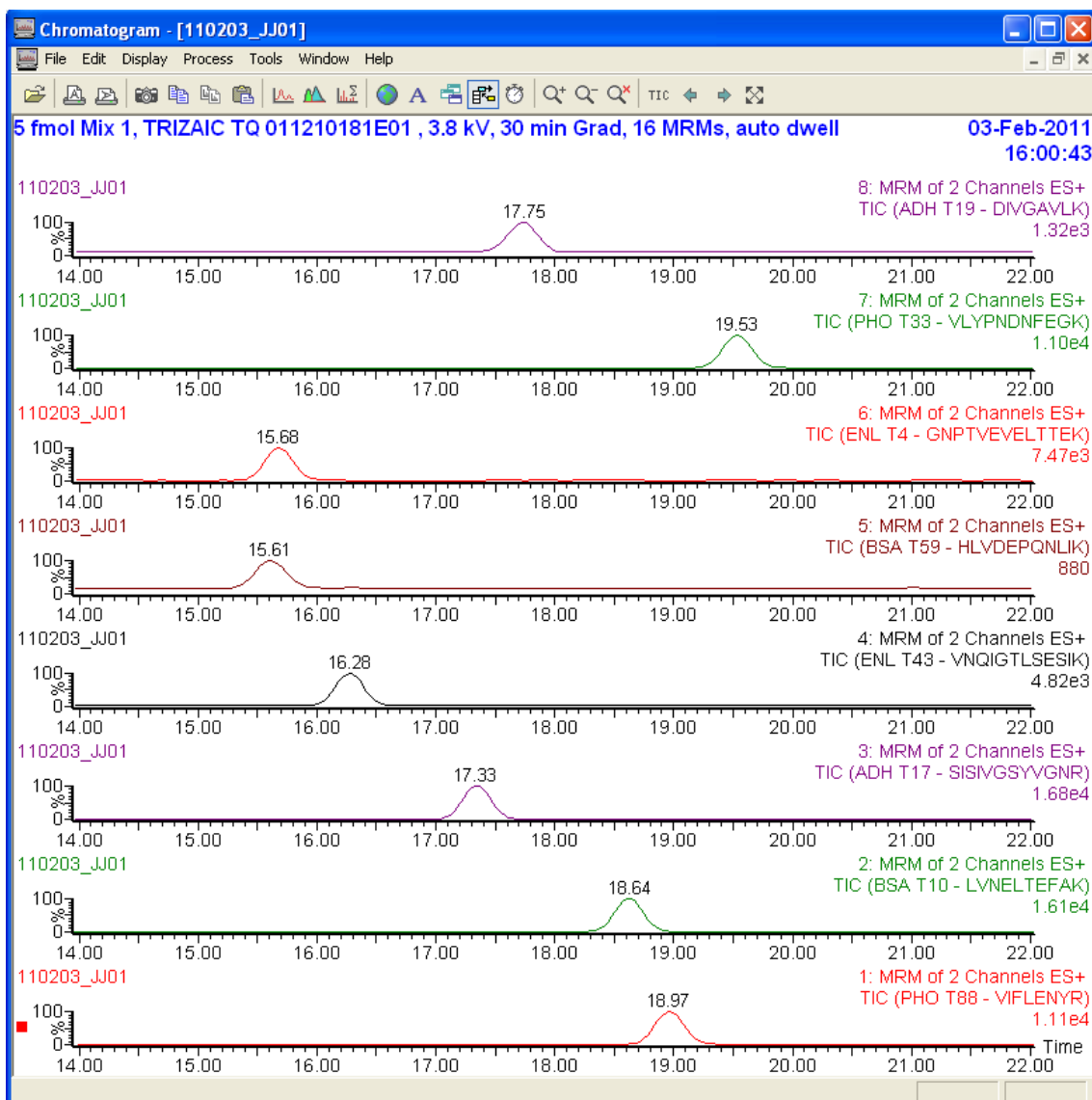


Figure 97 - 5 fmol Mix 1 injected onto tile

Completing the installation

Resetting the service interval

At the end of the installation, reset the MS Console service record.

1. Open the MS Console.
2. Click **Xevo TQ-S > Troubleshoot > Service Record**.
3. In the Service Record dialog box, enter the date of the instrument installation, the engineer's name and the date of the next scheduled service.

NOTE: *The next scheduled service is typically a PM visit, one year after the installation.*

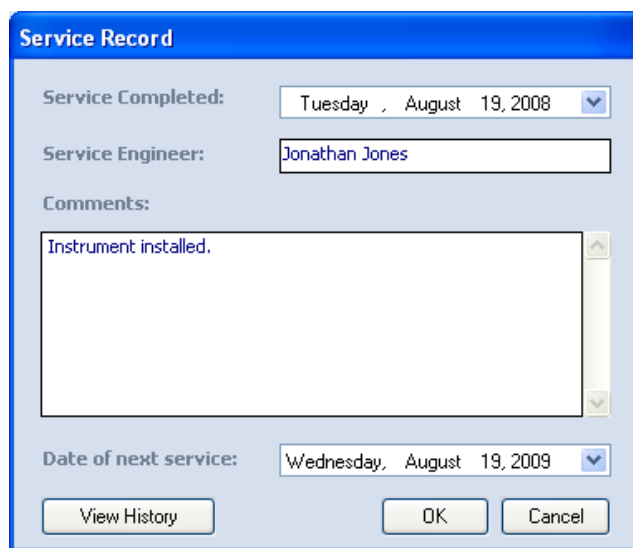
The image shows a 'Service Record' dialog box with a blue title bar. It contains several input fields: 'Service Completed:' with a date dropdown set to 'Tuesday, August 19, 2008'; 'Service Engineer:' with a text box containing 'Jonathan Jones'; 'Comments:' with a large text area containing 'Instrument installed.'; and 'Date of next service:' with a date dropdown set to 'Wednesday, August 19, 2009'. At the bottom, there are three buttons: 'View History', 'OK', and 'Cancel'.

Figure 98 - Service Record dialog box

4. Click **OK** when complete.

Removing the MS Tune page service views

At the end of the installation, remove the advanced views from the MS Tune page.

1. Open the MS Tune page.
2. Click **View > Normal** to set the MS Tune page back to the normal state.
3. Ensure all the advanced menus are not visible.

Saving installation data

1. Save copies of the installation and system QC projects to DVD.
2. Label the DVD with the date and instrument serial number.
3. Store the DVD with the installation signoff documentation and printouts.

Appendix A: Negative ion optimum ion energy

1. Optimize the ion energy in MS1 Negative Ion mode:
 - a. In negative mode, open a single MS1 Scan window to display mass 554 Da with a span of 3.
 - b. Optimize the MS1 ion energy to maximize the intensity of the m/z 554 ion.
 - c. Tune the ion energy so that the intensity of the m/z 554 ion drops to between 5 and 10% of the maximum intensity obtained in step b.
 - d. Add **0.5 V** to the current ion energy value. This is the optimal low mass ion energy for negative MS1.
2. Optimize the ion energy in MS2 negative mode:
 - a. In negative mode, open a single MS Scan window to display mass 554 Da with a span of 3.
 - b. Set the collision energy to 5 V.
 - c. Turn on the collision gas and set a flow to give a collision cell pressure of **3.5E-3 mbar**.
 - d. Optimize the MS2 ion energy to maximize the intensity of the m/z 554 ion.
 - e. Tune the ion energy so that the intensity of the m/z 554 ion drops to 10% of the maximum intensity obtained in step d.
 - f. Add **1 V** to the current ion energy value. This is the optimal low mass ion energy for MS2.

Appendix B: Using the Windows statistical calculator

This section describes using the Windows statistical calculator for calculating the average, standard deviation, and %RSD values.

Calculating the average and standard deviation

1. Click **Start > All Programs > Accessories > Calculator**. The Windows calculator appears.
2. Click **View > Statistics**. The calculator switches to statistical view.
3. Enter the first value, for example retention time, signal-to-noise ratio, or peak area/height.
4. Click **Add** in the lower right corner of the calculator.
5. Enter each of the remaining values, clicking **Add** after each to enter it in the statistics box.
6. Once all values are entered, click $\bar{x}$ to calculate the average value.
7. Click σ_n to calculate the standard deviation.

NOTE: Do not close the calculator; it will be used in the following section.

Calculating the %RSD

1. Click **Start > All Programs > Accessories > Calculator** to open a second Windows calculator.

NOTE: It is not necessary to switch the mode of the calculator; it is required only for basic calculation.

2. Calculate the %RSD using the formula in [Figure 99](#), referring to the other calculator for the average and standard deviation.

$$\%RSD = \frac{(\text{Standard Deviation} \times 100)}{\text{Average}}$$

Figure 99 - %RSD equation

Appendix C: Useful software tools

Waters DHCP server configuration

Waters DHCP server configuration.exe allows the user to determine the IP and MAC addresses of the Waters components connected to the host PC.

The software can be found at: C:\Program Files\Waters Instruments\



Figure 100 - Waters DHCP server configuration.exe icon

Stop ACQUITY Processes

Use Stop Acquity Processes.cmd when resetting the ACQUITY communications/software to terminate any processes which may be running in the background.

The software can be found at: C:\Program Files\Waters Instruments\Bin\



Figure 101 - Stop Acquity Processes.cmd icon

Appendix D: Solvent quality check (LC/MS system test)

The test provides a means of gauging system cleanliness so that quality items (such as solvent quality, LC system contamination, and source cleanliness, for example) can be monitored and optimized.

The level of sodium adducting will vary if, for example, the source or chromatographic conditions are changed from those specified in this test. The nature of the analyte also determines the degree of sodium adduct formed; the absence of a sodium adduct on a particular compound (for example hydroxyprogesterone) does not mean suppression is not taking place; the <10% value is therefore only relevant for sulfadimethoxine under the specified conditions/procedure.

The target solution contains the components shown in [Table 45](#).

Table 45: System test sample components

Component	Concentration (pg/ μ L)
Acetaminofen	0.2
Caffeine	0.2
Sulphadimethoxine	0.1
Verapamil	0.05
Chloramphenicol	0.05
17- α -hydroxyprogesterone	5

Preparing the test solution

Perform the following steps to prepare the test sample solution:

1. Add 100 μ L of Tuning Solution to a 10-mL volumetric flask and dilute to 10 mL with diluent.
2. Stopper and invert the flask several times to fully mix.

Preparing the system for testing

1. Use the LC conditions and methods and the MS conditions that were used for the ESI positive ion sensitivity test described earlier in this document.
2. For the MS acquisition create an MS full scan experiment file as shown in [Figure 102](#).

Function:1 MS2 Scan

Mass (m/z)

Start: 210

End: 410

Method

Ionization Mode: ES+

Data: Continuum

Time (Mins)

Start: 0

End: 2.5

Scan Duration (secs)

Scan Time: 0.1

Cone Voltage

☐ Use Tune Page

Cone Voltage (V): 40

☐ Use Cone Voltage Ramp

CV Ramp...

APCI Probe

☐ Use Tune Page Settings

Probe Temp: 500

OK Cancel

Figure 102 - MS method parameters for solvent quality check

NOTE: The cone voltage must be set to the value used for the positive ion sensitivity specification.

Test procedure

1. Acquire six replicate injections.
2. Create an extracted mass chromatogram for sulfadimethoxine (take the exact m/z value from the spectrum) for each of the last five injections.

NOTE: The first injection is a setup injection and is not used.

3. Right-click and drag across the peak to create a combined spectrum from the entire chromatographic peak.
4. Smooth the spectra using the parameters shown in Figure 103.

Spectrum Smooth

Peak width (Da): 0.5

Number of smooths: 2

Smoothing method

☐ Mean

☐ Median

☒ Savitzky Golay

OK Cancel

Figure 103 - Smoothing parameters

5. Calculate the mean sodium adduct % over the five replicates (height of m/z 333 Da as a % of m/z 311 Da). Example spectra are shown in [Figure 104](#).

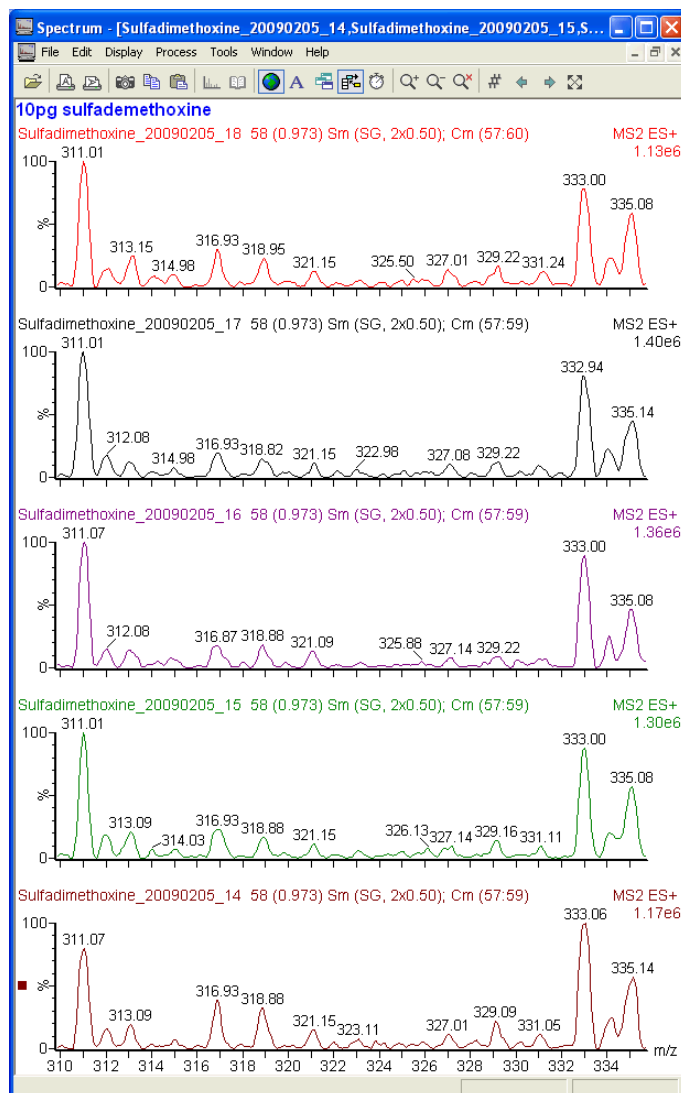


Figure 104 - Combined extracted sulfadimethoxine spectra from five injections

NOTE: The intensities of sodiated sulfadimethoxine peaks shown in these spectra are approximately equal to the protonated sulfadimethoxine signal, and are therefore unacceptably high.

Evaluation of results

The level of sodium adducting must be $<10\%$ to ensure minimal loss in performance. If the intensity is $>10\%$ then the mobile phase must be remade, preferably using solvents from different batches or sources.