Waters® Xevo® QTof MS

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 QTof MS system at a customer site. The FSE must have attended the official Xevo QTof MS training course and obtained the necessary certification before carrying out an installation.

The customer must have completed a *Xevo QTof MS Site Preparation Guide* (p/n 715001948) 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 cartons.

For instruments with no UPLC, the *Xevo QTof MS Installation Checklist* (p/n 715002003) must be completed during the course of the installation. If the qualification option has been purchased then the installation checklist is not required and instead the installation qualification and the operational qualification (document set p/n 715002075) documents must be completed.

> ***NOTE:*** *The Xevo QTof MS Installation Checklist (p/n 715002003) does not contain the ACQUITY UPLC or nanoACQUITY UPLC checklists and specifications.*
>
> *If the system includes an ACQUITY UPLC or nanoACQUITY UPLC then either the Xevo QTof MS ACQUITY UPLC System Installation Checklist (p/n 715002120) or Xevo QTof MS nanoACQUITY System Installation Checklist (p/n 715002121) must be completed when being installed with a Xevo QTof MS.*

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.

Ensure that the customer is fully aware of the advantages of the iAssist capability within Connections INSIGHT which allows remote instrument troubleshooting after installation. For more information refer to the latest service notes on Connections INSIGHT.

Figure 1 - Xevo QTof MS

Useful information and contacts

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

Toll free numbers are available for contacting Manchester GSS 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 main Atlas Park switchboard +44 (0) 161 435 4100.

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

Startup procedures

The startup procedures in the appropriate system checklists describe 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 the service and support area of the Portal.

Site preparation

The Xevo QTof MS requires specific laboratory facilities that must be in place prior to the installation commencing. These prerequisites are detailed in the *Xevo QTof MS Site Preparation Guide* (p/n 715001948) which is available from the service and support area of the Portal. 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

- Xevo QTof MS installation kit   (p/n 205000594)
- Waters QTof chemical kit   (p/n 700003276, 700004734 and 700004768)

In addition to the items above, the Xevo QTof MS includes several consumables spares kits.

Customer training

Initial customer training is expected to require 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 shipping cartons 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 Manchester.

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

Chemicals

The Xevo QTof MS uses the *Waters Q-Tof Products Chemical Kit* (p/n 700004734) which includes a set of instructions for preparing the required standard solutions (p/n 715001491).

The customer is required to supply HPLC grade solvents or better, and/or additives such as formic acid, for preparing the mobile phases for any options purchased. These requirements are detailed in the *Xevo QTof MS Site Preparation Guide* (p/n 715001948).

Table 1: Systems and required kit

System	Kit Required
Systems with ACQUITY UPLC	Waters ACQUITY UPLC MS Startup Solution kit (p/n 700002741)
Systems with nanoACQUITY UPLC	Waters MassPREP digestion standard Mixture 1 (p/n 186002865)

Waters does not provide full 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 commencing.

Unpacking the instrument

The Xevo QTof MS is delivered in a number of carton/boxes. The largest carton size has a width of 1000 mm (39.4 in.), a depth of 1140 mm (44.9 in.), and a height of 1235 mm (48.6 in.). The gross weight is approximately 250 kg (551 lbs). The large carton contains the instrument, and must be unpacked according to the instructions below.

A forklift truck or A-frame hoist is required for lifting and transporting the instrument.

> *CAUTION:*   *Read through the instructions before beginning to unpack the carton.*

Unpacking the transport cartons

> *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 lifting and positioning the instrument.*

1. Check all shock and tilt watches.
2. If any watchers have turned red, report to the local service manager and GSS before progressing any further.

Figure 2 - Checking the shock and tilt indicators

3. Carefully remove the packaging.
4. Remove the outer foil, but leave any inner protecting material in place.
5. Perform a visual check to ensure there is no damage to the packaging or instrument.

Lifting the instrument

The instrument is fitted with a lifting harness, which must be used to lift the instrument from the shipping carton onto the bench (Figure 3). The net weight of the Xevo QTof MS is approximately 200 kg (441 lbs).

> *WARNING:* **The instrument must only be lifted using lifting equipment capable of raising 200 kg (441 lbs) safely. The instrument must not be lifted manually. The lifting harness requires a minimum additional 300 mm (12 in.) above the highest point of the instrument. The lifting equipment must be capable of raising the instrument to sufficient height above the bench on which it is to be located.**
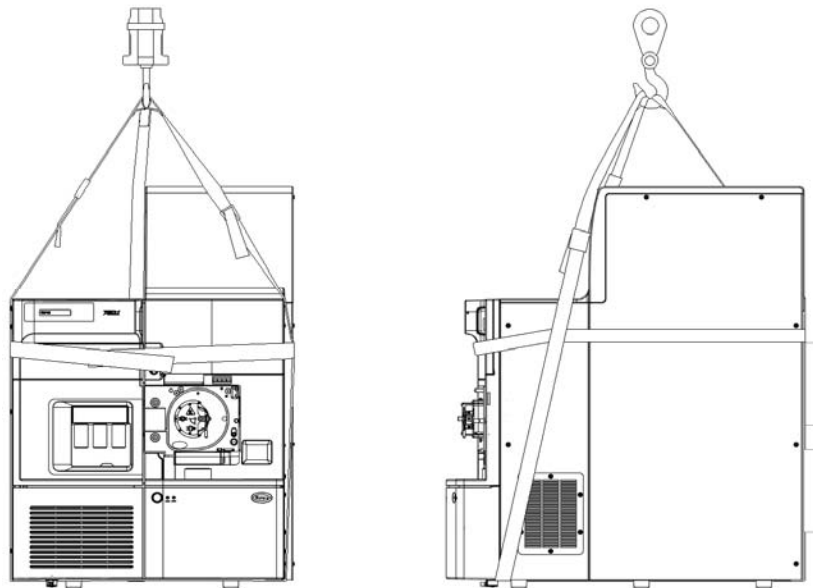
Figure 3 - Xevo QTof MS lifting harness

Positioning the instrument

1. Install the instrument upon a flat bench, preferably one that is movable.
2. Ensure the instrument has the following clearances:
   - 600 mm (23.6 in.) to the front, back, and right of the instrument
   - Permanent clearance of 30 mm (1.2 in.) to the left of the instrument
   - Temporary clearance of 1000 mm (39.4 in.) to the left of the instrument for service
3. If the instrument is placed on a moveable bench, allow 150 mm (6 in.) minimum clearance behind the instrument for service access and ventilation.

4. Install the external roughing pump on a level surface underneath or behind the Xevo QTof MS and within 1 m (3.3 ft) of the rear of the instrument chassis.

   CAUTION: **To prevent overheating it is not recommended that the external pump is installed behind closed doors. It is recommended that the external pump is elevated 150 to 200 mm (6 to 8 in) 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 in) in the bench top to allow the tubing to be passed through to the instrument (Figure 4).*

   The access slot may be positioned outside the footprint. The access slot must allow the vacuum tubes to follow a smooth radial bend when connected to the rear of the instrument; the diameter of the vacuum tubes must not be pinched or kinked in any way.

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

5. Once the instrument is in its final location, remove any remaining protective packing material.
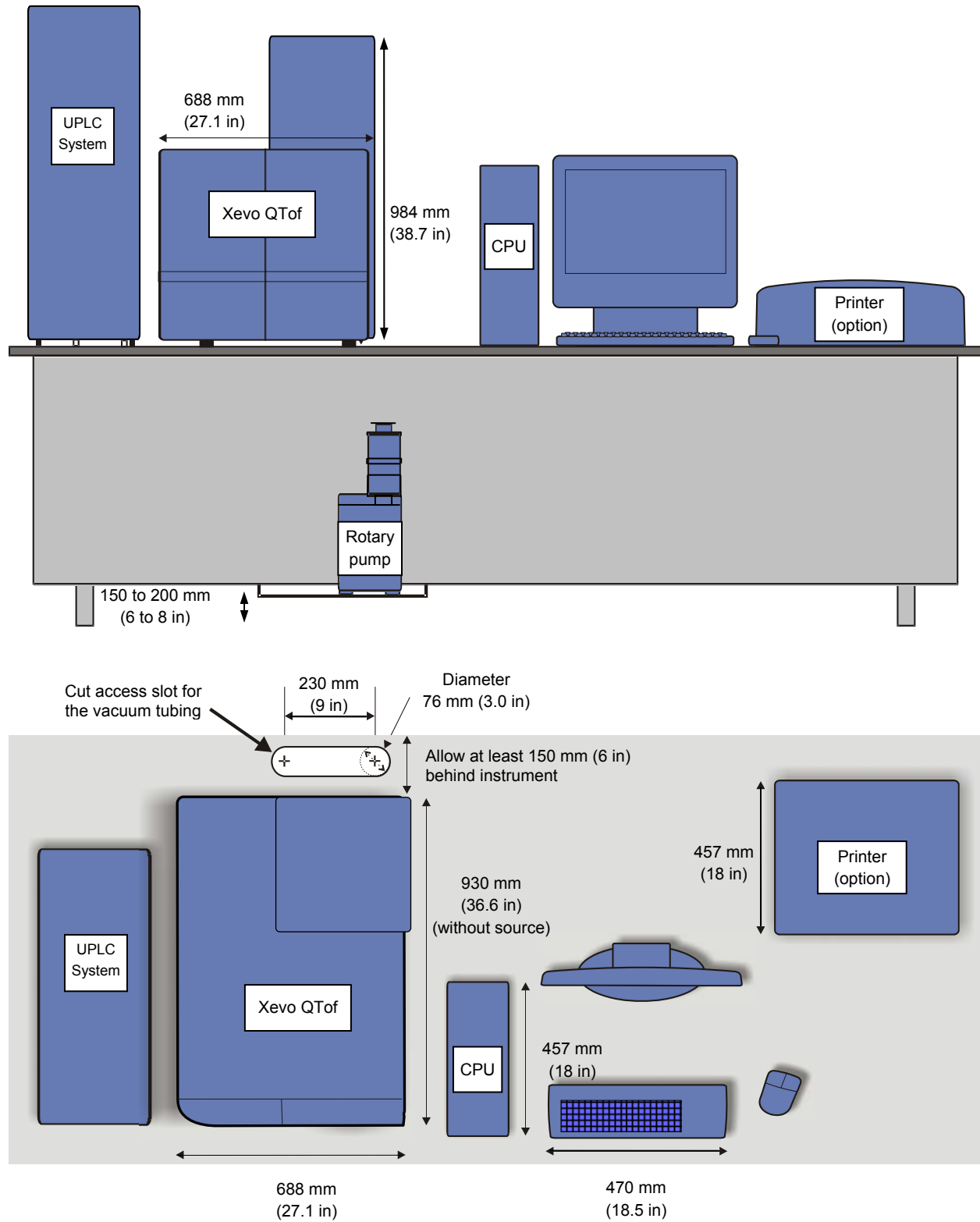
Figure 4 - Plan and front views showing a typical layout

Instrument setup

Setting up the system

Connecting the fluidics waste line

1. Connect the waste fitting (located under the instrument) to a suitable waste container.

2. Slide a 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 5 - Liquid waste line connection

3. To avoid fluid backup, perform the following procedure to 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.*
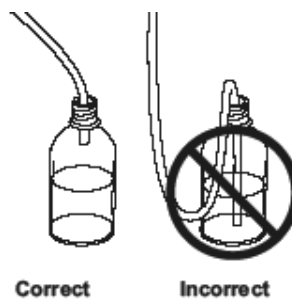
Figure 6 - Waste container connection

Rear panel connections
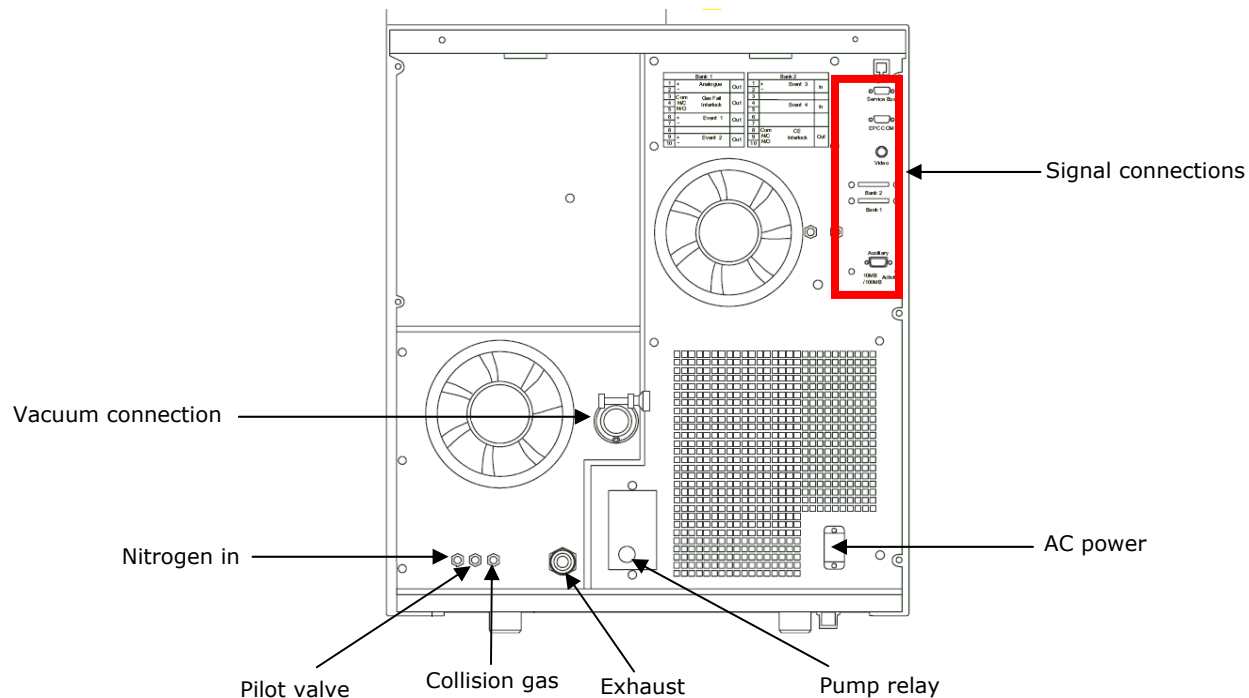
Figure 7 - Rear panel connections

Connecting the instrument exhaust line

The exhaust on the instrument rear panel is used to vent the source gases and must be connected to a suitable extraction system via the waste bottle (Figure 8). This waste system comprises a single continuous waste line between the source and the exhaust 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 using 12-mm PTFE tubing.

2. Connect the pilot valve on the waste bottle to the instrument using 4-mm PTFE tubing.

3. Connect the outlet of the trap bottle to the laboratory exhaust system, using the 12-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 millibar below atmospheric pressure (negative pressure). It must be capable of supporting a maximum instrument exhaust gas load of 2000 L/hour.*
>
> *The source exhaust line must be connected either to a laboratory fume hood or to an active exhaust system. Where a shared exhaust system is used, the source exhaust must be connected via its own exhaust spur.*
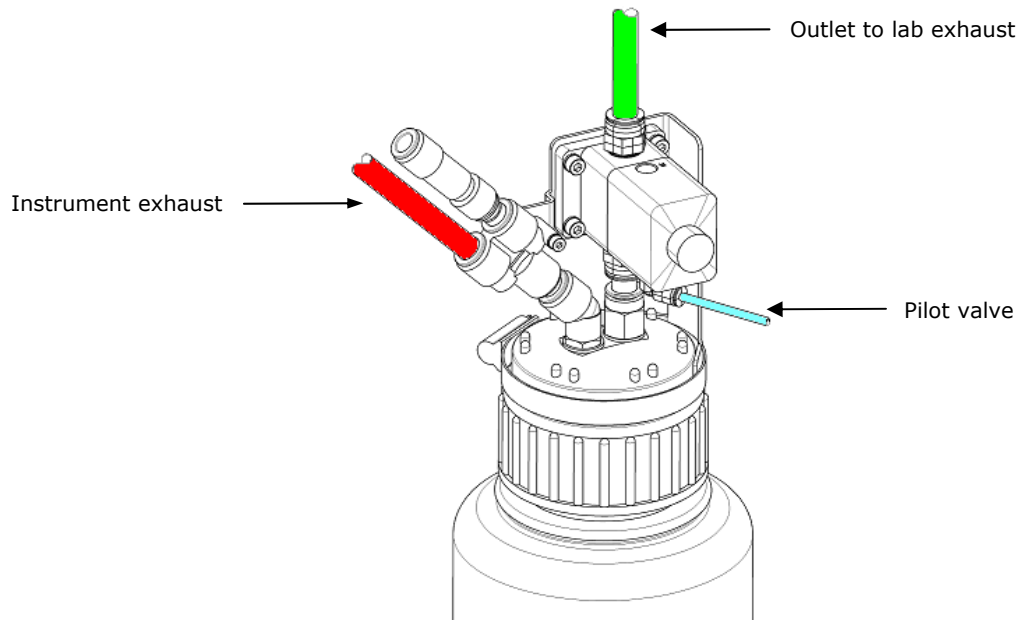
Figure 8 - Source waste trap bottle assembly

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

Connecting the API gas supply

The Xevo QTof MS requires a supply of dry, oil free nitrogen with a purity of at least 95%.

1. Ensure the nitrogen is regulated at 7 bar (100 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 (p/n 6060732) supplied in the installation kit.

> *WARNING:* **Use the entire 5 m length of supplied 6-mm tubing. This is a safety requirement and minimizes the rate of nitrogen escape into the laboratory in the event of a gas leak at the instrument.**

Connecting the collision gas supply

The Xevo QTof MS 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 supply to the collision gas port on the instrument rear panel, using the ⅛-inch medical grade stainless steel tubing and Swagelok fittings supplied with the installation kit.

Connecting the instrument power

1. Connect the 2.5 m (8 ft) power cable from the power connector on the rear panel of the instrument to the laboratory mains supply.

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

Setting up the external pump

The Xevo QTof MS ships with either a Varian HS602 rotary pump, or an Edwards XDS35i scroll pump. Follow the appropriate procedure to suit the configuration on site. See "Appendix A: Installing the Varian external oil mist filter" for details of fitting the oil return kit (mist filter).

Setting up a rotary pump (Varian HS602)

WARNING: **Always wear protective gloves when handling pump oil.**

1. Unpack the pump and place in the final location.

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

2. Install the exhaust filter and oil return tubing if not already present (see "Appendix A: Installing the Varian external oil mist filter").

3. Fill the pump with oil to just below the maximum level.

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

4. Connect the rotary pump exhaust to a suitable air extraction system using the 19-mm PVC hose supplied in the installation kit.

5. Connect the hose to the 25-mm nozzle on the pump.

6. Secure the hose using a hose clip supplied with the installation kit.

   WARNING: **The pump exhaust must be installed properly and must be monitored by the user for oil build up.**

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

7. Connect the pump control cable to the pump.

8. Connect the other end of the pump control cable to the pump relay port on the rear panel of the instrument.

9. Turn on the power switch on the pump.

10. Using the appropriate components, configure the vacuum hose as shown in Figure 9.

To exhaust system ⟶

⟵ To rear panel
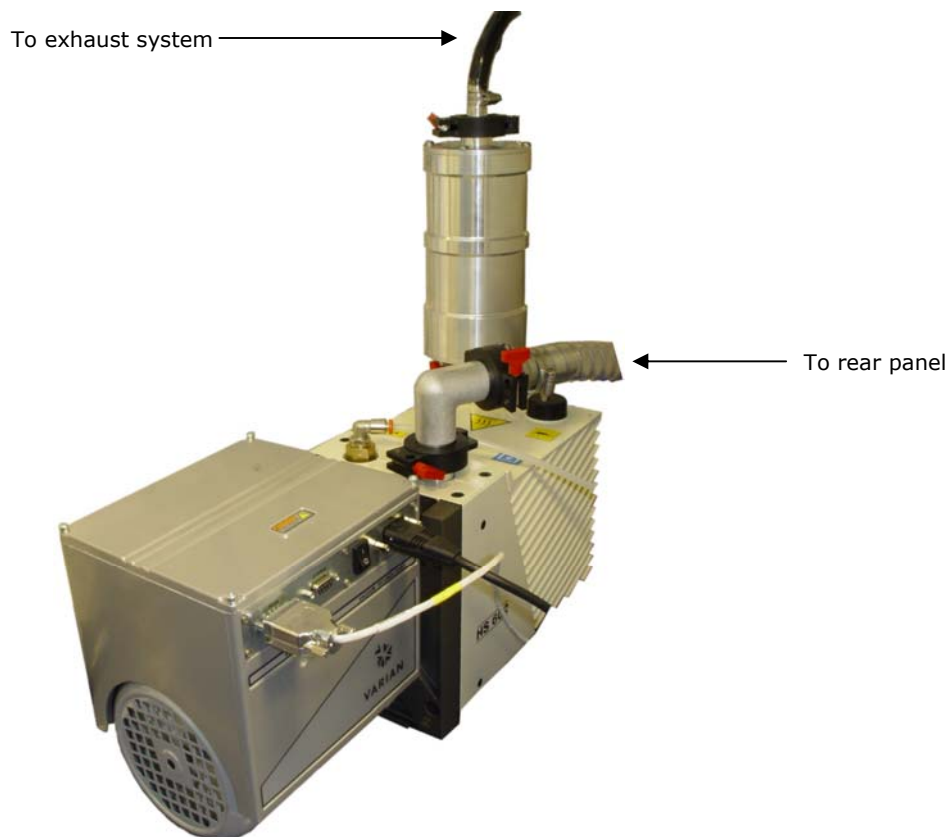
Figure 9 - Rotary pump tubing connections

Setting up a scroll pump (Edwards XDS35i)

Perform the following steps to unpack and setup the pump:

1. Gently roll the scroll pump out of its packaging to avoid unnecessary lifting.
2. Roll the polystyrene packing case onto its top.
3. Remove the bottom section.
4. Roll the pump back onto its rubber feet.

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

5. Connect the scroll pump exhaust to a suitable air extraction system using the 19 mm PVC hose supplied in the installation kit.
6. Connect the hose to the 25-mm nozzle on the pump.
7. Secure the hose using a hose clip supplied with the installation kit.

   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 also be monitored by the user for safe operation.

11. Connect the pump control cable to the pump.

12. Connect the pump control cable to the pump relay port on the rear panel of the instrument.

13. Ensure the ballast control on the scroll pump is set to position zero.

> **CAUTION:** **Do not configure the scroll pump to 120 V. It requires a 200 to 230 V, 50/60Hz supply.**

8. Before connecting the scroll pump to the mains, ensure that the mains switch on the pump is off.

> **NOTE:** *This ensures that the pump will only activate when requested by the instrument via the pump control cable.*

14. Using the appropriate components, configure the vacuum hose as shown in Figure 10.

To rear panel

To exhaust system
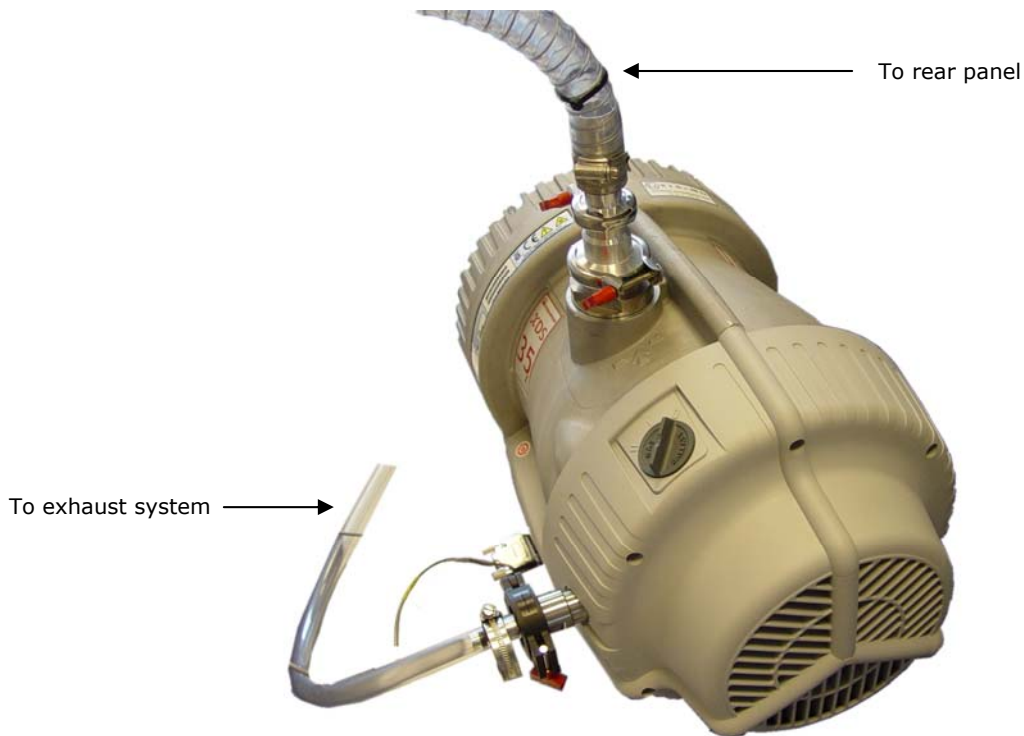
Figure 10 - Scroll pump tubing connections

Final pump configuration (scroll and rotary pumps)

1. On the rear panel, set the pumping override switch to the upper position, Auto.

2. Set the electronics switch to the upper position, Electronics On.

Switches in
upper
position
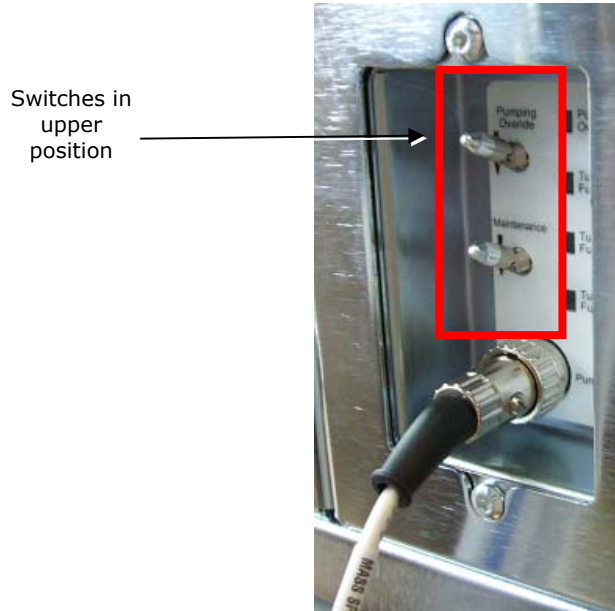
Figure 11 - Checking the rear panel switches

NOTE: *The instrument fans are not required at this time. If the fans start spinning, press the power button on the front panel while the remaining connections are made.*

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

3. Connect the vacuum hose to the port on the instrument rear panel, using the additional NW25 elbow fitting in the installation kit, where required.

4. Connect the power cable from the pump to the laboratory power supply.

Other cable connections

All cables inside the instrument are already connected, so the instrument panels do not need to be removed during installation.

A null modem cable is provided in the installation kit to connect to the service bus from the host PC for firmware upgrades. This does not need to be connected under normal circumstances.

CAUTION: *Once all cables are connected, use a cable tie to keep them tidy and prevent a trip hazard behind the instrument.*

Setting up the host PC

Basic setup and installing MassLynx

1. Unpack the host PC, LCD monitor, and other peripherals and place in the final location.

2. Connect the PC components together (such as keyboard, mouse and other hardware).

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 cables 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 = waters).

8. Install the MassLynx software from the supplied CD (see the release notes instructions).

9. If the Xevo QTof MS is not part of an ACQUITY or nanoACQUITY UPLC system, connect the Ethernet cable from the instrument LAN port directly to the host PC network card.

10. Install Connections INSIGHT® and set up the connection (if applicable).

    NOTE: *For details on installing Connections INSIGHT refer to the Connections INSIGHT Installation Guide (p/n 715001399) and Connections INSIGHT User Guide (p/n 715001400). Also refer to the latest service notes for more information.*

ACQUITY UPLC preparation (if applicable)

1. Ensure the ACQUITY UPLC system has been unpacked and placed in the final configuration.

2. Make all electrical connections as appropriate for the ACQUITY UPLC options purchased.

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

3. Connect the Ethernet cable from the instrument LAN port to the Ethernet switch on the rear panel of the high temperature column heater module or column manager, to which the other ACQUITY components are connected.

Figure 12 - Router for ACQUITY UPLC connections

4. Connect a stop flow contact closure cable to the gas fail output, external connections 2, pins 3 and 5, on the instrument rear panel.

5. Connect the other end of the cable to connector II, pins 3 and 4, on the ACQUITY BSM.

nanoACQUITY UPLC preparation (if applicable)

1. Ensure the nanoACQUITY UPLC system has been unpacked and placed in the final configuration.

2. Make all electrical connections as appropriate for the nanoACQUITY UPLC options purchased.

   NOTE: *Refer to the nanoACQUITY System Installation Checklist (p/n 715001015) for further details.*

3. Connect the Ethernet cable from the instrument LAN port to the Ethernet switch provided with the nanoACQUITY, to which the other nanoACQUITY components are connected.

Powering on and pumping the instrument

>*NOTE:*   *Ensure that the host PC is turned on, MassLynx has been installed, and that all Ethernet cables have been connected.*

Turning instrument power on
(without ACQUITY UPLC or nanoACQUITY UPLC)

1. Press the button on the front panel to switch on power to the instrument.
2. Check the instrument fans are spinning on the rear panel.
3. Allow up to ten minutes for the EPC to boot succesfully.

>*NOTE:*   *The first time the EPC boots it may need to download extra information from the Host PC. Subsequent boot ups will occur more quickly.*

Turning instrument power on (with ACQUITY UPLC)

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

>*CAUTION:*   **The ACQUITY UPLC module which contains the Ethernet switch must be turned on first to allow communications to be established.**

2. Press the button on the front panel of the Xevo QTof to switch on its power.
3. Check the instrument fans are spinning on the rear panel.
4. Switch on power to the binary solvent manager.
5. Switch on power to any additional UPLC modules.
6. Allow up to ten minutes for the EPC to boot succesfully.

Turning instrument power on (with nanoACQUITY UPLC)

1. Turn on power to the Ethernet switch.

>*CAUTION:*   **The nanoACQUITY Ethernet switch must be turned on first to allow communications to be properly established.**

2. Press the button on the front panel to switch on power to the instrument.
3. Check the instrument fans are spinning on the rear panel.
4. Ensure that power is on for the binary solvent manager.
5. Switch on power to any additional UPLC modules.
6. Allow up to ten minutes for the EPC to boot succesfully.

Launching MassLynx

> *NOTE:* *The system must complete at least one successful boot sequence before the Hosts file is updated with the correct IP address and "EPC" label. The EPC IP address may change from system to system, as the values are controlled by the DHCP server.*
>
> *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 beep is heard from inside the electronics unit.*

1. Launch the MassLynx software.

> *NOTE:* *The informational messages "Instrument Present", followed by "Not Scanning", appears at the bottom of the main MassLynx window.*

2. Ensure that MassLynx successfully establishes communications with the instrument.

3. Click **Start Menu > Run** on the host PC.

4. Type **ping epc –t** to display a command prompt window showing the communication status of the instrument.

Xevo QTof LEDs

The front panel LEDs display information regarding the power and pump status of the instrument.

Table 2: LED indications

LED Type	Mode	Color	Description
Power	Green	🟢	Instrument is in Operate
	Off	⚪	Instrument is in Standby
	Red	🔴	RF trip
	Flashing red (1 Hz)	⊘	Instrument is not at vacuum
	Amber	🟡	Source Standby *
	Flashing green	⊘	Instrument in Operate, with pump override on
	Flashing amber	⊘	Instrument in Standby, with pump override on

> *NOTE:* *The Source Standby indication is available only if the lens/control PCB firmware is version 4258827PDL or later.*

Fitting the source

1. Mount the source supplied with the instrument and fit the ESI probe/sample sprayer as applicable.

2. Connect all cables from the source to the instrument front panel.

3. Close the sliding panel.

4. Connect the outlet line from the sample fluidics to the sample (analyte) probe.

5. Connect the outlet line from the reference fluidics to the LockSpray probe.

6. Place the two long orange PEEK lines from the fluidics (reference valve port 6, analyte valve port 2) into a reservoir of a suitable, clean wash solvent (such as 50:50 methanol/water).

   CAUTION: ***Do not place the wash solvent reservoir, or any other solvents, on top of the instrument.***

7. Purge the fluidics a minimum of three times with the wash solvent.

Fluidics connections

NOTE: *Fluidics connections are complete when receiving an instrument for installation. It is still necessary to supply the wash reservoir, and connect the appropriate lines to the sprayers.*
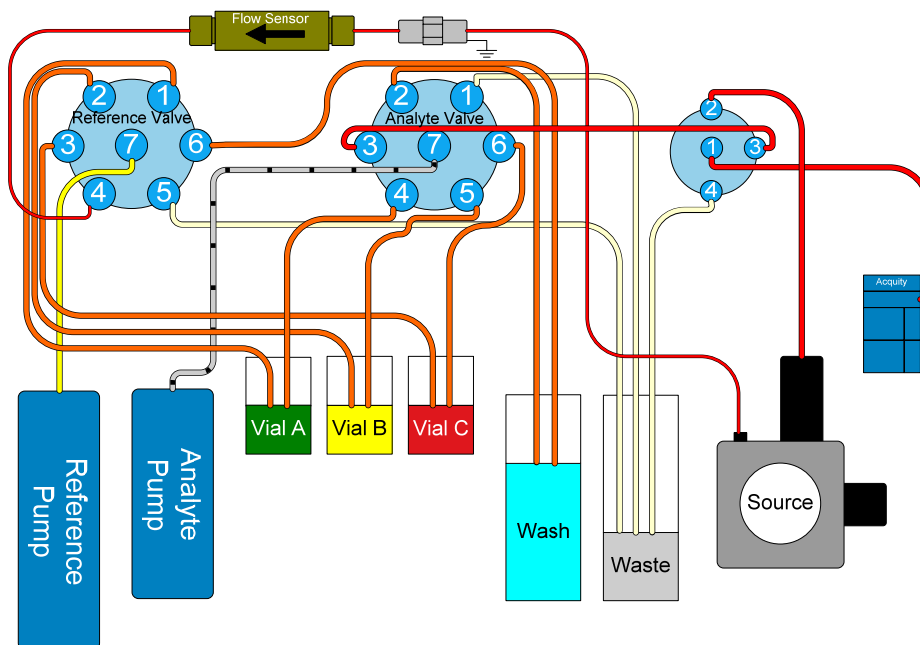
Figure 13 - Fluidic connections

Preparing the instrument for use

The instrument must always be started up through the MassLynx MS Console so that the proper sequence of events is observed. IntelliStart facilitates instrument start up.

1. Click MS Console in the main MassLynx window to launch the MS Console page.

2. Click in the MS Console page to prepare the instrument for use.

   NOTE: *There may be a delay of several seconds before the pump switches on.*

                  The instrument pumps down, and IntelliStart monitors the vacuum. Once the vacuum has reached the required level the MCPs are automatically conditioned. Once conditioning is completed, the instrument is in Operate. The API, collision gases, and source voltages are started automatically.

                  Turbo pump speeds are expected to reach 100% within 5 minutes.

   CAUTION: **The instrument must be started correctly, otherwise important software flags will not be set. This may cause the instrument to perform incorrectly during later tests.**

   NOTE: *On systems with a scroll pump, it is normal to see black powder collecting in the scroll pump exhaust line after initially turning the pump on. This is from the PTFE composite tip seals within the pump, which produce some dust during the initial run-in period.*

3. Open the MS Tune page from the Console window.
4. Check all Tune page readbacks are correct.
5. Check the capillary voltage readback is present.
6. Click on the **Source** menu and select the correct source type as supplied with the instrument.

Checking the source exhaust flow

To ensure that there is flow from the source exhaust, perform the following procedure.

1. Attach a spare length of tubing to the waste bottle exhaust.
2. Place the free end in a beaker of water.
3. Turn on the API gas using the MassLynx software to produce bubbles from the end of the tubing in the water.

   NOTE: *Ensure all sources and probes supplied pass the source pressure test run through IntelliStart (excludes the NanoFlow options).*

4. While the instrument attains a suitable vacuum, complete the plumbing of the ACQUITY or nanoACQUITY.

Performance tests

During the installation the instrument must be tuned and tested to provide the customer with the following items:

- documented evidence of the instrument performance (verification tests)
- a fully working tune file containing the optimized settings for both Positive and Negative Ion modes
- documented evidence of the system performance, including UPLC (where applicable)

> *CAUTION:* **The engineer must read this entire section carefully before progressing with the specifications.**

The instrument EPC contains a flash ROM which contains the optimized system setting tune parameters from factory test.

1. In the MassLynx Tune page, click **Setup > System View**.

2. Type **access** as the password.

3. Click **File > Factory Defaults Import** to load optimized system test tune parameters.

System settings are saved to the host PC registry and have independent settings for Positive Ion mode and Negative Ion mode. This structure then provides the following functionality:

- IPR file containing sample dependent parameters. These do not affect the TOF resolution and TOF sensitivity.
- System parameters containing instrument dependant parameters.

> *CAUTION:* **Instrument dependant parameters affect the TOF resolution and TOF sensitivity.**

Recalling a saved IPR file, or selecting a new IPR file, does not reset the TOF tuning, affect the Veff value or change parameters such as pusher offset. Therefore, unless the system settings have been altered, the instrument TOF tuning will remain stable.

> *CAUTION:* **The system settings must be checked for each of the primary modes as part of the installation.**

1. Name the working tune file that is to be used as YAAXXX.ipr (where XXX is the instrument serial number).

2. Save it within the Acqudb folder inside **Commission.pro**.

   NOTE: *For guidance on instrument tuning refer to "Appendix B: Instrument tuning".*

A Q-Tof Products Sample Kit (p/n 700004734) is shipped with the instrument. Refer to document p/n 715001491 to prepare the samples required for the installation.

For instruments purchased with the NanoLockSpray source, refer to the Xevo QTof MS Operator's Overview and Maintenance Guide (p/n 71500174502) for details on assembling the Sample (analyte) and LockSpray sprayers. This can be completed while the instrument is waiting for vacuum.

For systems with ACQUITY or nanoACQUITY configured, turn off the UPLC triggering while running the MS install tests, by performing the following procedure:

1. Open the Inlet Method editor

2. Click **Tools > Instrument**.

3. Click **Events and Triggering**.

4. Clear the **Event 1** checkbox.

   NOTE: *This must be re-enabled to run the LC-MS system tests.*

Setting up the Commission Project for installation

The Commission.pro project contains predefined sample lists in order to run the installation specifications.

1. Click File > Open Project…
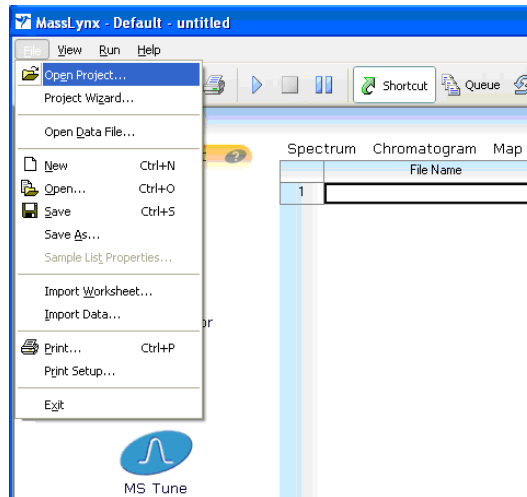
Figure 14 - Opening a project

2. Click **Yes** to the pop up message.
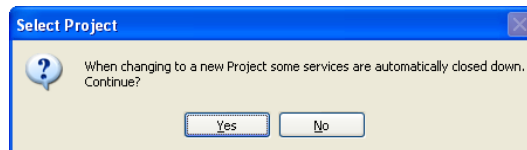
Figure 15 - Responding to message

3. Click **Commission.pro** and click **OK**.
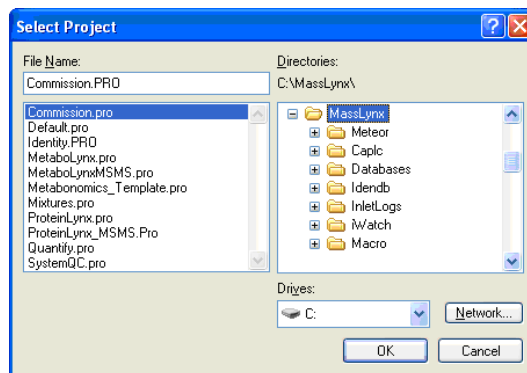
Figure 16 - Selecting a project

Initial setup

1. Click System > Flow Sensor Calibration.

2. Record the flow sensor calibration values in the *Xevo QTof MS Installation Checklist* (p/n 715002003)*.*

    NOTE: *The calibration may take a few moments to appear.*

3. Thoroughly rinse the 30-mL fluidics vials with methanol.

4. Ensure the fluidics tubing to the vials is pulled through the front panel sufficiently to reach the bottom of each vial.

5. Fill each vial with approximately 15 mL of methanol.

6. Install the vials in the instrument.

7. On the MS Console, click **Purge** to purge all fluidics lines to waste.

8. Remove the vials.

9. Refill the fluidics vials as follows:

    - A - [Glu[1]]-fibrinopeptide B 50 fmol/μL
    - B - leucine enkephalin 200 pg/μL

10. From the MS Console, purge the sample fluidics from vial A.

11. Purge the reference fluidics from vial B.

12. Set the sample and reference fluidics divert valves to **Infusion**.

13. Infuse [Glu[1]]-fibrinopeptide B and observe the resolution on the 785 Da peak (positive ion) in the non-storage peak display.

    NOTE: *If required, acquire **Instrument_Setup_Pos** in the sample list and measure the resolution in the spectrum window.*

    The positive ion resolution must be >9000.

14. Switch to Negative Ion mode.

15. Observe the resolution on the 783 Da peak.

    NOTE: *If required, acquire **Instrument_Setup_Neg** in the sample list and measure the resolution in the spectrum window.*

    The negative ion resolution must be >9000.

    If the resolution in the relevant mode is less than the expected value, instrument tuning may be required. Perform the relevant "Detector gain test", set the Veff value and refer to "Appendix B: Instrument tuning" for a guide to instrument tuning.

Detector gain test (Positive Ion mode)

> *NOTE:*   *The aim of this procedure is to verify that the signal threshold value set in the acquisition setup parameters enables a suitable detector plateau voltage to be achieved.*

1. Ensure the working tune file *YAAXXX.IPR* is open.

2. Select the non-storage TOF tune window.

3. Ensure the Tune page is in System View for the following test.

4. Click the Fluidics tab.

5. Click **LockSpray** in the **Sprayer Position** drop-down list to switch the baffle to the LockSpray position and infuse leucine enkephalin from bottle B.

6. Set the infusion flow rate to **20** µL/min.

7. Click **System > Acquisition Settings**, and ensure the signal threshold is set to a suitable value between 35 and 120 mV.

8. Select the **Enable Manual Controls** check box for the detector voltage.

> *NOTE:*   *A beam is visible on the TOF peak display.*

9. Increase the detector voltage from 1500 to 2200 V in 100 V increments, noting the voltage at which a beam can first be observed

10. At each increment ensure that the intensity of the leucine peak at 556 Da is less than 200 counts per second.

> *NOTE:*   *Reduce the capillary voltage if required to achieve a suitable number of counts.*

11. Acquire **MCP_Gain_Pos** from the sample list.

12. On the spectrum window, right-click and drag across the main leucine enkephalin peak to display the extracted mass chromatogram of that peak, as shown in Figure 17:

Figure 17 - Main leucine enkephalin peak

13. Reset the detector to the lowest voltage where a beam was observed.

14. Increase the detector to 2200 V, in 50 V increments, waiting approximately 30 seconds at each voltage.

> *NOTE:*   *Once the chromatogram reaches a plateau at 2200 V or less, the detector voltage is the minimum value to be used (Figure 18).*

Figure 18 - Example of detector gain measurement.

15. Print a labelled copy of the detector gain test as shown in Figure 19.

16. Record the detector value (along with the TDC signal threshold) to be used in the *Xevo QTof MS Installation Checklist* (p/n 715002003).

Figure 19 - Correctly labelled detector gain test

17. Click **System > Auto Values** to open the Auto Values dialog box (Figure 20).

18. Enter the chosen detector voltage for the Positive Ion.

19. Click **Update**, then **Close**.

Figure 20 - Auto values dialog box

> CAUTION: **Do not adjust Auto Values for Collision Energy and Collision Cell Gas Flow.**

20. On the Tune page, deselect **Enable Manual Controls** for the detector voltage and collision gas flow.

Checking Veff value (Positive Ion mode)

> *NOTE:*   *Continue to infuse leucine enkephalin through the LockSpray sprayer and continue to use the working tune file in System view.*

1. Setup the non-storage peak display to scan from 200 to 1000, with a scan time of 1 second.

2. Ensure a beam is visible on the TOF peak display.

3. If required, reduce the capillary voltage to avoid deadtime (average ion count of <200 counts per second).

4. Observe the nominal mass error on the first isotope of the singly charged leucine enkephalin peak (556.2771 Da).

5. If the error is less than 0.1 Da, no further action is required. Otherwise, perform the following steps.

6. Open the Acquisition Setup dialog box (Figure 21).

7. Select the **Calculate and set Veff** check box.

Figure 21 - Acquisition Setup dialog box

8. Enter the reference mass (556.2771) and measured mass.

9. Click **Calculate** to compute the new value automatically.

10. Click **Update**, then click **Close**.

11. Check the nominal mass error on the first isotope of the singly charged leucine enkephalin peak is less than 0.1 Da.

12. If required repeat steps 8 to 10 until the nominal mass accuracy is less than 0.1 Da.

Detector gain test (Negative Ion mode)

NOTE: *The aim of this procedure is to verify that the signal threshold value set in the acquisition setup parameters enables a suitable detector plateau voltage to be achieved.*

1. Ensure the working tune file *YAAXXX.IPR* is open.

2. Select the non-storage TOF tune window.

3. Ensure the Tune page is in System View for the following test.

4. Switch the baffle to **LockSpray** position to infuse leucine enkephalin from bottle B.

5. Click **System > Acquisition Setup**, and ensure the signal threshold is set to a suitable value between 35 and 120 mV.

6. Select the **Enable Manual Controls** check box for the detector voltage.

NOTE: *A beam is visible on the TOF peak display.*

7. Increase the detector voltage from 1500 to 2200 V in 100 V increments noting the detector voltage at which a beam is first observed.

8. At each increment ensure that the intensity of the leucine peak at 554.2615 Da is less than 200 counts per second.

   NOTE: *Reduce the capillary voltage if required to achieve a suitable number of counts.*

9. Acquire **MCP_Gain_Neg** from the sample list.

10. On the spectrum window, right-click and drag across the main leucine enkephalin peak to display the extracted mass chromatogram of that peak, as shown in Figure 22.
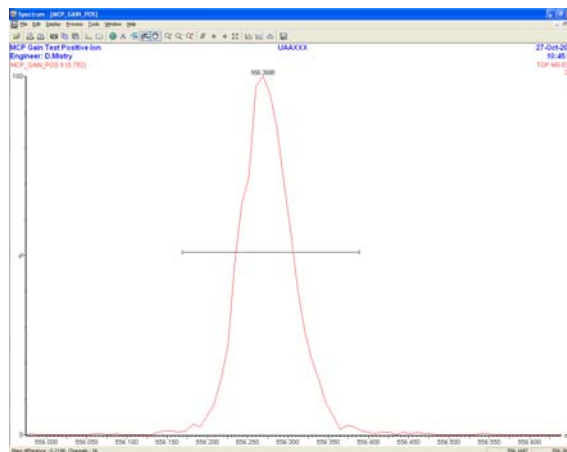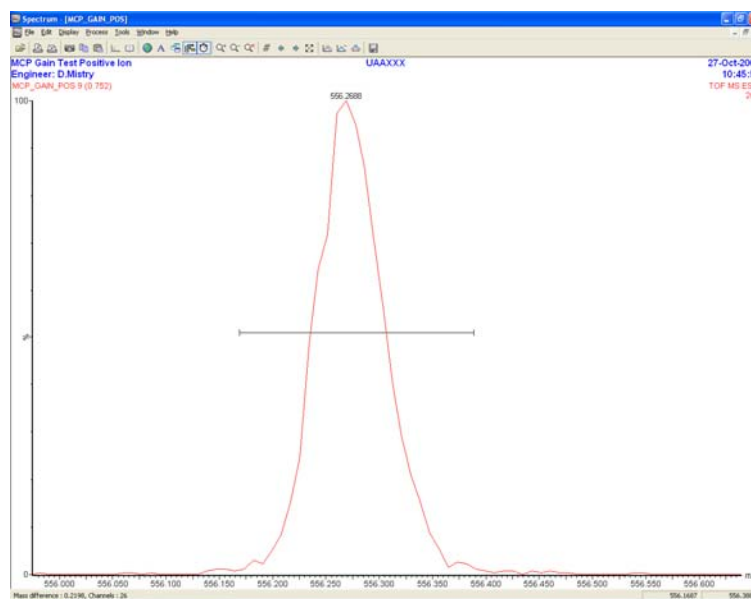

Figure 22 - Main leucine enkephalin peak

11. Reset the detector to the lowest voltage where a beam was observed.

12. Increase the detector to 2200 V, in 50 V increments, waiting approximately one minute at each voltage.

13. Once the chromatogram reaches a platea at 2200 V or less, the detector voltage on reaching the plateau is the minimum value to be used (Figure 23).
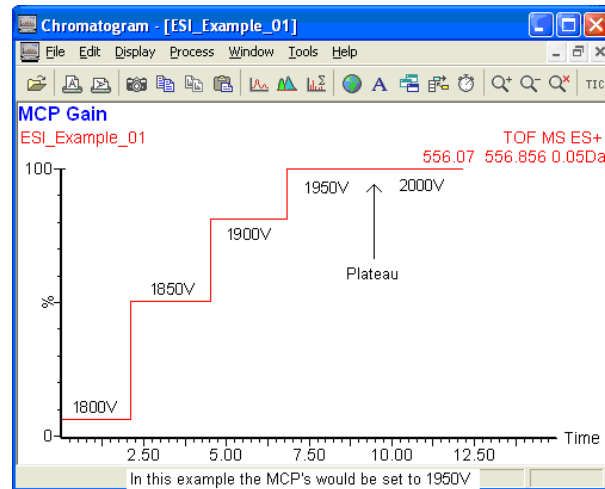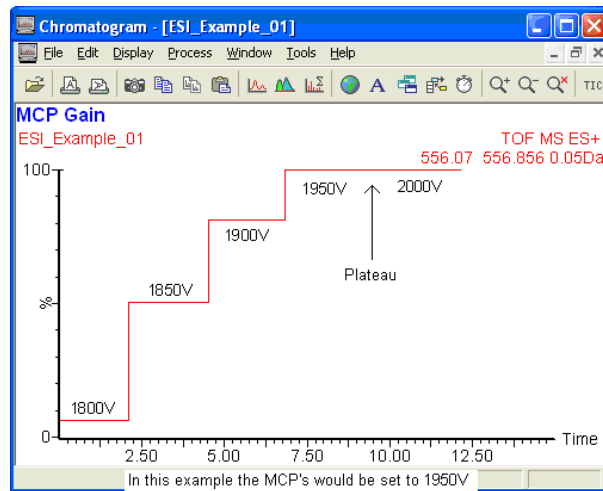
Figure 23 - Example of detector gain measurement

14. Print a labelled copy of the detector gain test as shown in Figure 24.

15. Record the detector value (along with the TDC signal threshold) to be used in the *Xevo QTof MS Installation Checklist* (p/n 715002003).

Figure 24 - Correctly labelled detector gain test

16. Click **System > Auto Values** to open the Auto Values dialog box (Figure 25).

17. Enter the chosen detector voltage for the positive ion.

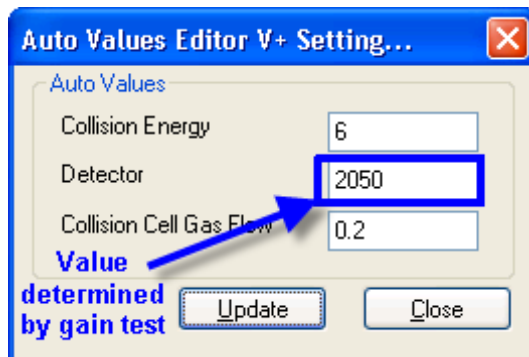18. Click **Update**, then **Close**.
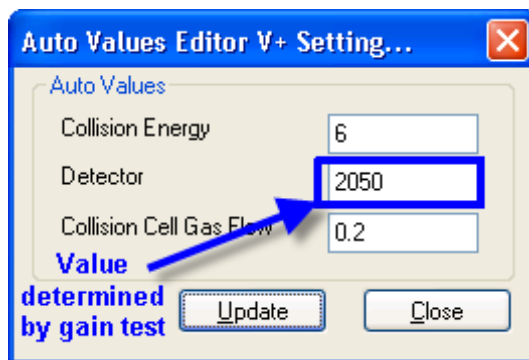
Figure 25 - Auto values dialog box

> **CAUTION:** **Do not adjust Auto Values for Collision Energy and Collision Cell Gas Flow.**

19. On the Tune page, deselect the **Enable Manual Controls** check box for the detector voltage and collision gas flow.

Checking Veff value (Negative Ion mode)

> *NOTE:* *Continue to infuse leucine enkephalin through the LockSpray sprayer and continue to use the working tune file in System view.*

1. Setup the non-storage peak display to scan from 200 to 1000, with a scan time of 1 second.

2. Ensure a beam is visible on the TOF peak display.

3. If required, reduce the capillary voltage to avoid deadtime (average ion count of <200 counts per second).

4. Observe the nominal mass error on the first isotope of the singly charged leucine enkephalin peak (554.2615 Da).

5. If the error is less than 0.1 Da, no further action is required. Otherwise, perform the following steps.

6. Open the Acquisition Setup dialog box (Figure 21).

7. Select the **Calculate and set Veff** check box for the negative ion.

8. Enter the reference mass (554.2615 Da) and measured mass.

9. Click **Calculate** to compute the new value automatically.

10. Click **Update**, then click **Close**.

11. Check the nominal mass error on the first isotope of the singly charged leucine enkephalin peak is less than 0.1 Da.

12. If required, repeat steps 8 to 10 until the nominal mass accuracy is less than 0.1 Da.

13. Exit System View.

14. Select the save option **Both Tuning Parameters and System Settings**.

Quad check

The procedure requires the samples described in Table 4.

Table 3: Quad check samples

Sample	Reservoir	Flow rate	Sprayer
50 fmol/µL [Glu[1]]-fibrinopeptide B	A	5 µL/min (0.5 µL/min for NanoFlow)	Sample sprayer
200 pg/µL leucine enkephalin	B		

> *NOTE: If quad tuning is required, use sodium iodide (2 µg/µL) provided in the sample kit.*

1. Ensure the instrument is in Positive Ion mode.

2. Ensure the API and collision gases are turned on.

3. Switch the baffle position to **Sample sprayer**.

4. Click the **Instrument** tab and ensure the LM Resolution slider is set to **4.7**.

5. Fill the sample fluidics from Vial B, and infuse 200 pg/µL leucine enkephalin at 5 µL/min.

6. Acquire **Quad_Check_1** from the sample list.

7. Compare the sensitivity of the 556 peak in function 1 and function 2.

> *NOTE: In MS/MS mode, the ion count must be >60% of the ion count in MS mode.*

8. Fill the sample fluidics from vial A, and infuse 50 fmol/µL [Glu[1]]-fibrinopeptide B through the sample sprayer at 5 µL/min.

9. Acquire **Quad_Check_2** from the sample list.

10. Compare the sensitivity of the 785 peak in function 1 and function 2.

> *NOTE: In MS/MS mode, the ion count must be >70% of the ion count in MS mode.*

11. If the ion count is >70% of the ion count, continue at step 28. Otherwise, continue by checking the sodium iodide peaks in the following steps.

12. Fill vial C with 2 µg/µL sodium iodide as provided in the sample kit.

13. Fill the sample fluidics (fill volume 250 µL) from vial C (containing sodium iodide) and infuse at 5 µL/min.

14. Ensure the sodium iodide signal is seen in the TOF non-storage peak display.

15. Click **Acq Mode > Quadrupole** to switch to the quad Tune page.

16. Set the masses on the Tune page as shown in Figure 26.

Figure 26 - Tune page quad mass selection

17. Click on the **Instrument** tab and set the LM Resolution and HM Resolution sliders to **15**.

18. Right-click in the peak display and select **Intensity > Normalise Data**.

19. Right-click and select **Intensity > Absolute Intensity***.

20. The four peaks appear as in Figure 27.
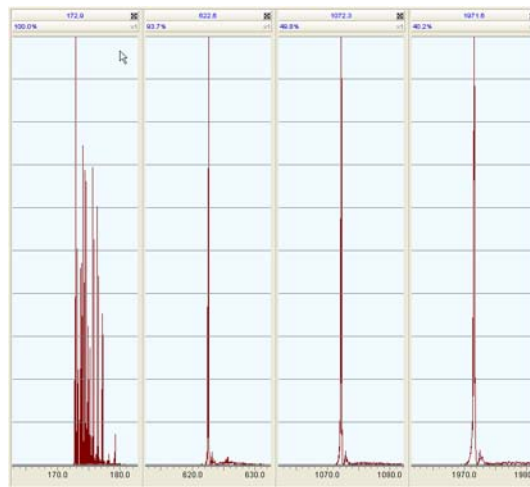
Figure 27 - Tune page peak display

21. Zoom in on the biggest peak in each window until only that peak is displayed.

22. Right-click on the peak display and deselect **Intensity > Normalise Data**.

23. Increase the gain of each window so the whole peak can be seen (Figure 28*)*.
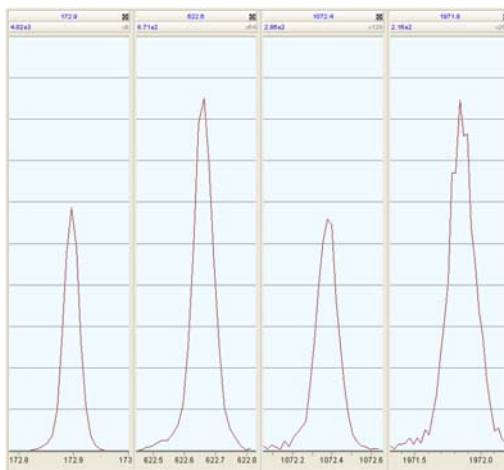
Figure 28 - Zoomed-in quad peak display

> *NOTE:*   *Veff must be set correctly for the peaks to appear at the actual mass values selected in the Tune page (the Tune page is displaying TOF data of four concurrent MS/MS functions). Peaks appearing within 0.4 to 0.7 Da of the correct mass are acceptable.*

24. If the ion count (measured at 10) is ≤70% of the ion count, tune the quad parameters.

> *NOTE:*   *Refer to the Xevo QTof Quad PCB Technical Reference sheet for further information on tuning the Quad.*

25. Ensure the MS/MS transmission is >70%.

> **CAUTION:**   **Do not adjust the Linearity slider. The default value of 52.70 must be used at all times.**

> *NOTE:*   *The data displayed is TOF data, so adjusting the Quad tuning sliders only increases or decreases the peak intensity. Do not expect the peak mass position to change as it would on a point detector.*

26. Repeat steps 1 to 10 to ensure the MS/MS transmission:
    - is >60% of the MS mode transmission (for leucine enkephalin)
    - is >70% of the MS mode transmission (for [Glu[1]]-fibrinopeptide B)

27. Click **Save Calibration**.

28. Print the Tune page and label with the Quad setup parameters on the Quadrupole tab.

29. Record the Quad setup parameters in the *Xevo QTof MS Installation Checklist* (p/n 715002003).

30. Click the **Instrument** tab.

31. Ensure the LM Resolution slider is set to **4.7**.

32. Click **Reservoir > Wash** to clean the infusion lines.

33. If sodium iodide has been used, fill vial C with 50:50 methanol/water and purge the sample fluidics line.

Positive Ion mode setup

This section defines the instrument dependant parameters used when performing the Positive Ion mode verification test.

For all procedures in this section, ensure these conditions are met:

- Instrument is in Operate
- API and collision gases are turned on
- Tune page is set to Positive Ion mode

These settings appear as in Figure 29*.*

Figure 29 - Positive Ion mode icons

The procedures require the samples described in Table 4.

Table 4: Positive Ion mode setup samples

Sample	Reservoir	Flow rate	Sprayer
50 fmol/µL [Glu^1]-fibrinopeptide B	A	5 µL/min (0.5 µL/min for NanoFlow)	Sample sprayer
200 pg/µL leucine enkephalin	B		LockSpray/nanoLockSpray sprayer
1 pmol/µL [Glu^1]-fibrinopeptide B	C	For calibration	

> ***NOTE:***   *The leucine enkephalin concentration can be adjusted to give a suitable number of counts, between 0.005 and 0.4 IPP.*

1. Set the Quad Profile as in Figure 30.
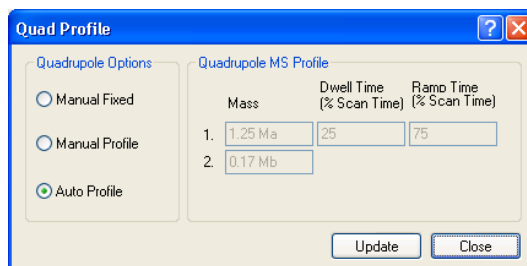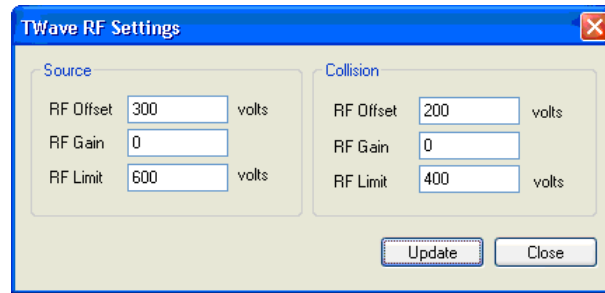
Figure 30 - Quad Profile
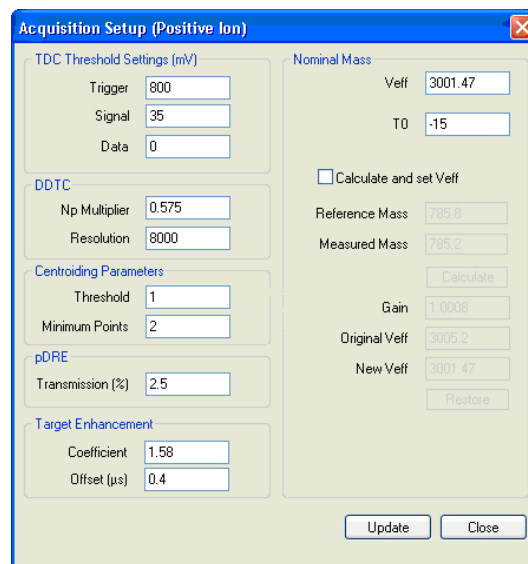
2. Set the RF Settings as in Figure 31.

Figure 31 - RF Settings

3. Set Acquisition Setup parameters as in Figure 32.

Figure 32 - Acquisition Setup parameters

CAUTION: ***Do not adjust the Target Enhancement parameters; these defaults are part of the system settings.***

4. Exit System View.

5. Select the save option **Both Tuning Parameters and System Settings**.

IntelliStart

LockSpray source setup (Positive Ion mode)

1. Ensure the baffle is in the LockSpray position.

2. Open the MS console, and click **Configure > Configuration Mode**.

3. Select the **LockSpray Source Setup** check box then click **Start**.

Figure 33 - Setting up LockSpray source set up in IntelliStart

4. On the initial wizard screen click **Next**.

5. Select the MS/MS lockmass compound **Leucine Enkephalin (system test)** from the drop-down list.

6. Ensure the leucine enkephalin lockmass sample is flowing through the LockSpray sprayer.

7. Click **Next**.

8. From the Setup Type window, select the **Automatic** check box to allow source conditions to be optimized automatically.

9. Click **Next**.

   NOTE: *For further information on the other choices, see "Appendix D: LockSpray source setup".*

10. Select the **Automatic** check box to allow IntelliStart to set the flow rate.

   NOTE: *Automatic sets the reference fluidics to flow at 50 µL/min. For nanoLockSpray, Automatic uses 0.5 µL/min.*

   For further information on the other choices, see "Appendix D: LockSpray source setup".

   The system is now ready to optimize the source conditions for the lockmass profile specified.

11. Click **Start**.

The IntelliStart software automatically sets up the source conditions for positive and Negative Ion modes. The values chosen are written automatically into the LockMass profile. This means that whenever the profile is used in an acquisition, these parameters are set on the LockMass sprayer.

> **NOTE:**   *The values saved in the LockMass profile can only be overwritten by repeating the whole of this procedure.*

When successfully completed, the MS Console displays the green check mark (Figure 34). The completed lockmass profile is available for selection in the LockMass window when creating new MS methods.

Figure 34 - Confirmation that source conditions are setup

Creating a calibration (Positive Ion mode)

Perform the following procedure to create an automatic calibration using IntelliStart.

1. Open the MS Console, and check **Configure > Configuration Mode**.

2. Select the **Create Calibration** check box.

3. Click **Start**.

Figure 35 - Creating calibration using IntelliStart

4. On the initial wizard screen, click **Next**.

5. Click **Calibration Profile Editor...**.
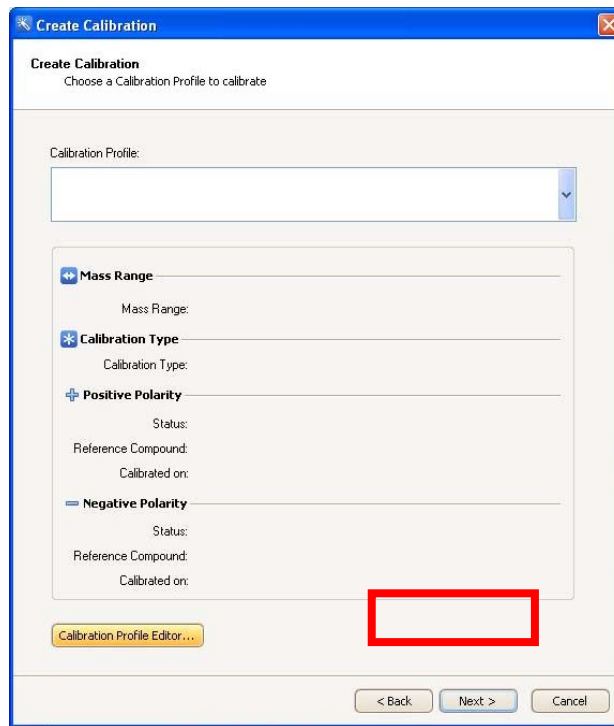
Figure 36 - Accessing the Calibration Profile Editor

6. In the Calibration Profile Editor window, click **File** > **New**.
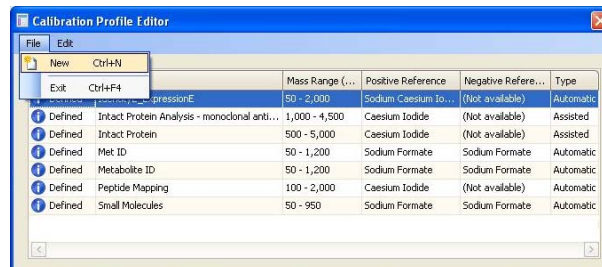
Figure 37 - Calibration Profile Editor window

7. Type **Commission** as the calibration profile name.

8. Set the parameters required:

    - Mass range 150 to 1500 Da
    - Automatic calibration
    - Positive polarity enabled, with reference compound [Glu^1]-fibrinopeptide B
    - Negative polarity disabled

9. Click **OK**.

10. Select the **Commission** calibration profile in the drop down list.

> *NOTE:*   *A red triangle indicates the calibration profile is defined, but has not yet been calibrated.*
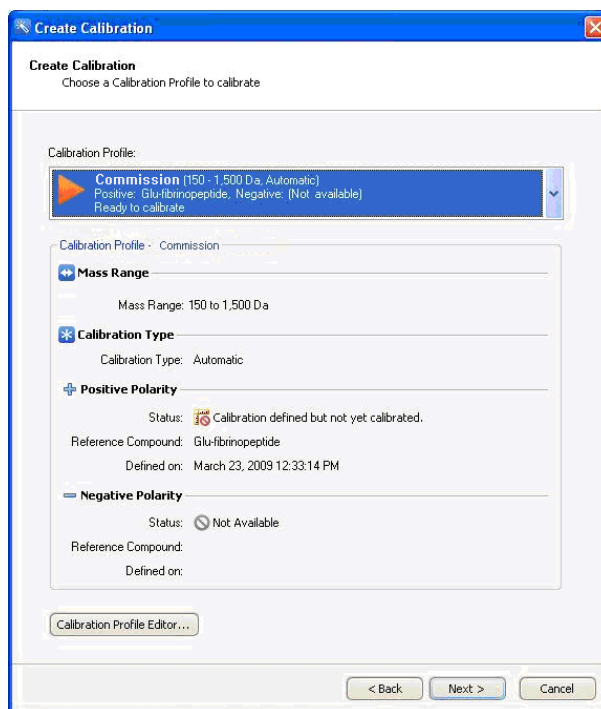
Figure 38 - Creating a calibration profile

11. Click **Next**.

12. On the Commission page, set the following parameters:
- Calibrate positive ion
- Display report
- Make Calibration profile Active

13. Click **Next** for the data acquisition options to be automatically populated by IntelliStart.

14. Click **Next**.

15. Select the **Automatic** check box to define how the fluidics will be controlled (Figure 39).

> *NOTE:*   *Automatic sets a default flow rate of 20 µL/min.*
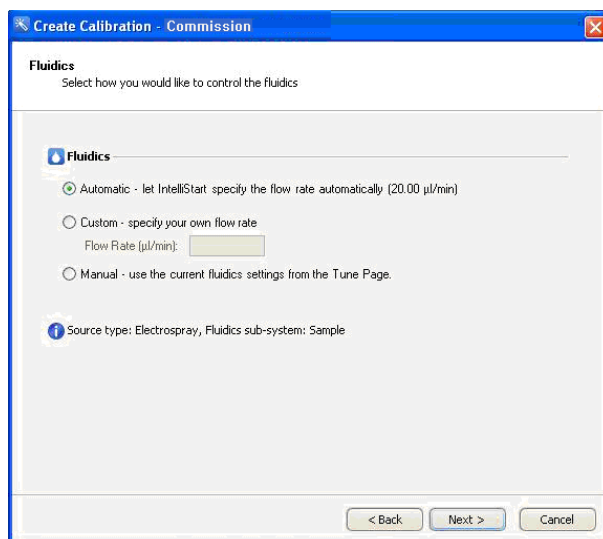
16. Click **Next**.

Figure 39 - Commission page

17. Set the acceptance criteria for the automatic calibration residual mass to **10** ppm.

18. Click **Next**.

19. Ensure the baffle is in the **Sample** position.

> **NOTE:**   *The system is now ready to perform automatic calibration.*

20. Ensure there is calibrant sample [Glu[1]]-fibrinopeptide B in vial C.

21. Click **Start**.

> **NOTE:**   *When starting an automatic or assisted calibration, IntelliStart automatically loads the blank calibration Uncal.cal before acquiring any new data.*

The IntelliStart window displays the current status of the calibration process during the following automatic steps:

- Previous calibration is removed by loading Uncal.cal.
- An intensity check is performed. If required, the instrument uses the DRE lens to achieve a suitable intensity (I.P.P. value) on the calibrant sample.
- Acquiring data. Once sufficient data is obtained on the least intense peak as defined in the calibrant reference file, the peak picking application creates the calibration.

A report screen is generated (Figure 40) displaying the result of the calibration process. A successful calibration (with a result inside the threshold acceptance criteria set above) produces a green tick in the header. Further details of the calibration data are provided further down the screen.
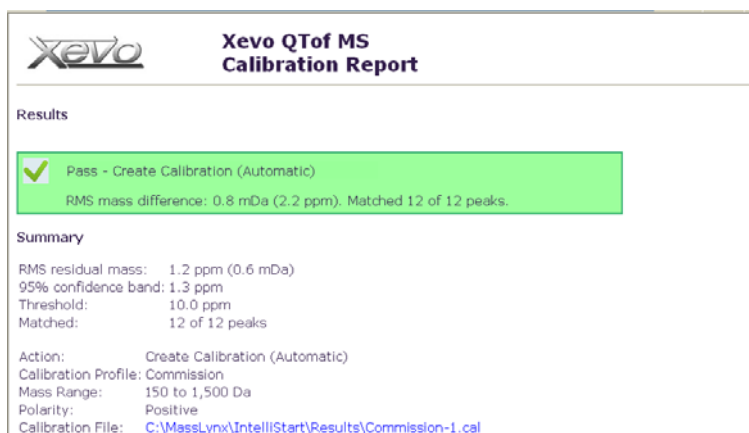
Figure 40 - Calibration details

When creating a calibration, the RMS mass difference achieved is expected to be approximately 1 ppm. If the RMS mass difference is much greater than this, investigate, then repeat the calibration process.

The IntelliStart window also details the successful completion of the calibration.
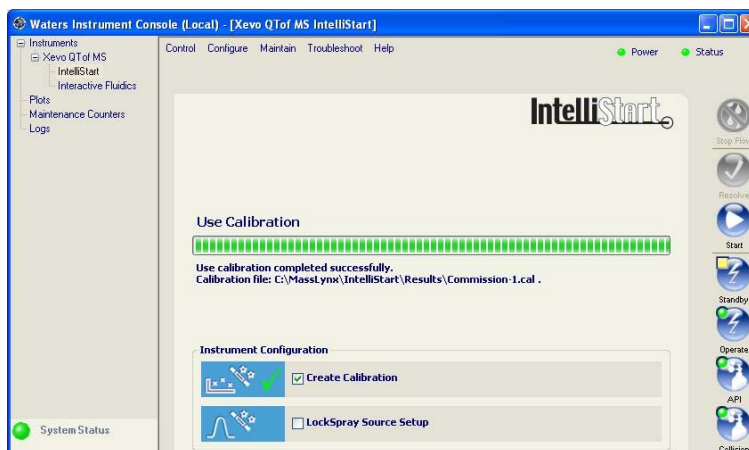
Figure 41 - Confirmation of calibration

Calibration check

Use the IntelliStart automatic Calibration Check to demonstrate the quality of the automatic calibration.

1. Open the MS Console, and click **Configure > Normal Mode**.

2. Select the **Calibration Check** check box.

3. Click **Start**.

4. Click **Next** on the initial screen.

5. Select the **Commission** calibration created in "Creating a calibration (Positive Ion mode)" from the drop-down list.

> *NOTE:*   *This selects which calibration profile to check. Only those calibration profiles that have already been calibrated (shown by a green tick) can be checked through IntelliStart. Manual calibrations (.cal files made in Custom mode) cannot be checked using IntelliStart.*

6. Click **Next**.

7. Ensure the **Positive Ion** check box is selected.

8. Click **Display Report**.

9. Click **Next**.

10. On the Check Calibration - Commission page, set the following parameters:
    - acquire data with LockMass
    - LockMass compound is leucine enkephalin (system test)
    - LockSpray fluidics set to Automatic

11. Click **Next**.

12. Set the Calibration Check Acceptance Criteria to **3.0** ppm RMS residual mass error.

13. Click **Next**.

> *CAUTION:*   ***Before IntelliStart checks the calibration, ensure the samples required are in the appropriate fluidics vials as described on screen.***

14. Click **Start**.

The IntelliStart window displays the current status of the calibration check process during the following automatic steps:
- An intensity check is performed. If required, the instrument uses the DRE lens to achieve a suitable intensity (I.P.P. value) on the calibrant sample.
- Acquiring sample and LockMass data. Once sufficient data is obtained, LockMass corrected data is presented in the IntelliStart report.

A report screen is generated (Figure 42) displaying the result of the calibration check. A successful calibration (with a result inside the threshold acceptance criteria set above) produces a green tick in the header. Further details of the calibration data are provided further down the screen.

Figure 42 - Calibration check details

Positive Ion mode MS system specification

The performance of the instrument is assessed using data from two MS/MS (switching) functions from a sample list acquisition; one with high collision energy and the other with low collision energy.

The procedures require the samples described in Table 5.

Table 5: Positive Ion mode setup samples

Sample	Reservoir	Flow rate	Sprayer
50 fmol/µL [Glu[1]]-fibrinopeptide B	A	5 µL/min	Sample sprayer
200 pg/µL leucine enkephalin	B	(0.5 µL/min for NanoFlow)	LockSpray sprayer

The resulting signal height is greater than 130 counts per second (centered data) on the most intense y sequence ion from the MS/MS spectrum of the doubly charged precursor ion (m/z 785.8).

The mass resolution is greater than 9000 (FWHM), measured on the doubly charged precursor ion (m/z 785.8).

The RMS error of the y sequence ions (free from interferences) in the MS/MS spectrum within the mass range 150 to 1500 is less than 3 ppm when using an external lockmass.

1. Open the following sample list: **Commission_ESI.SPL** (Figure 43).

   NOTE: *Pre-defined sample lists for installation are located in the folder C:\MassLynx\Commission.pro\Sampledb.*

   If the parameters in the Commission_Pos.exp are mistakenly altered, the default settings can be found in "Appendix E: ESI method parameters".

   Collision energy in function 2 is tunable (usually set with the value used during calibration above, between 28 to 32 V).

2. Open the **Commission_Pos.exp** file.

3. Select the Leucine (system test) Lockmass profile defined above before performing the specification.

8		GFP - Sample Fluidics, Leucine - Ref Fluidics				0.0
9						0.0
10	ESI_001_POS	ESI Positive Ion MS System Verification	Commission_Pos	DEFAULT	1	10.0
11	ESI_002_POS	ESI Positive Ion MS System Verification	Commission_Pos	DEFAULT	1	10.0
12	ESI_003_POS	ESI Positive Ion MS System Verification	Commission_Pos	DEFAULT	1	10.0
12						0.0

Figure 43 - Example sample list for ESI Specifications

4. Infuse 50 fmol/µL [Glu[1]]-fibrinopeptide B (bottle A) from the sample fluidics.

5. Infuse leucine (bottle B) from the reference fluidics.

> **CAUTION:** **The fluidics syringes require enough sample to allow at least 20 minutes of acquisition time.**

6. Highlight samples **ESI_001_POS** to **ESI_003_POS**.

7. Click ▷ and select the **Acquire Sample Data only** check box.

8. Wait for the acquisition process to complete.

> **NOTE:** *The instrument passes Positive Ion mode specification when the results meet the criteria specified.*

9. Display functions 1 to 3 in the chromatogram for file **ESI_001_POS**.

10. Combine all scans in function 1 and calculate the resolution (FWHM) at m/z 785.8.

> **NOTE:** *To pass the specification the FWHM resolution on the doubly charged precursor at 785.8 m/z must be greater than 9000.*
>
> *The ResCalc.exe program can be found in the following location: C:\MassLynx\SpectrumTools.*

11. Combine all scans in function 2 and save the spectrum.

12. In the spectrum window, click **Process > Automatic Peak Detection**.

13. Complete the display as shown in Figure 44.
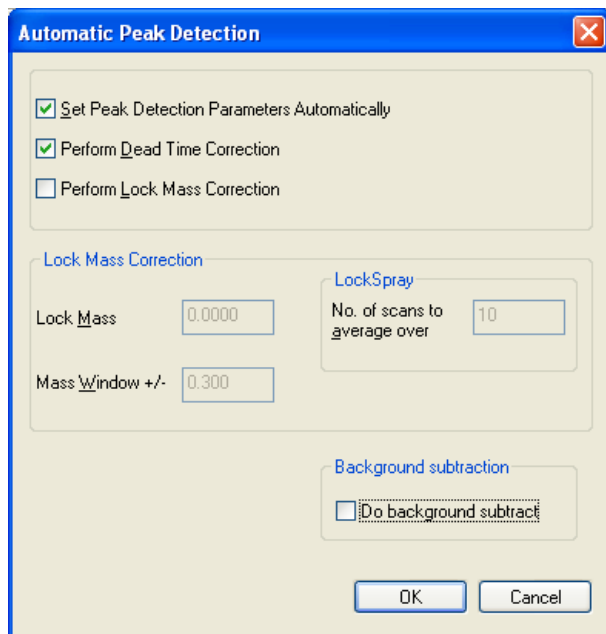
Figure 44 - Automatic Peak Detection

> **NOTE:** *The resulting signal height of the 684.3 m/z must be greater than 130 counts per second (centered data) to pass the specification.*

14. In the spectrum window, click **Edit > Copy spectrum list**.

15. Use the MassDiff.exe program to calculate the mass errors.

> *NOTE:*   *The MassDiff program can be found in the following location: C:\MassLynx\SpectrumTools.*

16. In the MassDiff program, click **Options > Input Options** and set the following parameters:
    - ☑ Use Mass Window
    - Low Mass 150
    - High Mass 1500
    - Peaks will be excluded 150 mDa
    - Ignore peaks below 100 counts

17. Click **Apply**, then **OK**.

18. Open **ESI_GFP_MSMS.ref** as the reference file to be used in the program.

> *NOTE:*   *The instrument meets the specification when the RMS error of the y sequence ions (free from interferences) in the MS/MS spectrum within the mass range 150 to 1500 is less than 3 ppm when using the external lockmass.*
>
> *The RMS error across all y sequence ions is the important result. Individual peaks must not be removed from the calculation to improve the result unless the data has been examined and there is a valid reason to do so, such as insufficient intensity or a peak interference. Annotated spectra showing any peaks removed and the reason for removing them must be printed and included with the specification result.*
>
> *The following equation calculates the RMS error, where Eppm is the ppm error, and n is the number of masses considered.*

$$RMS = \sqrt{\frac{\sum (E_{ppm})^2}{n}}$$

19. Click the Load from Clipboard icon and copy the peak list into the MassDiff program.

20. Repeat steps 4 to 18 for files **ESI_002_POS** and **ESI_003_POS**.

NOTE: *Record the results of all three runs in the performance specification in the appropriate system installation checklist, then print and annotate the data, as in Figure 45.*
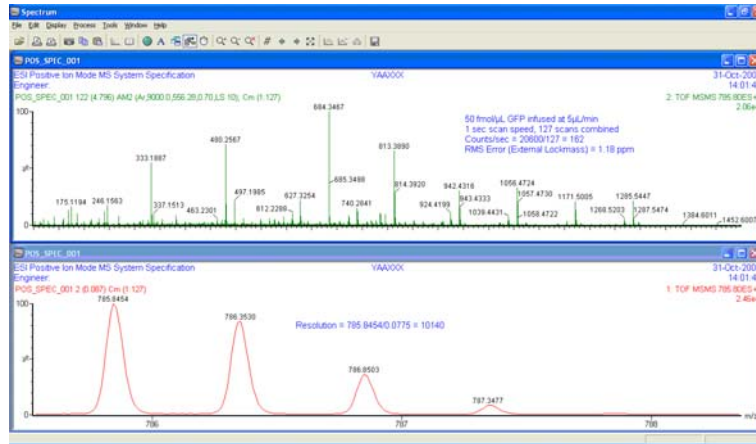
Figure 45 - Annotated spectra for Positive Ion mode MS system specification

Negative Ion mode setup

This section defines the instrument dependant parameters used when performing the Negative Ion mode verification test.

For all procedures in this section, ensure these conditions are met:

- Instrument is in Operate
- API and collision gases are turned on
- Tune window is set to Negative Ion mode

These settings appear as in Figure 46*.*

Figure 46 - Negative ion mode icons

The procedures require the samples described in Table 6.

Table 6: Negative ion mode setup samples

Sample	Reservoir	Flow rate	Sprayer
250 pg/µL raffinose	A	5 µL/min (0.5 µL/min for NanoFlow)	Sample sprayer
0.5 mM sodium formate	C	Defaults to 20 µL/min	Calibration only

1. Set the Quad Profile as in Figure 47.
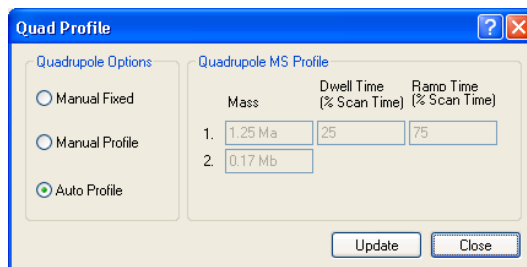
Figure 47 - Quad Profile

2. Set the RF Settings as in Figure 48.
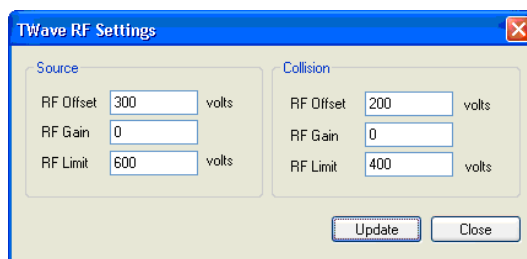
Figure 48 - RF Settings

IntelliStart

Creating a calibration (Negative Ion mode)

> **CAUTION:** **Calibrate the instrument in Negative Ion mode before running the specification.**

1. Purge the sample fluidics using the calibrant sample sodium formate in vial C.
2. Open the MS console, and click **Configure > Configuration Mode**.
3. Select the **Create Calibration** check box.
4. Click **Start**.

Figure 49 - Lockspray source setup in IntelliStart

5. On the initial wizard screen, click **Next**.

6. Select the **Small Molecules** calibration profile from the drop-down list (Figure 50).
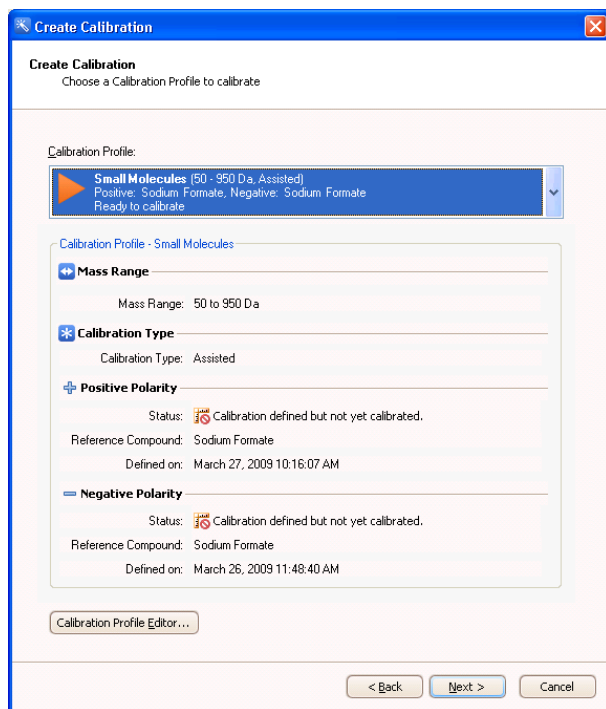
Figure 50 - Accessing the Calibration Profile Editor

NOTE: *A red triangle indicates the calibration profile is defined, but has not yet been calibrated.*

7. Click **Next**.

8. On the Callibration Options page, set the following parameters:
    - Calibrate negative ion
    - Display report
    - Make Calibration Profile Active

9. Click **Next**.

NOTE: *The data acquisition options are automatically populated by IntelliStart.*

10. Ensure **MS mode** and **Collision Energy** off are selected.
11. Click **Next**.
12. Click **Automatic** fluidics control (Figure 51).
13. Click **Next**.
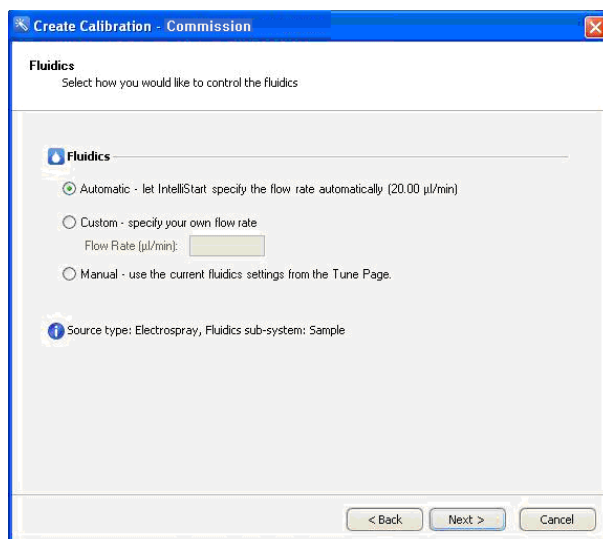
Figure 51 - Commission page

14. Set the acceptance criteria for the automatic calibration residual mass to **10** ppm.

15. Click **Next**.

NOTE: *The system is now ready to perform automatic calibration.*

16. Click **Start**.

NOTE: *When starting an automatic or assisted calibration, IntelliStart automatically loads the blank calibration Uncal.cal before acquiring any new data.*

The IntelliStart window displays the current status of the calibration process during the following automatic steps:

- Previous calibration is removed by loading Uncal.cal.
- An intensity check is performed. If required, the instrument uses the DRE lens to achieve a suitable intensity (I.P.P. value) on the calibrant sample.
- Acquiring data. Once sufficient data is obtained on the least intense peak as defined in the calibrant reference file, the peak picking application creates the calibration.

A report screen is generated (Figure 52) displaying the result of the calibration process. A successful calibration (with a result inside the threshold acceptance criteria set above) produces a green tick in the header. Further details of the calibration data are provided further down the screen.

Figure 52 - Calibration details

When creating a calibration, the RMS mass difference achieved is expected to be approximately 1 ppm. If the RMS mass difference is much greater than this, investigate, then repeat the calibration process.

The IntelliStart window also details the successful completion of the calibration.
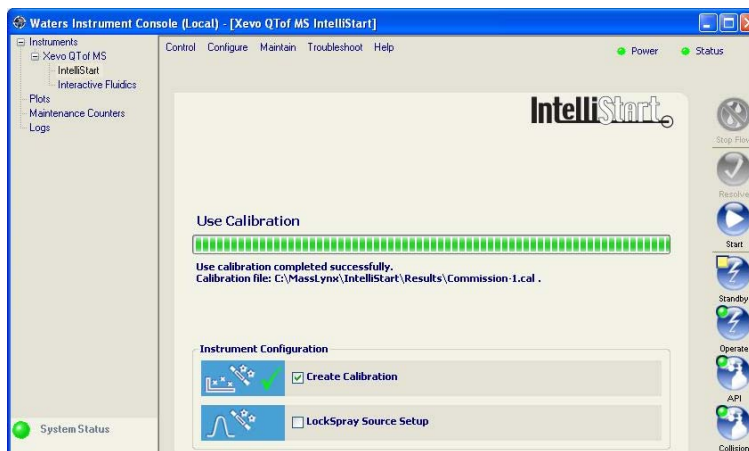
Figure 53 - Confirmation of calibration

NOTE: *If there is any issue passing the automatic calibration, ensure the sample cone voltage is set to give a good distribution of sodium formate peaks across the mass range, and that relative intensities are similar to those quoted in the reference file.*

Negative Ion mode MS system specification

The performance of the instrument is assessed using data from an MS/MS function with high collision energy (21 V) from a sample list acquisition.

The procedures require the sample described in Table 7.

Table 7: Negative Ion mode setup sample

Sample	Reservoir	Flow rate	Sprayer
250 pg/µL raffinose	A	5 µL/min (0.5 µL/min for NanoFlow)	Sample sprayer

The resulting signal height is greater than 130 counts per second (centered data) on the singly charged daughter ion (m/z 179.1).

The mass resolution is greater than 8000 (FWHM), measured on the singly charged raffinose ion (m/z 503.2).

1. Ensure the Tune page is in TOF MS mode and displays the image.

2. Set the non-storage peak display to scan from 200 to 1000, with a scan time of 1 second.

3. On the non-storage peak display, tune the source parameters to give maximum sensitivity on the singly charged precursor ion (m/z 503.2).

4. Check the resolution on the singly charged raffinose ion is >8000.

5. Ensure the fluidics syringe contains enough sample to allow at least 20 minutes of acquisition time.

6. In the sample list, highlight samples **ESI_004_NEG** to **ESI_006_NEG**.

7. Click .

8. Click **Acquire Sample Data only**.

   NOTE: *If the parameters in the Commission_Neg.exp are altered, the default settings can be found in "Appendix E: ESI method parameters".*

9. Once the acquisitions are complete, process the data as described in the following steps:

   NOTE: *The instrument passes Negative Ion mode specification when the results meet the criteria specified.*

10. Display the chromatogram for file **ESI_004_NEG**.

11. Combine all scans in function 1 and calculate the resolution (FWHM) at 503.2 Da.

NOTE: *To pass the specification the FWHM resolution must be greater than 8000.*

              The ResCalc.exe program can be found in the following location: C:\MassLynx\SpectrumTools.

12. In the spectrum window, click **Process > Automatic Peak Detection….**

13. Complete the display as shown in Figure 54.
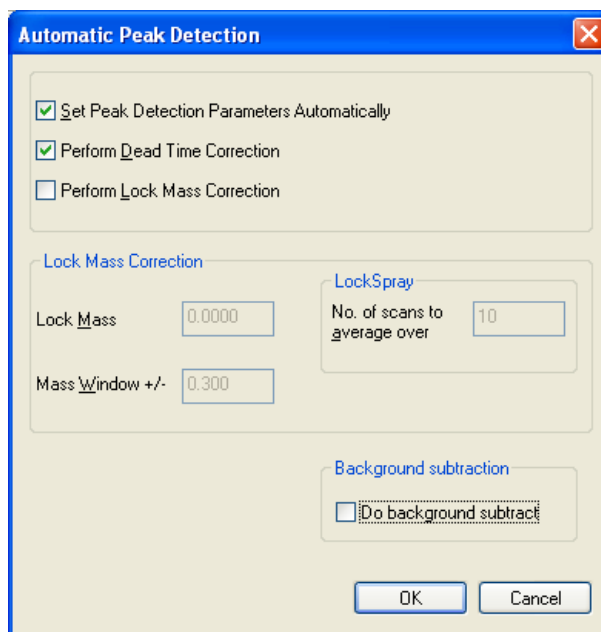
Figure 54 - Automatic Peak Detection

NOTE: *The resulting signal height of the 179.1 m/z must be greater than 130 counts per second (centered data) to pass the specification.*

14. Repeat steps 11 to 13 for files **ESI_005_NEG** and **ESI_006_NEG**.

NOTE: *Record the results of all three runs in the performance specification in the appropriate system installation checklist with the data properly printed and annotated, as in Figure 55.*
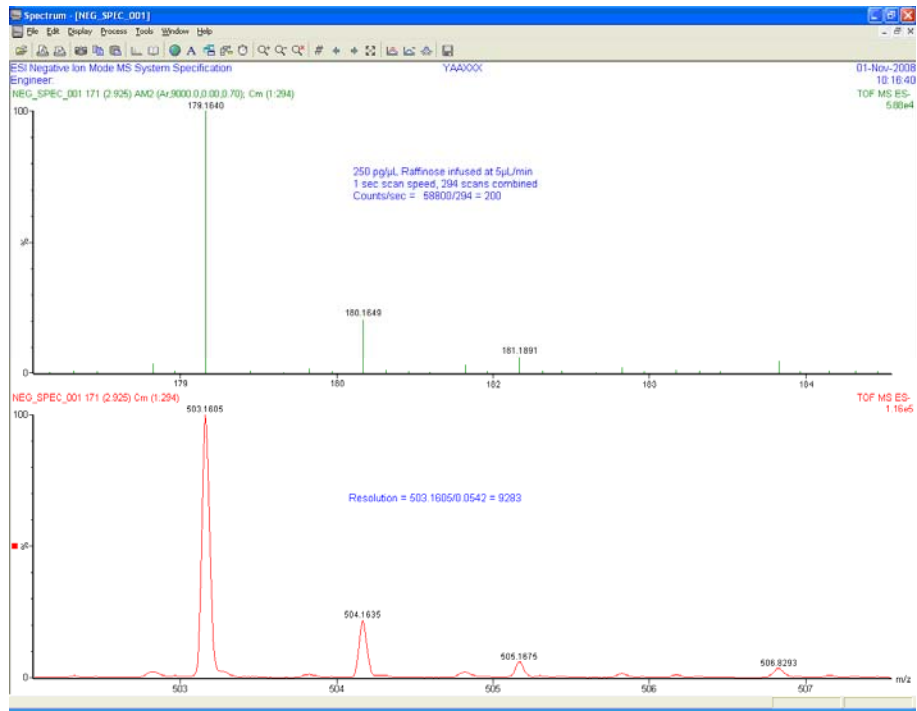
Figure 55 - Annotated spectra for Negative Ion mode MS system specification

Xevo QTof MS ACQUITY UPLC System

When installing a Xevo QTof MS with an ACQUITY UPLC system, these system precision and gradient performance tests must be performed.

Mobile phase preparation

Due to the increased sensitivity that both the ACQUITY UPLC system and the Xevo series of mass spectrometers offer, all solvents including water and additives must be of the highest chemical purity (at a minimum of HPLC grade). Failure to use high quality solvents will result in high background contamination, low signal-to-noise, and loss of sensitivity. Waters recommends using LC/MS grade solvents and reagents.

> **CAUTION:**   *To prevent contamination of glassware, solvent bottles must not be washed with detergents, or washed with other general glassware. The glassware must be rinsed with the high purity solvents to be used.*
>
> *For detailed information on guidelines for the prevention of system contamination, refer to the document Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems (p/n 715001307) located in the Waters Knowledge Center.*
>
> *Always wear suitable gloves to avoid contact with solvents and eliminate possible system contamination when performing any maintenance to ACQUITY system components.*

1. Prepare the following mobile phases and wash solvents:
   - Mobile phase A1   Water + 0.1% formic acid
   - Mobile phase B1   Acetonitrile + 0.1% formic acid
   - Weak wash   10:90 acetonitrile/water
   - Strong wash   50:50 acetonitrile/water
   - Seal wash   10:90 methanol/water

Sample preparation

To prepare the samples, use the Waters ACQUITY UPLC MS Startup Solution kit
(p/n 700002741).

Table 8: Solution kit 700002741 components

Solution	Description	Qty
Solution 1	1 mg/mL (±0.5%) sulfadimethoxine in methanol	1 x 10-mL ampoule
Solution 2	1 mg/mL (±0.5%) acetominophen in acetonitrile + 1 mg/mL (±0.5%) sulfadimethoxine + 1 mg/mL (±0.5%) caffeine + 1 mg/mL (±0.5%) reserpine + 1 mg/mL (±0.5%) terfenadine	1 x 10-mL ampoule
Solution 3	Formic acid, ACS reagent (96%) (used in mobile phase, wash solvent and diluent preparation)	1 x 10-mL brown Nalgene bottle

Preparing the diluent

1. Use a 100-mL graduated cylinder and measure 50 mL of LC/MS grade acetonitrile.

2. Transfer the acetonitrile to a 1-L reservoir bottle.

3. Use a 1000-mL graduated cylinder and measure 450 mL of LC/MS grade water.

4. Transfer the water to the same 1-L reservoir bottle.

5. Cap the reservoir bottle and mix well.

6. Label the reservoir bottle as "10:90 acetonitrile/water, diluent".

Preparing test sample 1 for system precision (2 ng/µL)

1. Add 100 µL of solution 1 (sulfadimethoxine) to a 50-mL volumetric flask and dilute to 50 mL with diluent.

2. Stopper and invert the flask several times to fully mix.

3. Label the flask as "System precision test sample (2 ng/µL)".

Preparing test sample 2 for gradient performance (2 ng/µL)

1. Add 100 µL of solution 2 (mixture) to a 50-mL volumetric flask and dilute to 50 mL with diluent.

2. Stopper and invert the flask several times to fully mix.

3. Label the flask as "Gradient performance test sample (2 ng/µL)".

Preparing test sample 3 for TUV/PDA system precision (0.1 mg/mL)

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

2. Stopper and invert the flask several times to fully mix.

3. Label the flask as "0.1 mg/mL TUV/PDA test solution".

System performance test preparation

1. Place the A1 and A2 solvent lines into the mobile phase A1 reservoir.

2. Place the B1 and B2 solvent lines into the mobile phase B1 reservoir.

3. Place the weak and strong wash solvent lines into their respective reservoirs.

4. Place the seal wash solvent line into the seal wash reservoir.

   NOTE: *Use the 10:90 acetonitrile/water diluent for weak wash.*

5. Place the ACQUITY UPLC outlet tubing into a suitable waste container.

6. Prime each solvent line of the binary solvent manager.

7. Prime the binary solvent manager seal wash lines.

8. Prime the sample manager with at least five cycles.

9. Click **Maintain > Characterise > Needle Seal** in the Sample Manager Console to perform the needle seal calibration procedure.

10. Click **Maintain > Characterise > Needle and loop Volumes** in the Sample Manager Console to perform the system volume calibration procedure.

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

11. Flush the system to waste using a 50:50 mixture of mobile phase A1 and B1 at 2 mL/min for 10 minutes.

12. Repeat the flush for solvent lines A2 and B2 using the same conditions.

13. Attach the inlet of the ACQUITY UPLC BEH C_{18} (2.1 x 50 mm, 1.7 µm) column and place it in the column heater.

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

14. Where a new column has been fitted, flush it to waste with 100% acetonitrile for at least one hour before connecting the outlet to the optical detector or mass spectrometer.

15. Set the initial mobile phase conditions of 80:20 water/acetonitrile.

16. Set a flow rate of 0.3 mL/min and then start the LC flow.

17. Equilibrate the column until the binary system manager system pressure is stable.

18. Place a mobile phase blank (diluent) into position 1:1 in the sample manager.

19. Place the system precision test sample into position 1:2 in the sample manager for the system precision test.

20. Place the gradient performance test sample into position 1:3 in the sample manager for the gradient performance test.

21. Place the TUV/PDA test sample into position 1:4 of the sample manager for the TUV/PDA system precision test (if applicable).

Setting up the inlet

System precision test

1. If no inlet method is present in the Commission project, create one by performing the following steps:

   a. Create the inlet method for the system precision test using the settings displayed in Figure 56 and Figure 57.
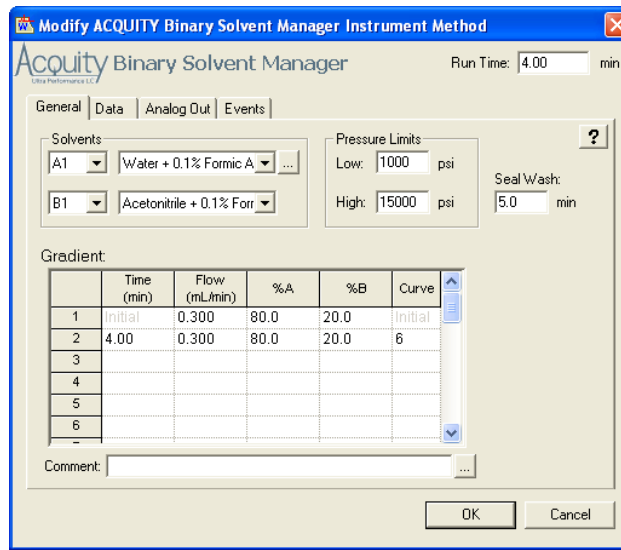
Figure 56 - Binary solvent manager parameters for system precision test
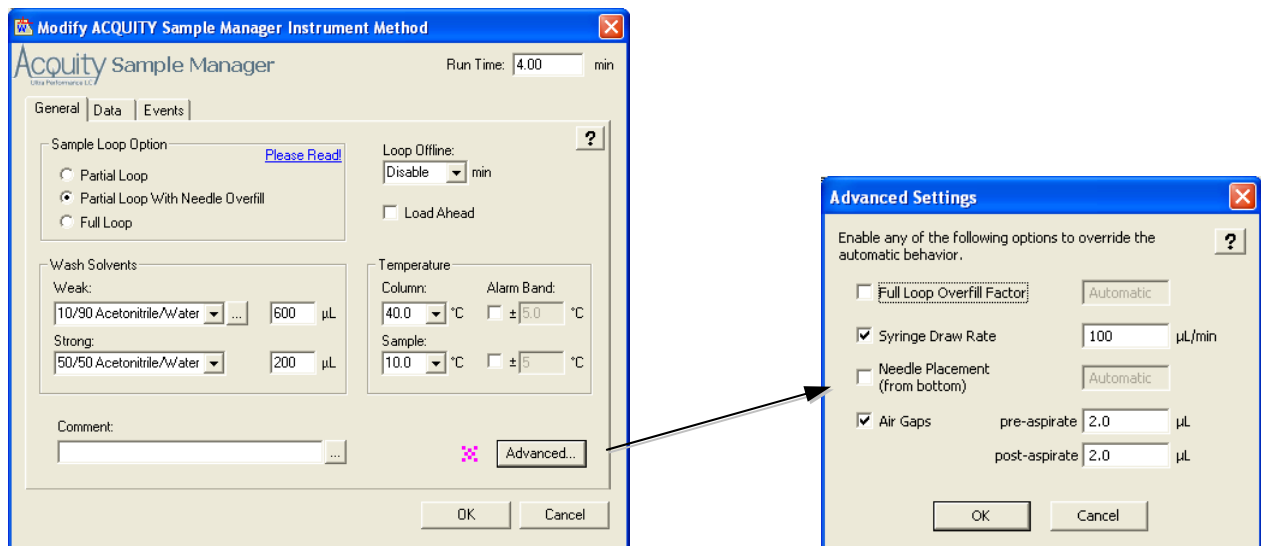
Figure 57 - Sample manager parameters

   b. Click **File > Save As** in the inlet editor.

   c. Save the inlet method using the filename **System Precision**.

2. Click [icon] to load the inlet method to the ACQUITY UPLC system.

Gradient performance test

1. If the inlet method required is not present in the Commission project, create one by performing the following steps:

   a. Create the inlet method for the gradient performance test with the parameters displayed in Figure 58, and the same sample manager parameters used in the system precision test (Figure 56).

Figure 58 - Binary solvent manager parameters for gradient performance test

   b. Click **File > Save As** in the inlet editor.

   c. Save the inlet method using the filename **Gradient**.

Setting up the mass spectrometer

Table 9: Expected [M+H]$^+$ values

Component	Expected [M+H]$^+$ ion
Caffeine	m/z 195.0882
Sulfadimethoxine	m/z 311.0814
Reserpine	m/z 609.2812
Terfenadine	m/z 472.3216

1. On the MS Console page, ensure the mass spectrometer is in Positive Ion mode.

2. Infuse 2 ng/µL leucine enkephalin solution into the LockSpray probe.

3. Connect the ACQUITY UPLC outlet tubing to the Xevo QTof MS fluidics system.

4. Set a flow rate of 80:20 water/acetonitrile at 0.3 mL/min and allow the baseline to stabilize.

5. Ensure that a spectrum of leucine enkephalin is observed on the MS Tune page.

6. With the ACQUITY UPLC flowing through the sample probe, perform a Lockmass setup through the MS console.

Creating the MS method

1. If the MS method required is not present in the Commission project, create one by performing the following steps:

   a. Create an MS^E Centroid method using the parameters shown in the following table:

Table 10: Entry data for MS^E method

Tab	Field	Values
Acquisition	Acquisition time	Start time = 0 End time = 4
	Source	Source = ES
	Acquisition mode	Positive polarity Extended dynamic range
TOF MS	Low energy range	Start = 85 Da End = 1000 Da
	High energy range	Start = 85 Da
	Scanning conditions	Scan time = 0.08 sec
Collision energy	Expression collision energy	Low energy = 6 V Ramp high energy from 20 to 30 V
Cone Voltage	n/a	Override set Cone voltage = 24 V

   b. Save the MS method using the filename **Acquity_Xevo_QTof_MS**.

Acquiring sample data

1. A Commission ESI sample list is provided to perform the ACQUITY system tests. If the list is not present, set up a sample list as shown in Figure 59.

2. Select the appropriate inlet and MS method files created previously.

3. Save the sample list as **SPT_DDMMYY.spl** (where DDMMYY = date).

4. Calibrate the instrument using sodium formate over the mass range 85 to 1000 Da, by acquiring the ESI_MS_NaFormate entry in the sample list.

5. Use the IntelliStart 'small molecules' calibration procedure as described previously.

6. Start the leucine enkephalin infusion to the LockMass probe through the LockMass fluidics.

> **CAUTION:** **_The Lock mass fluidics vial must contain enough sample for the entire sample list acquisition._**

	File Name	File Text	MS File	Inlet File	Bottle	Inject Volume	Control
25						0.000	
26		Acquity Xevo QTof MS System Verification				0.000	
27						0.000	
28	ESI_MS_NaFormate	Acquity Xevo QTof MS Calibration	Acquity_Xevo_QTof_MS_CAL	DEFAULT	1	10.000	
29	Precision Blank 1	System Precision Blank	Acquity_Xevo_QTof_MS	System Precision	1:1	1.000	
30	Precision Blank 2	System Precision Blank	Acquity_Xevo_QTof_MS	System Precision	1:1	1.000	
31	System Precision 1	Sulfadimethoxine	Acquity_Xevo_QTof_MS	System Precision	1:2	1.000	
32	System Precision 2	Sulfadimethoxine	Acquity_Xevo_QTof_MS	System Precision	1:2	1.000	
33	System Precision 3	Sulfadimethoxine	Acquity_Xevo_QTof_MS	System Precision	1:2	1.000	
34	System Precision 4	Sulfadimethoxine	Acquity_Xevo_QTof_MS	System Precision	1:2	1.000	
35	System Precision 5	Sulfadimethoxine	Acquity_Xevo_QTof_MS	System Precision	1:2	1.000	
36	System Precision 6	Sulfadimethoxine	Acquity_Xevo_QTof_MS	System Precision	1:2	1.000	
37	Gradient Blank 1	Gradient Performance Blank	Acquity_Xevo_QTof_MS	Gradient	1:1	0.000	
38	Gradient Blank 2	Gradient Performance Blank	Acquity_Xevo_QTof_MS	Gradient	1:1	0.000	
39	Gradient Performance 1	Gradient Performance	Acquity_Xevo_QTof_MS	Gradient	1:3	0.000	
40	Gradient Performance 2	Gradient Performance	Acquity_Xevo_QTof_MS	Gradient	1:3	0.000	
41	Gradient Performance 3	Gradient Performance	Acquity_Xevo_QTof_MS	Gradient	1:3	0.000	

Figure 59 - Commission ESI sample list

7. Highlight all 13 samples and click **Run > Start**

8. Click **Acquire Sample Data** only.

9. Click **OK** to perform the analysis.

Processing the results

> *NOTE:*   *Calculate the system precision results for all detectors in the system.*
>
>   *Where there is a need for calculation in this section, see "Appendix F: Using the Windows scientific calculator" for instructions.*

System precision test (mass spectrometer)

Calculating the standard deviation of the peak retention times

1. Display the TOF MS chromatogram for each injection.

2. Click **Display > Mass** and enter **311.0814** to create an extracted mass chromatogram for sulfadimethoxine for each injection, with a window of 0.05 Da.
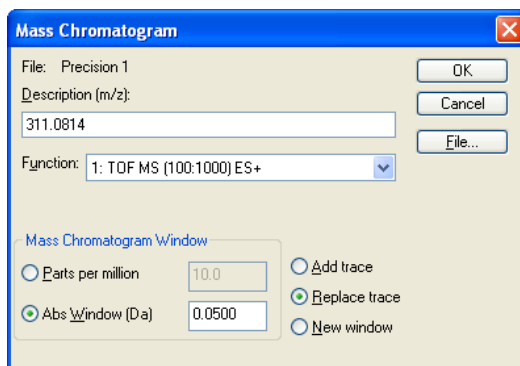
Figure 60 - Displaying the mass chromatogram

3. Click **Display > Peak Annotation** in the Chromatogram window.

4. Select the **Peak Response Area** and **Peak Response Height** check boxes.

5. To integrate the extracted mass chromatogram, click **Process > Integrate** and set the following parameters:
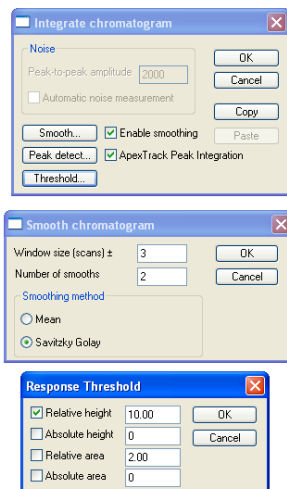
Figure 61 - Integration parameters
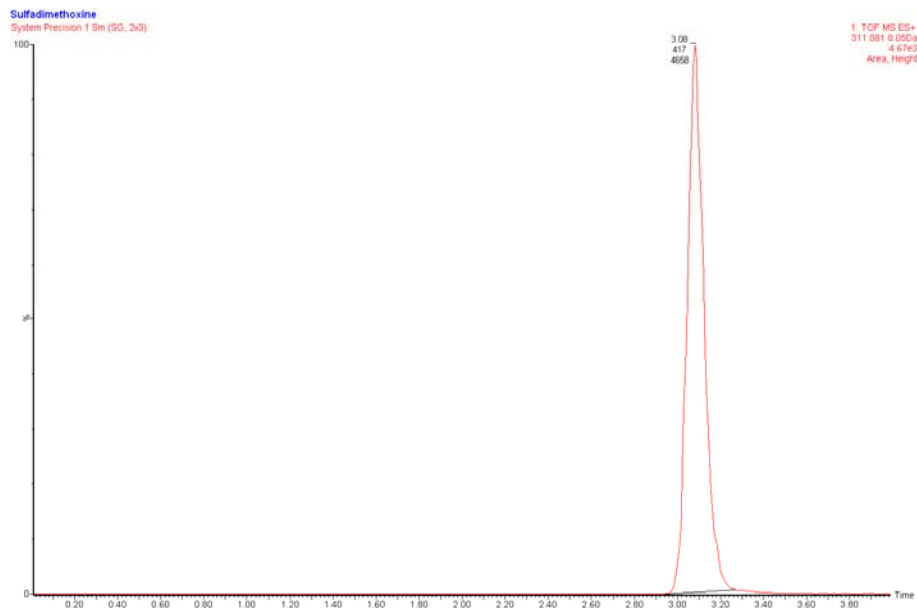
Figure 62 - Example system precision test results

> ***NOTE:*** *Example peak heights are for indication only.*

6. Use the Windows scientific calculator to calculate standard deviation.

   > ***NOTE:*** *The standard deviation must be ≤0.047 minutes.*

7. Record the retention time standard deviation in the appropriate section of the *Xevo QTof MS ACQUITY UPLC System Installation Checklist* (p/n 715002120).

Calculate the %RSD for the peak areas and heights

1. Calculate the %RSD for the peak areas and heights over six replicate injections.

   - For peak areas, the %RSD must be ≤3.0%.
   - For peak heights, the %RSD must be ≤4.5%

2. Record the %RSD for peak areas and heights in the appropriate section of the *Xevo QTof MS ACQUITY UPLC System Installation Checklist* (p/n 715002120).

Calculating the exact mass measurement accuracy

1. Click **Process > Combine Spectra** in the Chromatogram window.

2. Right-click and drag across the eluted 311.0814 peak from baseline to baseline.

3. Set the peak separation to **0.05** and click **OK** (Figure 63).
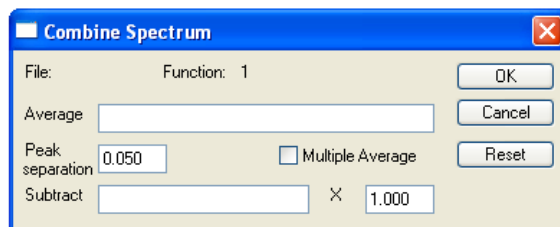
Figure 63 - Setting the peak separation to 0.05

> **NOTE:** *This process sets the stored peak separation value appropriate to the resolution. Subsequent spectrum combine actions can be performed using right-click and drag only. If the peak separation window is not set correctly, ions of a similar mass may be incorporated during the spectrum combine, producing incorrect masses.*

4. Click **Display > Peak Annotation**.

5. Set the spectrum annotation to four decimal places and click **OK** (Figure 64).
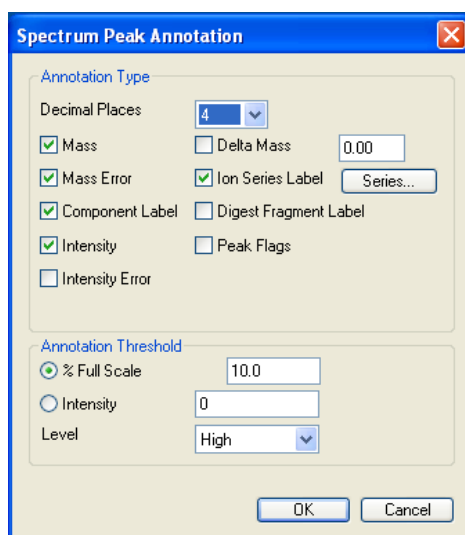
Figure 64 - Peak annotation parameters

6. For each injection, combine the entire sulfadimethoxine chromatographic peak to produce the exact mass DRE spectrum.

7. Determine the mass measurement error (mDa) for each injection using the expected [M+H]+ value from Table 9.

8. Calculate the overall RMS mass error over the six replicate injections.

> **NOTE:** *The RMS mass error must be ≤1.2 mDa.*

9. Record the RMS mass error in the appropriate section of the *Xevo QTof MS ACQUITY UPLC System Installation Checklist* (p/n 715002120).

Gradient performance test

> ***NOTE:*** *Calculate the gradient performance results for the mass spectrometer only.*

Perform the following procedure to calculate the standard deviation of the peak retention times for all four components, over the three replicate injections.

1. Display the chromatogram for each of the gradient performance samples.

2. Verify that all peaks have eluted from the ACQUITY UPLC system.

3. For each injection, create four extracted mass chromatograms (using the expected masses in Table 9) and a window of **0.05 Da** (Figure 65).
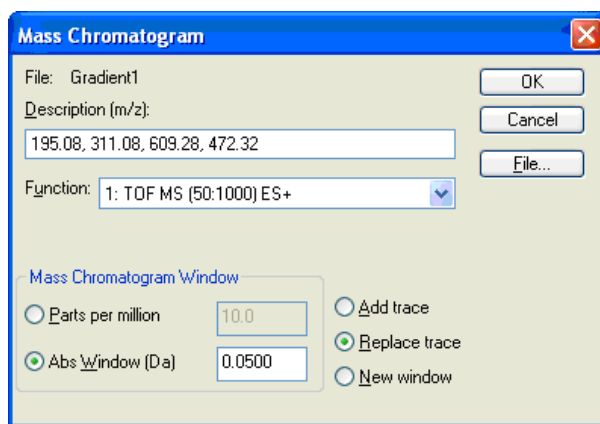
Figure 65 - Mass chromatogram parameters

4. Record the retention times for each eluted peak.

5. Repeat steps 3 and 4 for the remaining two gradient performance samples.

6. Use the Windows scientific calculator to calculate standard deviation for each component.

> ***NOTE:*** *The standard deviation must be ≤0.047 minutes.*

7. Record the standard deviation in the appropriate section of the *Xevo QTof MS ACQUITY UPLC System Installation Checklist* (p/n 715002120).
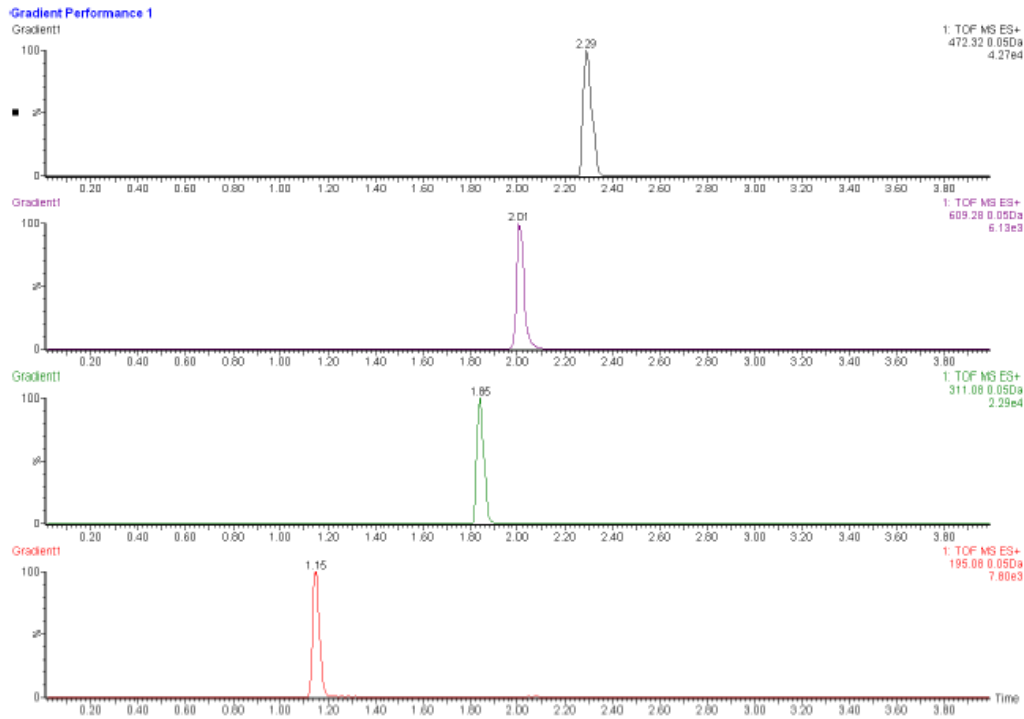
Figure 66 - Example gradient performance chromatograms

CAUTION: **Example peak heights and areas are for indication only.**

System precision test (TUV or PDA detector, if applicable)

Setting up the TUV or PDA detector

1. On the MS Tune page, ensure the YAAXXX.ipr file is open.

2. Set the capillary voltage to **0**.

3. Click **File > Save As...** and save the amended MS Tune page as **UV_Only.ipr.**

4. Fit the backpressure regulator to the outlet of the TUV or PDA flow cell and plumb to a suitable waste container.

5. Create a TUV or PDA detector method in MassLynx for the system precision test using the following settings:
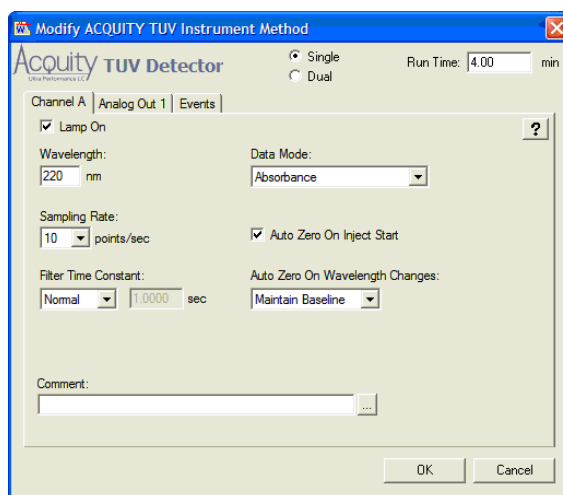
Figure 67 - TUV detector settings
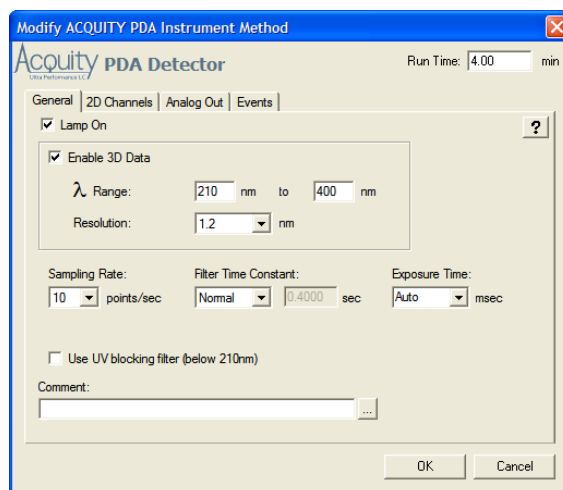
Figure 68 - PDA detector settings

6. Using the inlet editor, click **File > Save As** and save the inlet method using the format **SPT_PREC_DDMMYY** (where DDMMYY = date).

Acquiring sample data

> **NOTE:** Where there is a need for calculation in this section, see "Appendix F: Using the Windows scientific calculator" for instructions.

1. Set up a sample list as shown in Figure 69.

2. Select the appropriate inlet and MS method files created previously.

3. Save the sample list as **UV_DDMMYY.spl (**where DDMMYY = date).

4. Set a flow rate of **80:20 water/acetonitrile** at **0.3 mL/min** and allow the baseline to stabilize.

> **CAUTION:** **Ensure that the outlet from the TUV or PDA is disconnected from the Xevo QTof fluidics and/or ESI probe before performing this test, otherwise contamination of the mass spectrometer may occur.**

	File Name	File Text	MS File	Inlet File	Bottle	Inject Volume	MS Tune File
1	UV Precision Blank 1	System Precision Blank	SPT_PREC_091008	SPT_PREC_091008	1:1	5.000	UV_ONLY
2	UV Precision Blank 2	System Precision Blank	SPT_PREC_091008	SPT_PREC_091008	1:1	5.000	UV_ONLY
3	UV System Precision 1	Sulfadimethoxine 0.1mg/mL	SPT_PREC_091008	SPT_PREC_091008	1:4	5.000	UV_ONLY
4	UV System Precision 2	Sulfadimethoxine 0.1mg/mL	SPT_PREC_091008	SPT_PREC_091008	1:4	5.000	UV_ONLY
5	UV System Precision 3	Sulfadimethoxine 0.1mg/mL	SPT_PREC_091008	SPT_PREC_091008	1:4	5.000	UV_ONLY
6	UV System Precision 4	Sulfadimethoxine 0.1mg/mL	SPT_PREC_091008	SPT_PREC_091008	1:4	5.000	UV_ONLY
7	UV System Precision 5	Sulfadimethoxine 0.1mg/mL	SPT_PREC_091008	SPT_PREC_091008	1:4	5.000	UV_ONLY
8	UV System Precision 6	Sulfadimethoxine 0.1mg/mL	SPT_PREC_091008	SPT_PREC_091008	1:4	5.000	UV_ONLY

Figure 69 - Sample list for TUV or PDA detectors

5. Highlight all eight samples then click **Run > Start**.

6. Click **Acquire Sample Data** only.

7. Click **OK** to perform the analysis.

Calculating the standard deviation of the peak retention times

1. Display the detector chromatogram for each injection.

2. Click **Display > Peak Annotation** in the Chromatogram window.

3. Select the **Peak Response Area** and **Peak Response Height** check boxes.

4. To integrate the chromatogram, click **Process > Integrate**, and set the parameters shown in Figure 61.

5. Use the Windows® scientific calculator to calculate standard deviation.

> **NOTE:** The standard deviation must be ≤0.047 minutes.

6. Record the retention time standard deviation in the appropriate section of the *Xevo QTof MS ACQUITY UPLC System Installation Checklist* (p/n 715002120).

Calculate the %RSD for the peak areas and heights

1. Calculate the %RSD for the peak areas and heights over six replicate injections, using the formula:

$$\%RSD = \frac{(\text{Standard deviation} \times 100)}{\text{Mean}}$$

- For peak areas, the %RSD must be ≤0.5%.
- For peak heights, the %RSD must be ≤0.9%

2. Record the %RSD for peak areas and heights in the appropriate section of the *Xevo QTof MS ACQUITY UPLC System Installation Checklist* (p/n 715002120)*.*

System precision test (ELS or FLR detector)

1. For the ELS or FLR detectors, perform the system precision tests as described in the procedure *ACQUITY UPLC System Performance Tests* (p/n 715001134).

Calculating the standard deviation of the peak retention times

1. Process the results as described in the procedure *ACQUITY UPLC System Performance Tests* (p/n 715001134).

    NOTE: *The standard deviation must be ≤0.047 minutes.*

2. Record the retention time standard deviation in the appropriate section of the *Xevo QTof MS ACQUITY UPLC System Installation Checklist* (p/n 715002120)*.*

**Calculating the %RSD for the peak areas and heights**

1. Process the results as described in the procedure *ACQUITY UPLC System Performance Tests* (p/n 715001134).

2. Calculate the %RSD for the peak areas and heights over six replicate injections, using the formula:

$$\%RSD = \frac{(\text{Standard deviation} \times 100)}{\text{Mean}}$$

- For peak areas, the %RSD must be ≤5% (ELSD) or ≤3% (FLR).
- For peak heights, the %RSD must be ≤5% (ELSD) or ≤4.5% (FLR).

3. Record the %RSD for peak areas and heights in the appropriate section of the *Xevo QTof MS ACQUITY UPLC System Installation Checklist* (p/n 715002120)*.*

Xevo QTof MS nanoACQUITY UPLC System

When installing a Xevo QTof MS with a nanoACQUITY UPLC system, perform the following system tests. Refer to the *nanoACQUITY Operator's Guide* (p/n 71500097502) or *Quick Start Guide* (p/n 71500097503) if required.

For those systems purchased with optional ProteinLynx Global Server (PLGS) software, complete the included Identity^E test.

Mobile phase preparation

Due to the increased sensitivity that both the nanoACQUITY UPLC system and the Xevo series of mass spectrometers offer, all solvents including water and additives must be of the highest chemical purity (at a minimum of HPLC grade). Failure to use high quality solvents will result in high background contamination, low signal-to-noise, and loss of sensitivity. Waters recommends using Fisher Optima MS grade solvents and reagents.

> *CAUTION:* ***To prevent contamination of glassware, solvent bottles must not be washed with detergents, or washed with other general glassware. The glassware must be rinsed with the high purity solvents to be used.***
>
> ***For detailed information on guidelines for the prevention of system contamination, refer to the document Controlling Contamination in Ultra Performance LC/MS and HPLC/MS Systems (p/n 715001307) located in the Waters Knowledge Center.***
>
> ***Always wear suitable gloves to avoid contact with solvents and eliminate possible system contamination when performing any maintenance to nanoACQUITY system components.***

1. Before setting up to run the system test, prime and flush the system with 25:25:25:25 ACN/MeOH/IPA/H_2O with 0.2% formic acid.

2. Flush to waste for 300 minutes, at 50 µL/min.

3. Prepare the following mobile phases and wash solvents:

   - Mobile phase A1   Water + 0.1% formic acid
   - Mobile phase B1   Acetonitrile + 0.1% formic acid
   - Seal wash   10:90 acetonitrile/water + 0.1% formic acid

Sample preparation

To prepare the sample, use the Waters MassPREP digestion standard (MPDS) Mixture 1 (p/n 186002865).

Table 11: Solution kit 186002865 components

Solution	Description	Qty
Mixture 1	Alcohol dehydrogenase (ADH) Glycogen phosphorylase B (GPB) Bovine serum albumin (BSA) Enolase	1

Preparing the diluent

1. Use a 100-mL graduated cylinder and measure 50 mL of MS grade acetonitrile.

2. Transfer the acetonitrile to a 1-L reservoir bottle.

3. Use a 1000-mL graduated cylinder and measure 450 mL of MS grade water.

4. Transfer the water to the same 1-L reservoir bottle.

5. Use a calibrated pipette to add 0.1% (500 µL) of formic acid to the same 1-L reservoir bottle.

6. Cap the reservoir bottle and mix well.

7. Label the reservoir bottle as "10:90 acetonitrile/water + 0.1% formic acid, diluent".

Preparing Mixture 1 for system test (25 fmol/µL, each component)

1. Add 200 µL of diluent to the Mixture 1 vial to create a stock solution of 250 fmol/µL of each Mixture 1 component.

2. Stopper and invert the vial several times to fully mix.

3. Label this vial as "Mixture 1 stock solution".

4. Using a calibrated pipette, add 10 µL of Mixture 1 stock solution to a new vial.

5. Add 90 µL of diluent to the same vial, to create the Mixture 1 Test sample.

6. Cap the vial and mix well.

7. Label this vial as "Mixture 1 Test solution".

8. This solution contains 25 fmol/µL of each Mixture 1 component.

Preparing the reference solution for auxiliary solvent manager line B1 (if required)

1. Prepare the desired volume of 200 fmol/µL (or suitable concentration) solution of [Glu^1]-fibrinopeptide B.

   NOTE: *Additional [Glu^1]-fibrinopeptide B sample may be required if larger volumes of reference solution are to be installed on the nanoACQUITY.*

2. Place the reference solution on top of the nanoACQUITY with the other solvent bottles.

3. Insert auxiliary solvent manager line B1 and prime.

Calibrant sample

1. Install a 30-mL fluidics vial containing a 1 mg/mL sodium caesium iodide solution to fluidics position C for calibration.

System performance test preparation

MassLynx configuration

1. Click **Inlet Method** in the MassLynx window.

2. Click **Tools > Instrument Configuration...** and select **Configure** to launch the Configuration wizard.

3. Click **Next** and select Waters ACQUITY options for pump and autosampler.

4. Click **Finish**.

5. Click **OK** to install the Instrument Control Option Pack (ICOP).

6. Select **Install software support for one or more instruments**, then click **Next**.

7. Select the relevant modules (binary solvent manager and sample manager) then click **Next**.

   NOTE: *The relevent modules' control software will now be installed.*

8. Once the Results are shown, click **Finish**.

9. Click **Tools > Instrument Configuration...** and then select **Events & Triggering**.

10. Click **Next** and select **Event In 1**.

11. Click **Finish**.

12. Reboot the host PC before turning on the power to any of the nanoACQUITY modules.

13. Switch on each module, starting with the binary solvent manager.

14. Update the firmware for each module if required.

15. Update the ICOP software to the correct version for nanoACQUITY if required.

    NOTE: *The following procedures are all based on ICOP 1.41.*

    CAUTION: ***Where the TRIZAIC source is used, nanoACQUITY must use ICOP 1.46 or later.***

nanoACQUITY configuration

1. Place the binary solvent manager and auxiliary solvent manager A1 solvent lines, and the sample manager weak wash line, into the mobile phase A1 reservoir.

2. Place the binary solvent manager B1 solvent line, and the sample manager strong wash line, into the mobile phase B1 reservoir.

3. Place auxiliary solvent manager B1 solvent line into the reference solution reservoir.

4. Place the binary solvent manager and auxiliary solvent manager seal wash solvent lines into the seal wash reservoir.

5. Connect the weak needle wash (WNW) and strong needle wash (SNW) lines to the degasser outlets.

6. Place the ACQUITY UPLC waste tubing into a suitable waste container.

7. Prime the binary solvent manager seal wash line.

8. Prime the auxiliary solvent manager seal wash line.

9. Prime solvent lines A and B, A1 and B1 of the binary solvent manager for 10 minutes.

10. Prime solvent line B, B1 of the auxiliary solvent manager for 10 minutes.

11. Prime the sample manager syringes with at least five cycles.

> *NOTE:*   *Each prime takes approximately four minutes.*

12. Click **Control > Wash Needle** in the Sample Manager Console to perform the needle wash procedure. Enter 2000 µL for both strong and weak washes.

13. Click **Maintain > Characterise > Needle Seal** in the Sample Manager Console to perform the needle seal calibration procedure.

14. Record the results in the *Xevo QTof MS nanoACQUITY Installation Checklist* (p/n 715002121).

15. Click **Maintain > Characterise > Needle and Loop Volumes** in the Sample Manager Console to perform the system volume calibration procedure.

16. Record the results in the *Xevo QTof MS nanoACQUITY Installation Checklist* (p/n 715002121).

> *CAUTION:*   *Calibrate the system volume whenever there is a change to the mobile phase, wash solvents, sample loop, needle, or any of the syringes.*
>
> *If the needle fails the test, check all fittings for leaks. Also check the needle to see if it is bent, broken or blocked. If the sample loop fails the test, the syringe draw (aspiration) rate may be too high. Also examine all fittings for leaks, or determine whether the loop is blocked or leaking. Check the configuration and make sure the needle and loop sizes are correct.*

Calibrating the XYZ mechanism

1. Open the sample manager door and remove the sample plate tray holders.

2. Remove the teach block from the sample manager floor.

3. Insert the teach block into the locating holes.

4. In the Sample Manager console, click Troubleshoot > Calibrate XY and Zp axes.

5. Click **Start**.

> *NOTE:*   *A warning appears prompting you to keep clear of the sample chamber.*

6. Click **OK**.

> *NOTE:*   *The XYZ mechanism positions the puncture needle above the teach block.*
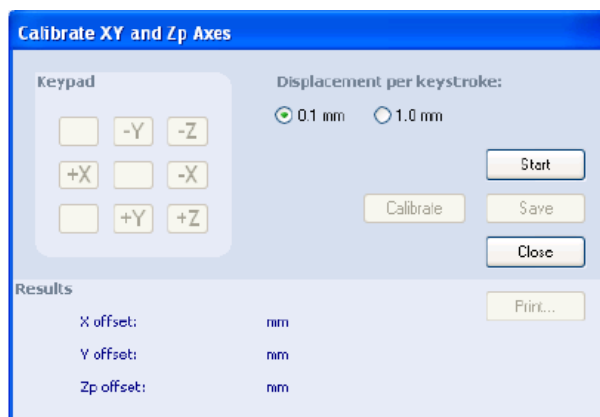
Figure 70 - Calibrate XY and Zp axes dialog box

7. Examine the location of the puncture needle relative to the needle hole in the teach block.

8. Select **1.0 mm** increments and then select +Z to position the XYZ mechanism close to the teach block.

9. Select **0.1 mm** increments to fine tune the position so that the XYZ mechanism foot is just above the teach block, but not touching it.

10. Use the +X and −X buttons to adjust position left and right.

11. With the aid of a dental mirror or similar, use the +Y and −Y buttons to adjust front-to-back position so that the needle is centered over the hole in the teach block.

12. Using 0.1 mm increments, lower the needle into the teach block hole to confirm accurate X and Y alignment.

13. When the alignment is satisfactory, click **Calibrate**.

    NOTE: *A warning appears prompting you to keep clear of the sample chamber.*

14. Record the results in the Xevo QTof MS nanoACQUITY Installation Checklist (p/n 715002121).

15. Click **Save**, then click **Yes** when the confirmation window appears.

16. Remove the teach block and place it back in its storage location.

17. Re-insert the sample tray holders.

18. Click **Maintain > Calibrate needle Z axis**.

19. Click **Start**, then **OK** in the confirmation window.

20. Click **+Z** to drive the sample needle down to within 1 mm of the tray surface.

21. Place a business card under the tip of the needle.

22. Change the increments to 0.1 mm, and lower the needle until it is lightly touching the card (but not restricting its movement).

23. Click **Save**, then **Yes** in the confirmation window.

24. Record the result in the *Xevo QTof MS nanoACQUITY Installation Checklist* (p/n 715002121).

Auto zeroing the flow control module

1. Select the binary solvent manager within the nanoACQUITY console.

2. Set a flow of 50% A, at 50 µL/min on the binary solvent manager.

3. Ensure liquid flows out of the binary solvent manager flow controller module.

4. Stop the flow on the binary solvent manager.

5. Insert a pin plug into the output of the binary solvent manager flow controller module.

6. Click **Binary Solvent Manager > Troubleshoot > Auto zero flow transducers**.

7. Select **Pump A and Pump B**.

8. Click **Start**, then click **Results** to check whether the test has been successful.

   NOTE: *The test outcome is indicated by **Passed** displayed for both Pump A and Pump B in the Results section.*

nanoACQUITY tubing connections and set pressure diagnostics

Refer to document *nanoACQUITY UPLC™ 10k psi plumbing* (p/n 715001069) and also the *nanoACQUITY UPLC System Operator's Guide* (p/n 71500097502) if required.

Table 12: Set pressure diagnostics tests

Test	Component under test	Measured leak rate criteria at 10,000 psi (µL/min)	Location to plug	Position of injection valve	Position of trapping valve
A	Flow control module	0.015	Outlet mixing tee	N/A	N/A
B	Flow control module Injection valve	0.015	N/A	Blocked	N/A
C1*	Flow control module Injection valve Sample loop off-line Trap column with tee	0.018	Nano tee (two PEEK plugs)	Load	N/A
C2*	Flow control module Injection valve Sample loop in-line Trap column with tee	0.018	Nano tee (two PEEK plugs)	Inject	N/A
D*	Flow control module Injection valve Trap column with tee Trap (HTM) valve	0.020	Nano tee (one PEEK plug: vent line installed)	Inject	Analytical

NOTE: ** These tests are not required for the TRIZAIC.*
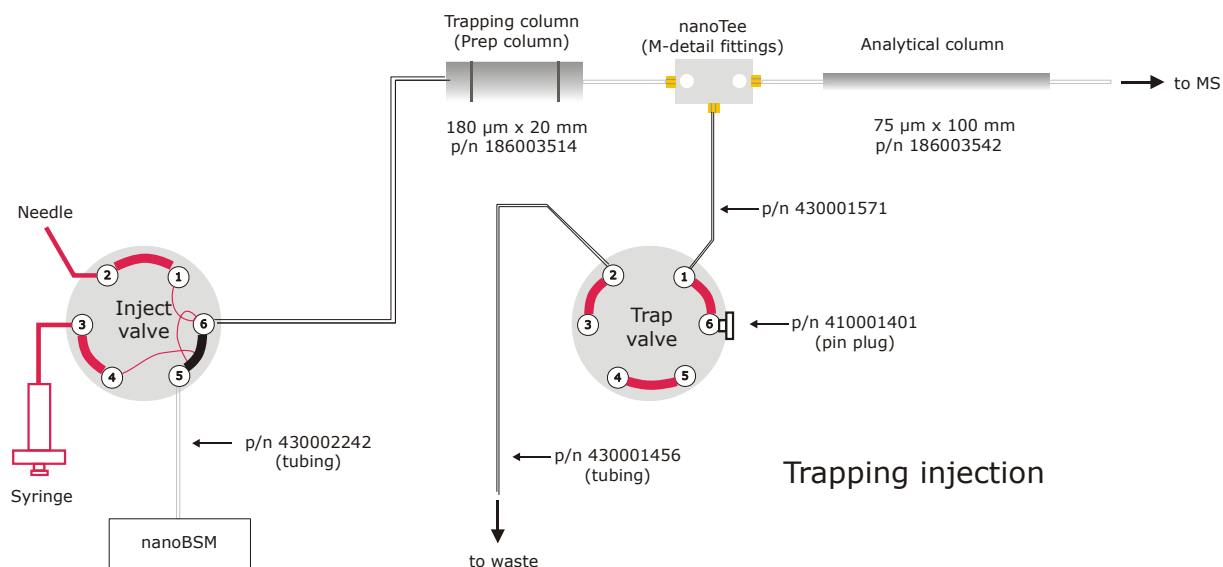
Figure 71 - Diagram of connections (ICOP 1.41)
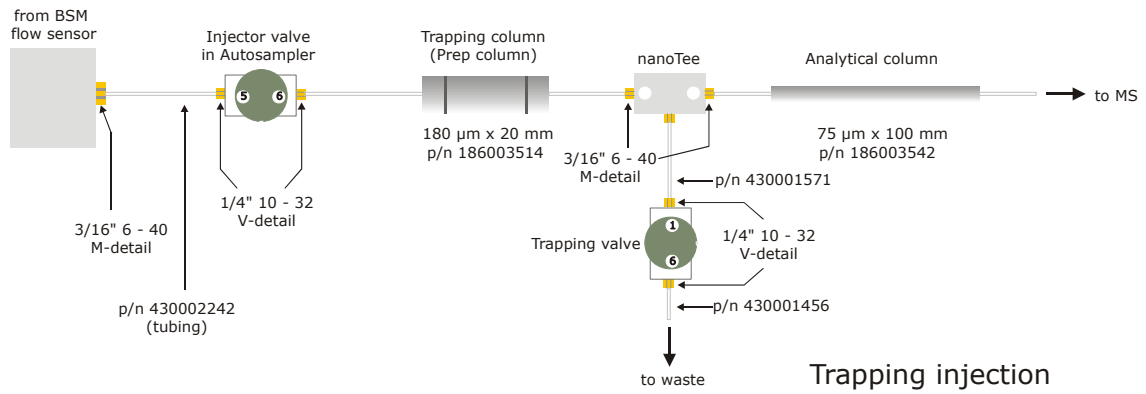
Figure 72 - Diagram of connections (pre-ICOP 1.41)
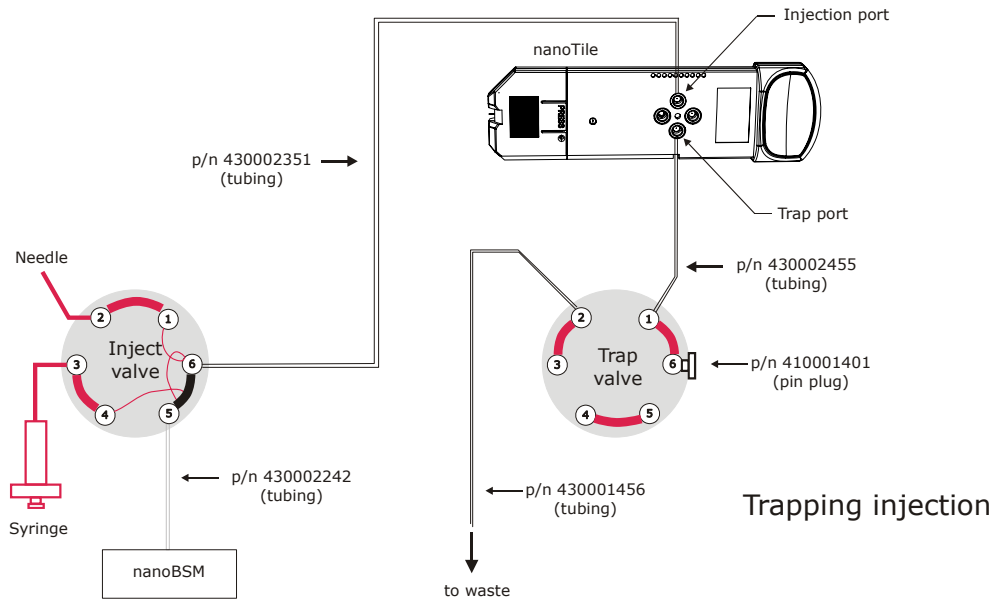
Figure 73 - Diagram of connections (TRIZAIC)
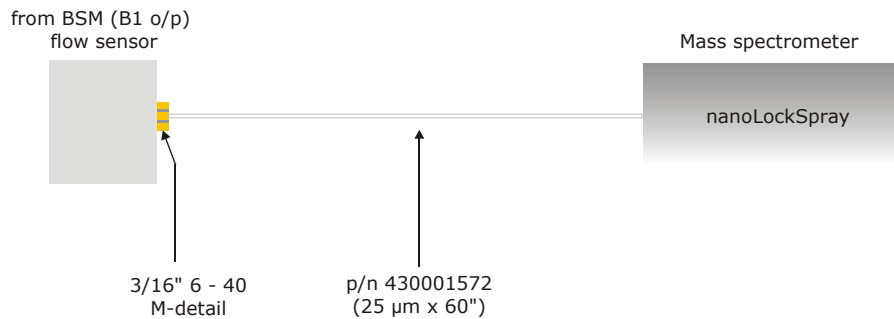
Figure 74 - Flow sensor and instrument connections

Table 13: Recommended tightening

Fitting	Using a new fitting	Using a previously installed fitting
$^3/_{16}$" 6-40 M-detail	Snug plus ½-turn (180°)	Hand-tight plus ⅛-turn (45°)
¼" 10-32 V-detail	Hand-tight plus 1 turn (360°)	Hand-tight plus $^1/_6$-turn (60°)

> *NOTE:* *In the above table, 'snug' is defined as finger-tight until the ferrule makes contact with the bottom of the port; a spanner is required to complete the additional ½ turn.*

Before commencing tests, flush the BSM flow control module with a 50:50 (A:B) mix at 50 µL/min to remove particulates.

Test A

Perform Test A from Table 12 using the following steps.

1. Stop the flow.

2. In the nanoACQUITY console, click **BSM**.

3. Click **Troubleshoot** > **Set Pressure Diagnostic**, to access the Set Pressure Diagnostic dialog box (Figure 75).
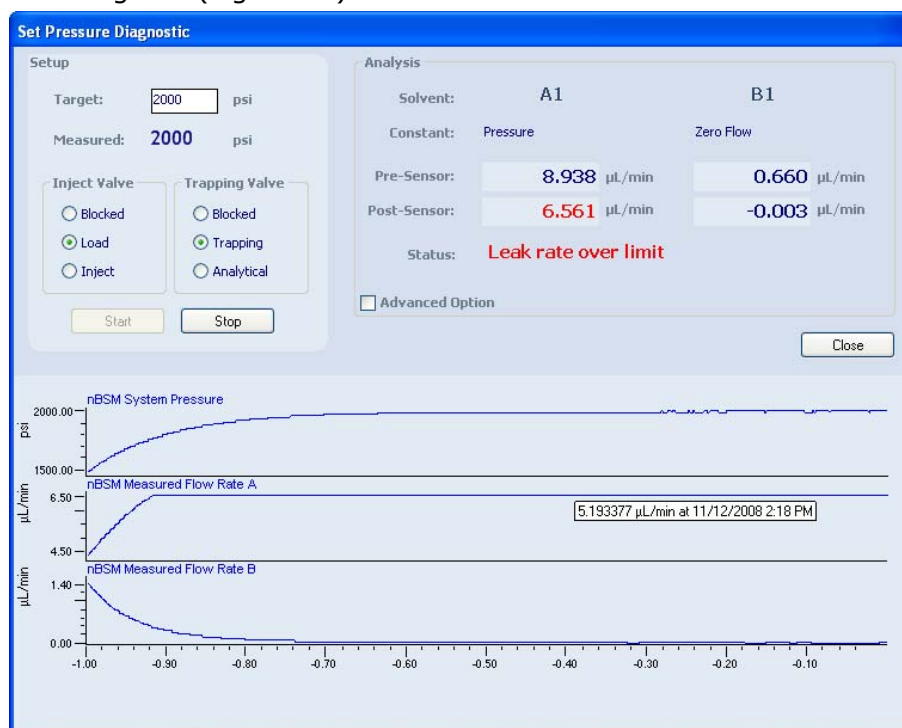
Figure 75 - Set Pressure Diagnostic dialog box

4. Install the pin plug into the outlet of the BSM flow control module.

5. Ensure the pressure is set to **0**.

6. Click **Start**.

7. Monitor the A and B post-sensor flow readings. If peak-to-peak readings exceed ±0.007 µL/min, auto zero the flow control module.

8. Increase the pressure in steps of 2000 psi up to 10,000 psi.

    NOTE: *Allow the pressure to stabilize at each step before increasing to the next pressure.*

9. When system reaches the set pressure (10,000 psi), monitor the measured flow (leak criteria) of pump A and ensure it passes the test (0.015 µL/min or less).

10. Lower the set pressure in steps of 2000 psi back to 0 psi

11. Click **Stop**.

    CAUTION: **If the results for Test A are significantly above the leak rate criteria, contact Waters technical support.**

Test B

Perform Test B from Table 12 using the following steps.

1. Remove the pin plug from the outlet of the BSM flow controller.

2. Connect the capillary tubing (p/n 430002242) to the outlet on the BSM flow controller.

    NOTE: *Refer to Table 13 for tightening procedures.*

3. Flush the capillary tubing with 50:50 (A:B) mix at 10 µL/min to remove particulates.

4. Connect the other end of the tube to port #5 of the sample manager's inject valve.

    NOTE: *Refer to Table 13 for tightening procedures.*

5. Flush through the valve with a 50:50 (A:B) mix at 10 µL/min to remove particulates.

6. Click **Stop**.

7. Set Inject Valve to **Blocked**.

8. Ensure the pressure is set to **0**.

9. Click **Start**.

10. Increase the pressure in steps of 2000 psi up to 10,000 psi.

    NOTE: *Allow the pressure to stabilize at each step before increasing to the next pressure.*

11. When system reaches the set pressure (10,000 psi), monitor the post-sensor flow (leak criteria) of pump A and ensure it passes the test (0.015 µL/min or less).

12. Lower the set pressure in steps of 2000 psi back to 0 psi.

13. Click **Stop**.

    NOTE: *If the results for Test B are significantly above the leak rate criteria, perform Test A. If flow control module is acceptable, tighten or replace the capillary tubing from the flow control module to the injection valve.*

                    When using TRIZAIC, no further tests are required.

Test C1

Perform Test C1 from Table 12 using the following steps.

1. Connect the trapping column assembly to port #6 of the sample manager's inject valve.

    NOTE: Refer to Table 13 for tightening procedures.

2. Flush/condition the trapping column for a minimum of 30 minutes at 10 µL/min using a 15:85 (A:B) mix to remove particulates.

3. Click **Stop**.

4. Connect the trapping column to the nanoTee.

5. Put PEEK pin plugs into the additional two ports of the nanoTee.

6. Set Inject Valve to **Load**.

7. Ensure the pressure is set to **0**.

8. Click **Start**.

9. Increase the pressure in steps of 2000 psi up to 10,000 psi.

    NOTE: Allow the pressure to stabilize at each step before increasing to the next pressure.

10. When the system reaches the set pressure (10,000 psi), monitor the post-sensor flow (leak criteria) of pump A and ensure it passes the test (0.018 µL/min or less).

11. Lower the set pressure in steps of 2000 psi back to 0 psi

12. Click **Stop**.

    NOTE: If the results for Test C1 are significantly above the leak rate criteria, perform Test B. If Test B passes, the trap column or nanoTee may need replacing. If Test B fails, perform Test A.

Test C2

Perform Test C2 from Table 12 using the following steps.

1. Set Inject Valve to **Inject**.

2. Ensure the pressure is set to **0**.

3. Click **Start**.

4. Increase the pressure in steps of 2000 psi up to 10,000 psi.

    NOTE: Allow the pressure to stabilize at each step before increasing to the next pressure.

5. When the system reaches the set pressure (10,000 psi), monitor the measured flow (leak criteria) of pump A and ensure it passes the test (0.018 µL/min or less).

6. Lower the set pressure in steps of 2000 psi back to 0 psi

7. Click **Stop**.

   NOTE: *If Test C2 fails while Test C1 has passed, check the sample loop connections.*

Test D

Perform Test D from Table 12 using the following steps.

1. Connect capillary tubing (p/n 430001571) to the top nanoTee connection.
2. Flush/condition the capillary tubing to waste at 10 µL/min using a 50:50 (A:B) mix to remove particulates.
3. Attach the other end to port #1 of the trapping valve (Figure 76).
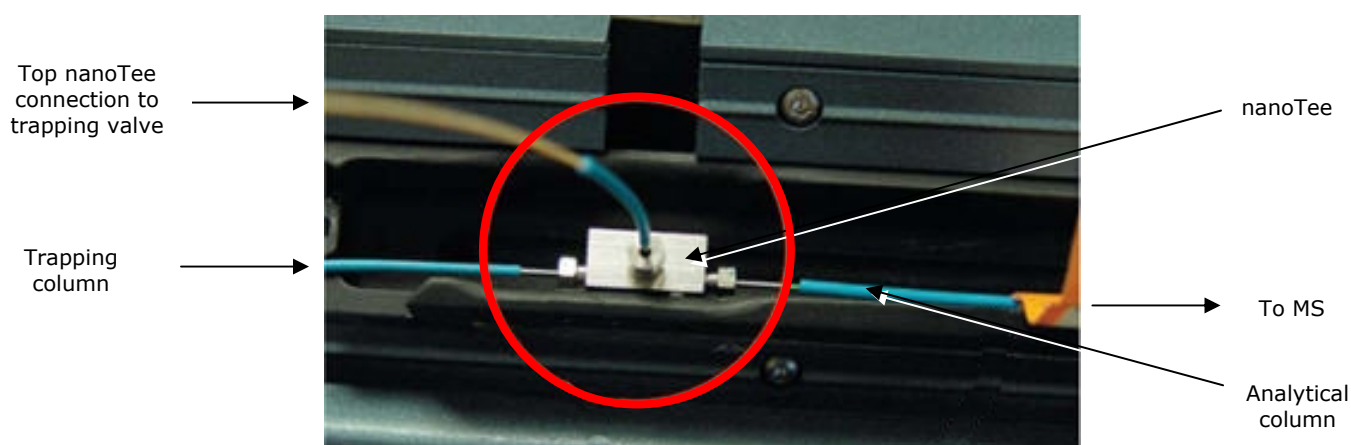
Figure 76 - nanoTee connections

4. Ensure the output port of the nanoTee is plugged.
5. Set Inject Valve to **Inject**.
6. Set Trapping Valve to **Analytical**
7. Ensure the pressure is set to **0**.
8. Click **Start**.
9. Increase the pressure in steps of 2000 psi up to 10,000 psi.

   NOTE: *Allow the pressure to stabilize at each step before increasing to the next pressure.*

10. When the system reaches the set pressure (10,000 psi), monitor the measured flow (leak criteria) of pump A and ensure it passes the test (0.020 µL/min or less).
11. Lower the set pressure in steps of 2000 psi back to 0 psi.
12. Click **Stop**.

   NOTE: *If the results for Test D are significantly above the leak rate criteria, perform Test B. If Test B passes, perform Test C1. If Test C1 passes, the capillary tubing from the nanoTee to the trap valve may need replacing.*

13. Remove the pin plug from the nanoTee.

14. Connect the 75 µm x 100 mm analytical column (p/n 186003542) to the outlet of the nanoTee.

> *CAUTION:*   ***Flush the analytical column with a 15:85 (A:B) mix at 0.4 µL/min for at least one hour before continuing this procedure.***

15. Connect a waste line (p/n 430001456) to port #2 of the trapping valve, and a pin-plug to port #6 (for ICOP 1.41).

16. Connect a waste line (p/n 430001456) to port #6 of the trapping valve (for pre-ICOP 1.41).

17. Where applicable, connect tubing (p/n 430001572) from auxiliary sample manager B1 output (this will connect to the reference sprayer) and set the flow to 0.5 µL/min.

ProteinLynx Global SERVER (PLGS)

Setting up PLGS

> *NOTE:*   *PLGS version 2.3 or higher is required for Identity based installations.*

1. Insert the CD into the host PC to start the Installation wizard.
2. Click **Next** to start installation.
3. Accept the terms of the license agreement, then click **Next**.
4. Click **Next** to install to the directory given as default. Click **Browse** to install the software in a different location.
5. When prompted to install the processor and search engine as services, select **No**.

> *NOTE:*   *This ensures the processor and search engine services must be started manually.*

6. Read the summary and ensure the software issue and location is correct.
7. Click **Install**.
8. Click the check box to reboot the PC later.
9. Once the Installation wizard has finished, click **Finish**.

> *CAUTION:*   **Once PLGS has been installed, ensure the PC is rebooted in order for the software to operate correctly.**

Setting the number of processors

If the computer on which ProteinLynx Global SERVER is installed has more than one processor, it is possible to take advantage of the additional power with PLGS.

> *NOTE:*   *If one processor only is to be used, no changes are required.*

Specify the number of processors used for each of three different circumstances:
- DDA data processing
- Electrospray MS data processing
- DDA data bank searching

Setting the number of processors for DDA data processing

> *CAUTION:*   **Copy the file before editing. Making changes other than those explicitly outlined may prevent PLGS from operating properly.**
>
> **Ensure the data bank format is specified correctly, or the data bank will not be added correctly. This will cause problems with PLGS.**
>
> **Do not set the number of processors to a value greater than the number of processors on the system.**

1. Navigate to the PLGS directory **Installation** > **Lib**.
2. Open the **process.cfg** file.

   > *CAUTION:*   **If it does not exist, create a text file called process.cfg.**

3. Add the following lines to the file where <number> is the number of processors DDA is to use:

   [MULTITASKING]

   Number of Processors=<number>

4. Save the file.

Setting the number of processors for electrospray MS data processing

> *CAUTION:* **Copy the file before editing. Making changes other than those explicitly outlined may prevent PLGS from operating properly.**

1. Navigate to the PLGS directory **Installation** > **Lib**.
2. Open the **process.cfg** file.

> *CAUTION:* **If it does not exist, create a text file called process.cfg.**

3. Add the following lines to the file where <number> is the number of processors electrospray MS data is to use.

   [EKL Processing]

   Number of Processors=<number>

4. Save the file.

Setting the number of processors for DDA data bank searching

> *CAUTION:* **Copy of the file before editing. Making changes other than those explicitly outlined may prevent PLGS from operating properly.**

1. Navigate to the PLGS directories **Installation** > **Config** > **Micro**.
2. Open the **mkconfig.cfg** file. This contains the following lines:

```
0
0
1.8 100000
8192
..\\config\\micro\\mod_list.txt
..\\config\\micro\\BLOSUM62.txt
1
```

Number of processors ⟶ (line with `1`)

Figure 77 - Extract of mkconfig.cfg file

3. On the seventh line of the file, type the number of processors that you want data bank searching to use.
4. Save the file.
5. Reboot the host PC to complete the installation.

> *NOTE:* *Refer to the PLGS-Help.pdf file for further information.*

Identity^E specification

Calibrating the mass spectrometer

> **CAUTION:**   **When the TRIZAIC is fitted, the calibration tile must be fitted during this procedure.**

Creating a calibration using IntelliStart

1. Ensure the baffle is set to the Sample position.
2. Connect the sample fluidics to the universal sprayer.
3. Set the flow to Infusion from vial C, sodium caesium iodide calibrant.
4. Open the MS console, and click **Configure > Configuration Mode**.
5. Start the flow at 0.5 µL/min through the sample sprayer.
6. Select the **Create Calibration** check box, then click **Start**.

Figure 78 - Setting up calibration in IntelliStart

7. On the initial wizard screen click **Next**.
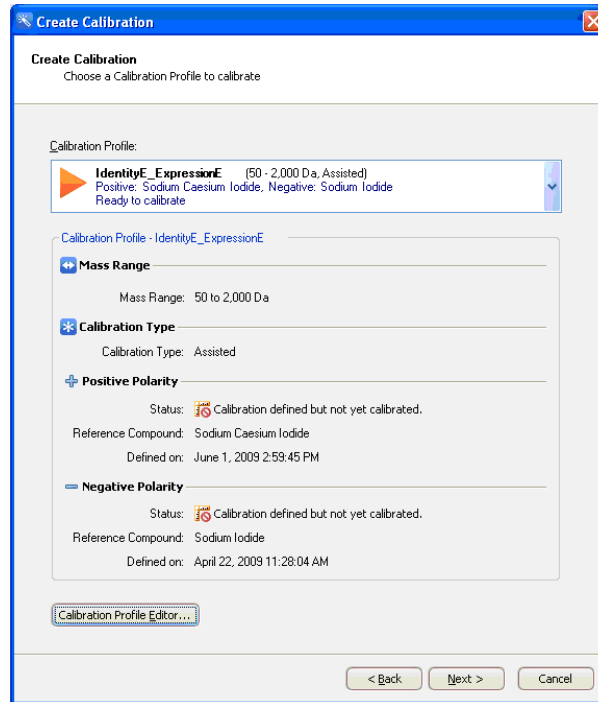
Figure 79 - Calibration profiles

8. Select the **IdentityE_ExpressionE** calibration profile from the drop-down list.

> *NOTE:*   *An orange triangle indicates the calibration profile is defined, but has not yet been calibrated.*

9. Click **Next**.

10. On the Callibration Options page, select the following check boxes:
    - **Positive**
    - **Display Report**
    - **Make the Calibration Profile Active**
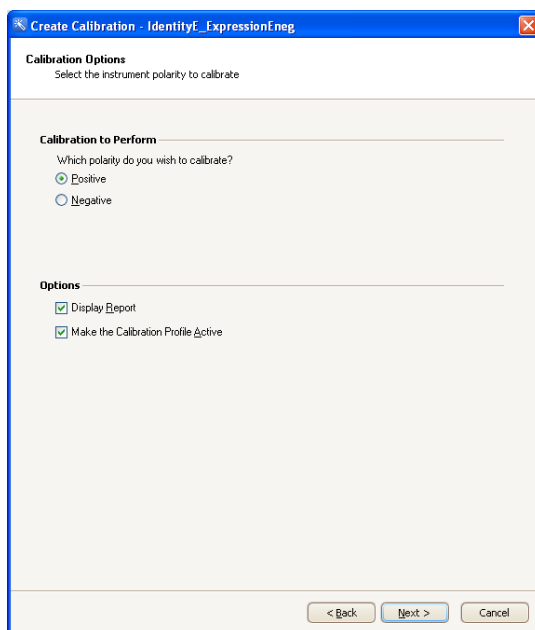
Figure 80 - Create Calibration window: Calibration Options

11. Click **Next**. The data acquisition options (Figure 81) are automatically populated by IntelliStart.

12. Click **Next**.
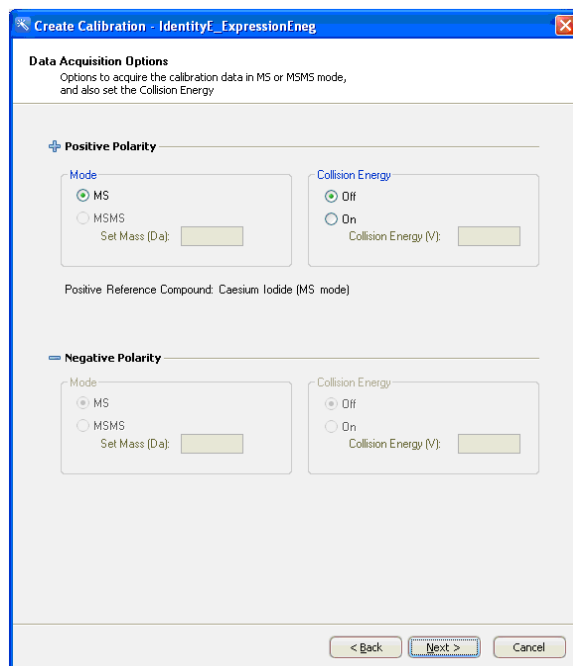
Figure 81 - Data acquisition options

13. Select the **Manual** check box to define how the fluidics will be controlled (Figure 82).
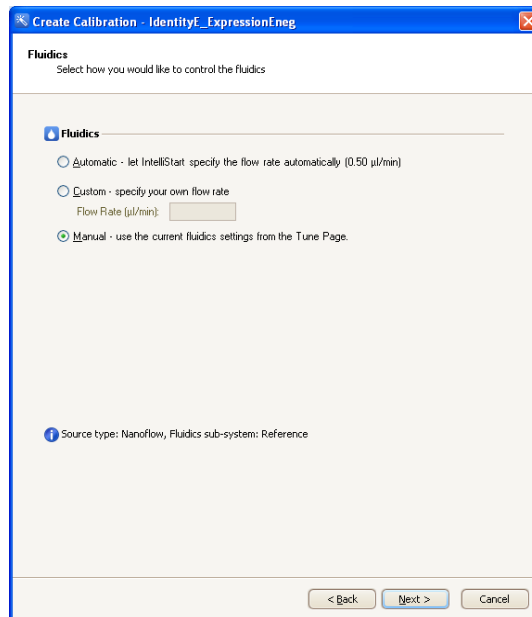
Figure 82 - Fluidics control

14. Click **Next**.

15. Use the default Acceptance Criteria for the Automatic Calibration as shown in Figure 83.
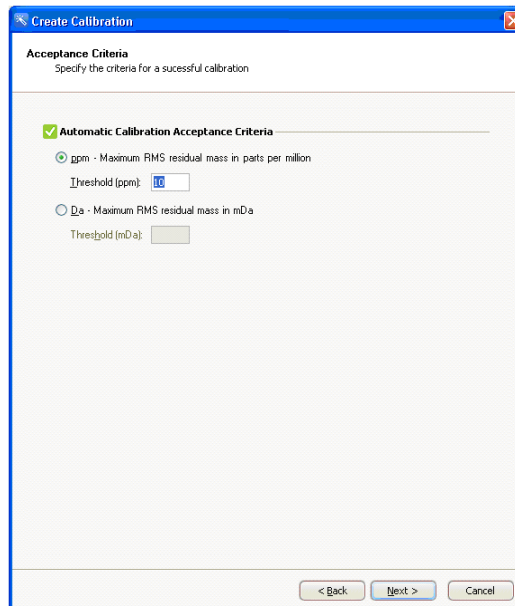
Figure 83 - Acceptance criteria

16. Click **Next**.

   NOTE: *The system is now ready to perform automatic calibration.*

17. Ensure there is calibrant sample (sodium caesium iodide) in vial C as instructed.

18. Click **Start**.

> *NOTE:*   *When starting an automatic or assisted calibration, IntelliStart will automatically load the blank calibration Uncal.cal before acquiring any new data.*

The IntelliStart window will now display the current status of the calibration process. The automatic steps are as follows:

- Removes any previous calibration by loading Uncal.cal
- Performs intensity check – if required, the instrument will use the DRE lens to achieve a suitable intensity (I.P.P. value) on the calibrant sample
- Acquires data – once sufficient data is obtained on the *least intense* peak as defined in the calibrant reference file, the peak picking application attempts to create the calibration.
- Assisted Calibrations – launches the peak picking window and requires the user to manually accept the calibration.

A report screen is generated (Figure 52) displaying the result of the calibration process.

A successful calibration (with a result inside the threshold acceptance criteria set above) produces a green tick in the header. Further details of the calibration data are provided further down the screen.
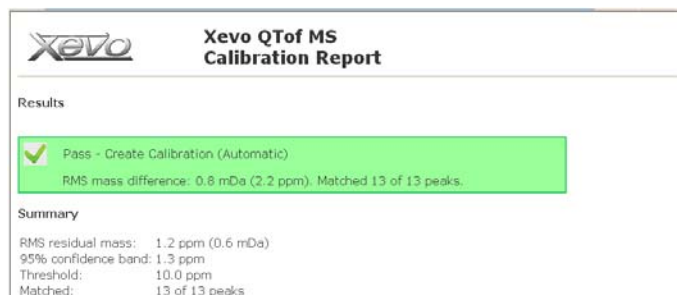
Figure 84 - Calibration detail

The IntelliStart window also details the successful completion of the calibration.
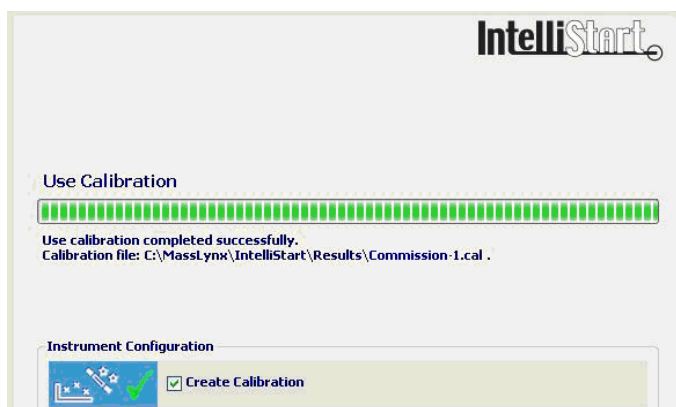
Figure 85 - Confirmation of calibration

19. Flush the universal sprayer by infusing deionized water until NaCsI peaks are no longer observed.

20. Infuse a sample of 200 fmol/µL [Glu^1]-fibrinopeptide B (in 80:20 water/acetonitrile + 0.1% formic acid) at 0.3 µL/min through the universal sprayer and tune the sprayer position for maximum sensitivity.

> *NOTE:* *Tuning the universal sprayer for maximum sensitivity under these conditions helps maximise sensitivity on peptides in the LCMS runs which follow.*

21. Set the analyte divert valve to LC, in order for the fluidics to deliver column flow to the universal sprayer.

22. Infuse 200 fmol/µL [Glu^1]-fibrinopeptide B (or suitable concentration) through the LockSpray sprayer at 0.5 µL/min.

23. Switch the baffle position to LockSpray to observe the GFP signal in the Tune page.

24. Adjust the sample cone voltage to give approximately 200 counts/second (continuum) on the 785 peak.

Minimizing the in-source fragmentation

In-source fragmentation is unavoidable when operating the instrument to analyze a diversity of peptides. It is managed and minimized by performing the following procedure:

1. Infuse a solution of angiotensin II (Sigma Aldrich p/n A9525) at 300 nL/min through the NanoLockSpray sample probe.

2. Acquire some continuum data from the MS Tune page, mass range 350 to 1500, scan time 1 second.

3. Combine twenty scans.

4. In the Spectrum window, click **Process > Smooth**.

5. Enter the following parameters in the Smooth dialog box:

Table 14: Smooth parameters

Parameter	Value
Smooth window	4
Number of smooths	2
Smoothing method	Savitsky Golay

6. Click **Process > Center** and enter the following parameters in the Center dialog box:

Table 15: Center parameters

Parameter	Value
Min peak width at half height (channels)	4
Centroid top (%)	80
Create centered spectrum	Areas

7. Ensure the two fragment ions at m/z 263.1395 and m/z 784.4106 are <5% of the intensity of the doubly charged parent ion at m/z 523.7751.

   NOTE: If the fragment ions are >5% of the intensity of the parent ion, there is too much in-source fragmentation. Reduce the cone voltage, extraction cone or collision energy as required, then repeat the procedure.

   If there is still too much fragmentation, the source assembly may need cleaning. See the Xevo QTof MS Operator's Overview and Maintenance Guide (p/n 71500174502) for details.

8. In the MS Tune page, click **File > Save** to save the .ipr file.

Using IntelliStart for LockSpray source setup

IntelliStart can determine suitable source parameters for a specified lock mass compound automatically to ensure sufficient Lock mass intensity is achieved during later acquisitions.

The recommended operating procedure is to run an initial LockSpray Source Setup to set and store source parameters in the lock mass profile, then continue to use the Lock mass Check within IntelliStart to confirm suitable lock mass performance is maintained.

1. From the main MassLynx window, open the MS Console.

2. Select **Configure > Configuration Mode** to access the instrument configuration display (Figure 86).

3. Select the **LockSpray Source Setup** check box, then click **Start**.

Figure 86 - Instrument configuration display

4. Click **Next** on the initial wizard screen, and then select **Lock mass Editor**.

5. Complete the details for the required lock mass.

   NOTE: *For the purpose of this document, the example Lock mass will be based on [Glu[1]]-fibrinopeptide B (GFP).*

6. Define the Lock mass parameters for [Glu[1]]-fibrinopeptide B (GFP) (m/z 785.8426), as displayed in Figure 87.

7. Click **OK** to display the Lock Mass Editor table (Figure 87).
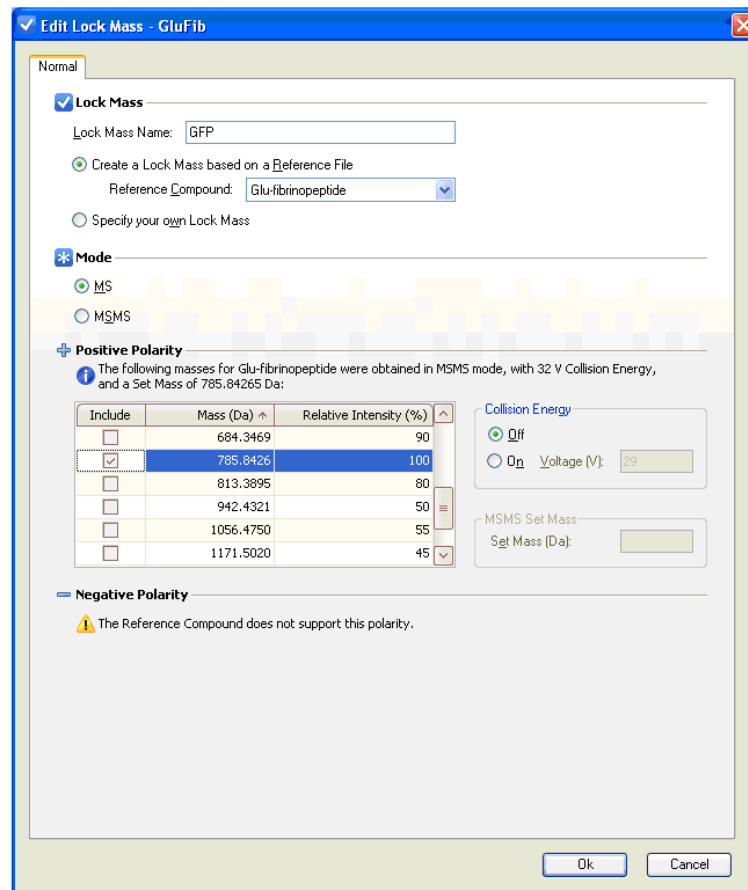
Figure 87 - Creating the GFP Lock mass in the Lock mass editor function

8. Close the Lock Mass Editor table to return to the IntelliStart LockSpray source setup.

Figure 88 - Lock mass editor table

9. Ensure the [Glu^1]-fibrinopeptide B Lock mass is selected as the sample.

10. Ensure that the sample is flowing through the LockSpray sprayer.

11. Click **Next**.
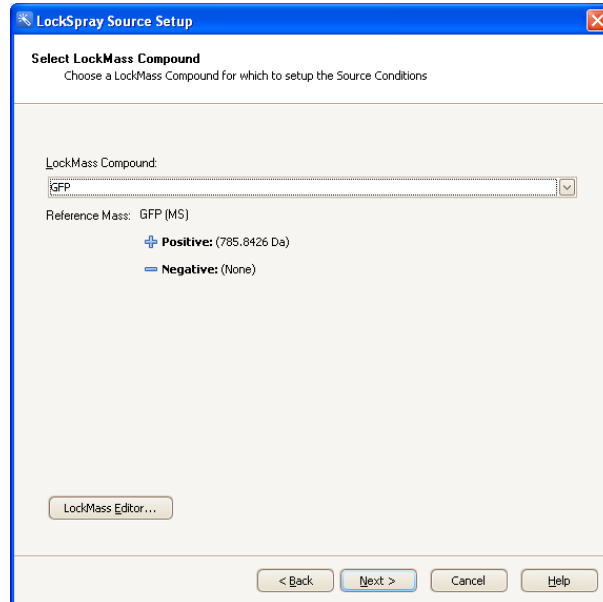
Figure 89 - LockSpray Source Setup display

12. Click **Automatic**, then click **Next**.
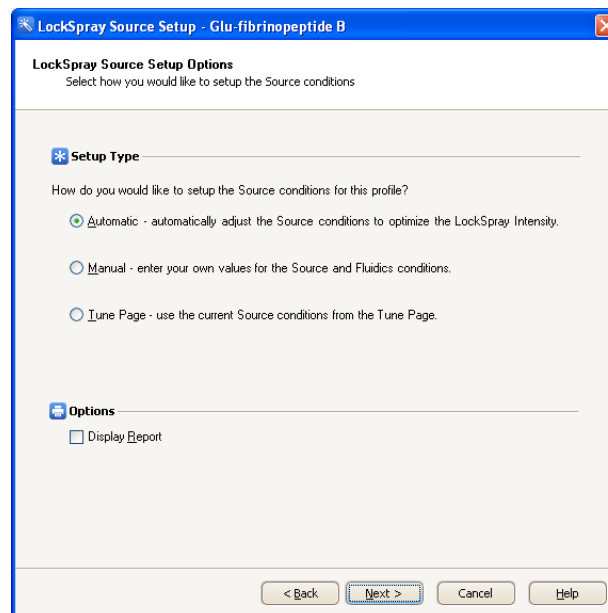
Figure 90 - LockSpray Source Setup Options display

13. Select **Manual** for the reference fluidics to be controlled from the MassLynx Tune page.

> *NOTE:*   *The Manual option is selected for the fluidics section, in case the Lock mass is provided from the ASM of the nanoACQUITY.*
>
> *If there is no ASM available, ensure that the reference fluidics are flowing at 0.5 μL/min.*
>
> *The system is now ready to optimise the source conditions for the Lock mass profile specified.*

14. Click **Next**.
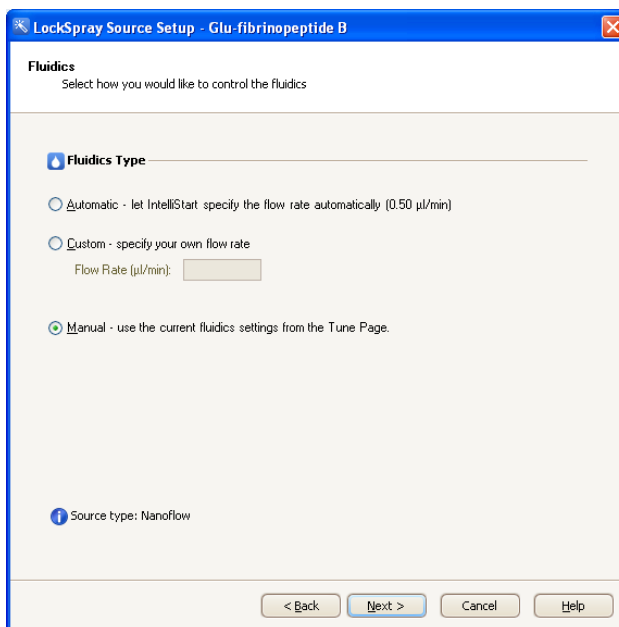
Figure 91 - Fluidics control

15. Click **Start** to begin the process.

The IntelliStart software automatically adjusts the source conditions. The values chosen are written automatically into the Lock mass profile, so whenever the profile is used in an acquisition, these parameters are set on the Lock mass sprayer.

> *NOTE:*   *Because the values are saved into the Lock mass profile, they can not be overwritten without repeating the "Using IntelliStart for LockSpray source setup" section.*

When successfully completed, the MS Console displays the green check mark.
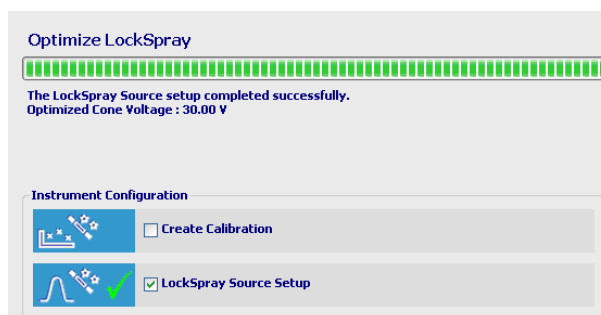
Figure 92 - Setup successful icon

16. The completed Lock mass profile is available for selection in the LockSpray window when creating new MS methods.

17. Update and save the sample list MS method Commission_Identity.exp to use the newly defined lock mass profile.

Acquiring sample data

1. Insert the vial of Mix 1 in position 1:A,1 (1:1) on tray 1 in the sample manager.

2. Place a vial of water with 0.1% formic acid (same as mobile phase A1) in position 1:B,1 (1:2) on tray 1 in the sample manager. This is used for blank injections.

3. Open the low pressure Identity^E inlet method (LP_Identity) in the inlet method editor and click ![icon] to download the method to the nanoACQUITY.

4. Allow the system pressure to stabilize on the binary solvent manager.

5. Select all 15 nanoACQUITY runs on the Commission_nanoflow sample list, and click ![icon] to start the acquisitions.

	File Name	File Text	MS File	Inlet File	Bottle	Inject Volume	Control
26		NanoAcquity Xevo QTof MS System Verification				0.000	
27						0.000	
28	Pos_NaCsl_cal	NaCsl, Manual Calibration	NaCsl_Cal	Default	1	0.000	
29	Blank_001	Blank Run	Blank	LP_Identity	1:2	2.000	
30	Identity_001	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.000	
31	Identity_002	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.000	
32	Identity_003	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.000	
33	Identity_004	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.000	
34	Identity_005	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.000	
35	Identity_006	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.000	
36	Identity_007	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.000	
37	Identity_008	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.000	
38	HP_Mix1_001	Commission High Pressure Test	Commission_HP	HP_Identity	1:1	2.000	
39	HP_Mix1_002	Commission High Pressure Test	Commission_HP	HP_Identity	1:1	2.000	
40	HP_Mix1_003	Commission High Pressure Test	Commission_HP	HP_Identity	1:1	2.000	
41	Blank_002	Blank run	Blank	LP_Identity	1:2	2.000	
42	Blank_003	Blank run	Blank	LP_Identity	1:2	2.000	
43						0.000	

Figure 93 - Example of a sample list

Processing the results

When all data have been acquired, process as described below to determine if the system test has passed successfully.

Table 16: Peptide values for test

ENOLASE	ADH	GPB	BSA
407.76	418.73	527.75	459.76
472.75	447.28	573.29	844.50

1. Highlight samples **Identity_004** to **Identity_008** in the MassLynx sample list.

2. Click **Chromatogram** to open the five consecutive acquisitions in the chromatogram display.

3. Ensure the display is set to **BPI** for function 1.

4. Record the retention times for each of the eight peptides as listed in Table 16, for all five acquisitions.

   NOTE: *The standard deviation of the retention times for each of the eight specified peptide masses must be <0.25 minutes.*

5. Record the results in the *Xevo QTof MS nanoACQUITY Installation Checklist* (p/n 715002121).

   NOTE: *For the BEH column, chromatographic peak width at half-height should typically be between 0.08 and 0.15 minutes.*

Identity^E specification (for PLGS installations only)

The specification is derived from a 2 µL infusion of Mix 1 sample (comprising ADH, GPB, enolase and BSA), which must meet the following criteria:

- Peptide coverage >25%
- Average precursor mass error <10 ppm

1. Open the PLGS browser.

2. Import the Identity^E (ZIP file) project from the CD into PLGS.

3. If the project is not available, create a new file; click **File** > **New Project**.
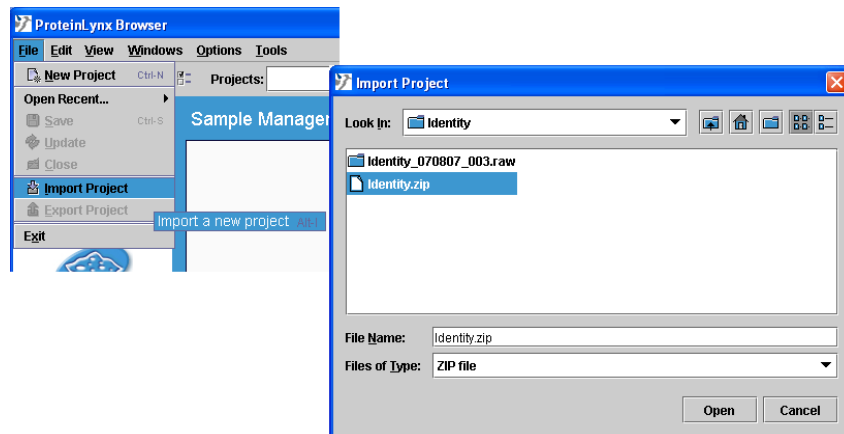
Figure 94 - Importing Identity.ZIP project

4. Click **Workflow Designer**, to access the Workflow Designer window.

5. Click **File** > **New** and select **Electrospray-MS^E** (Figure 95).
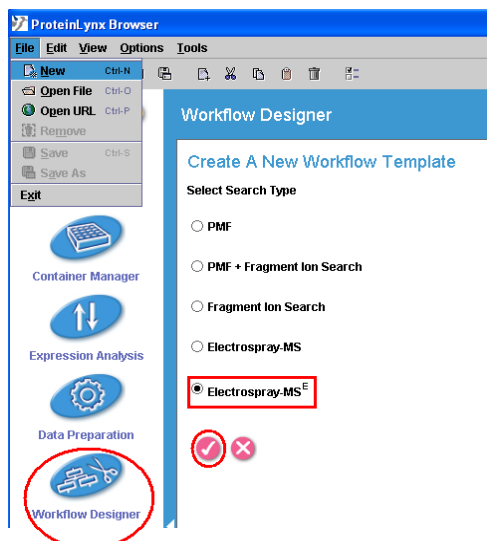
Figure 95 - Workflow Designer pop-up

6. Click the **tick** button.

7. Type an appropriate template name in the Title.

8. Right-click in the Workflow window.

9. Click **Workflow** in the right-hand window.

10. Right-click **Workflow**.

11. Click **Add** > **Databank Search** to open the Databank Search Query window.

12. Ensure the **Databank SWISSPROT – 1.0** is selected and the parameters are set as shown in Figure 96.
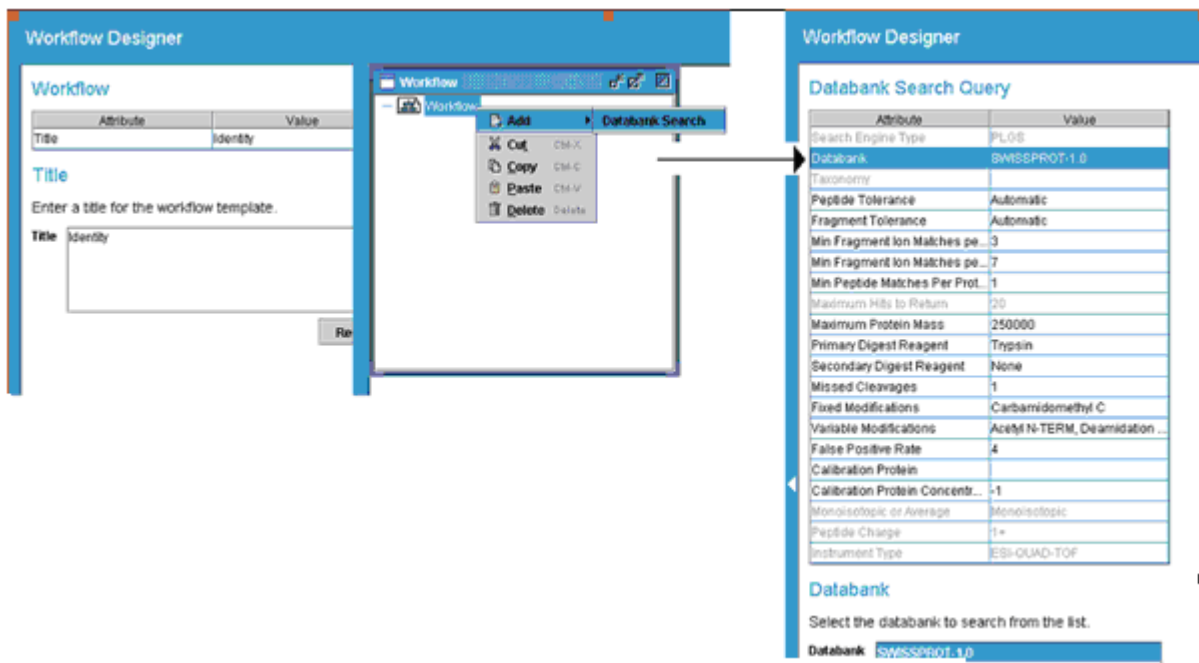
Figure 96 - Template parameters

13. Click **File** > **Save As** and give the template an appropriate name.
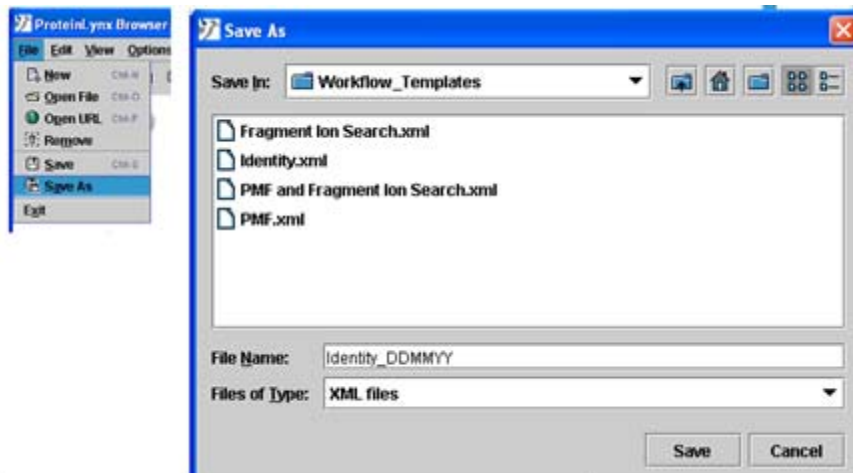
Figure 97 - Naming the workflow template

14. Click **Data Preparation** to access the Data Preparation window (Figure 98).

15. Click **File** > **New**.

16. Select the **Electrospray-MS^E** check box.

17. Click the **tick** button.

18. Type an appropriate name in the title box.

19. In the right-hand window, click **Apex3D**.

20. Ensure the Apex3D parameters are set as shown in Figure 98.
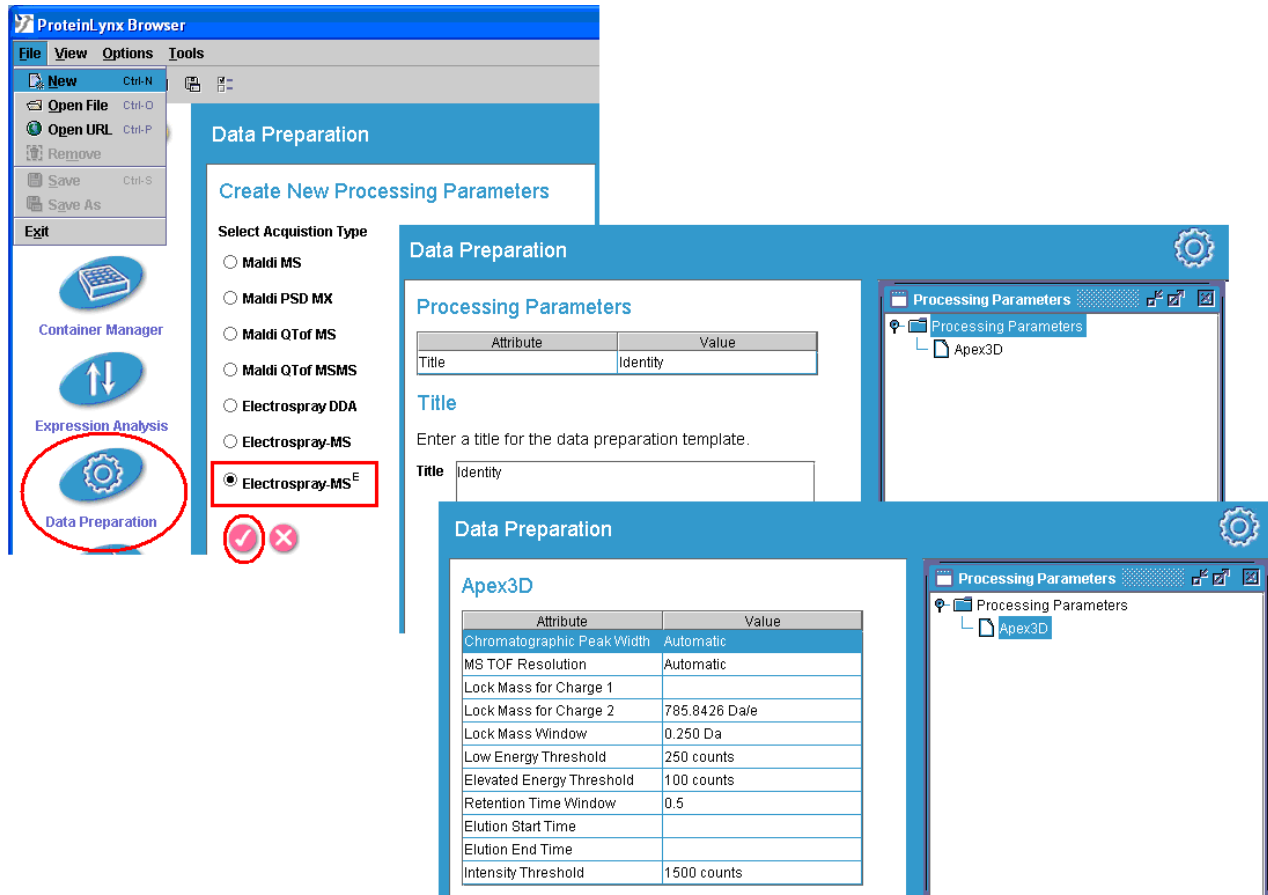
Figure 98 - Processing parameters

21. Click **File** > **Save As** and enter an appropriate name for the Processing Parameters (Figure 99).

22. Click **Save**.
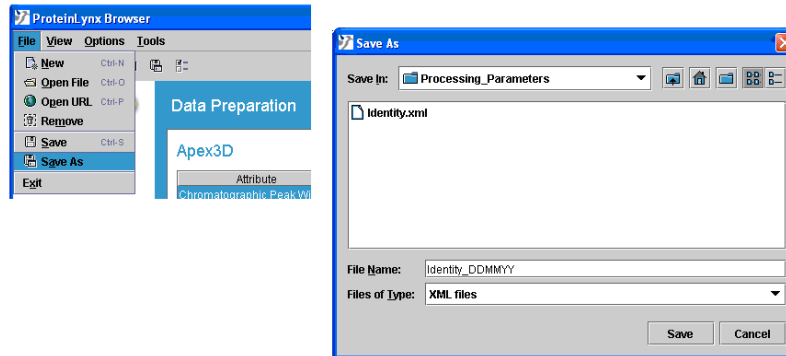
Figure 99 - Processing parameter

23. Click **Container Manager** on the left-hand side.

24. In the navigator tree, click **Microtitre Plate** (Figure 100).

25. Right-click and click **New Microtitre Plate**.

26. Type an appropriate name and click **OK**.
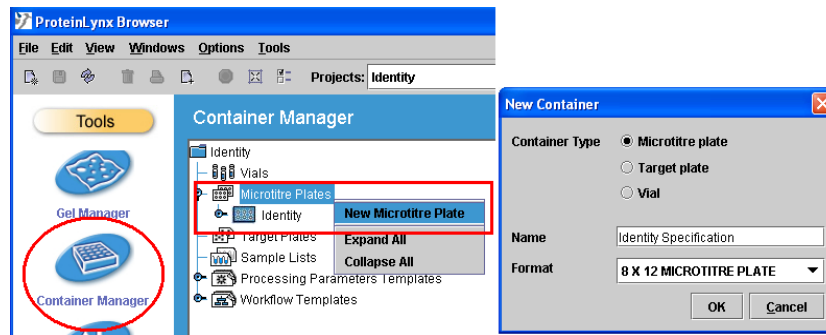
Figure 100 - Addition of a new microtitre plate

27. In the Container Manager window, click the name of the newly-created microtitre plate.

28. Click and drag from **A1** to **A5** (in the lower window) so that the spots turn black.

29. Right-click on one of the spots, and select **Set Raw Data File** (Figure 101).
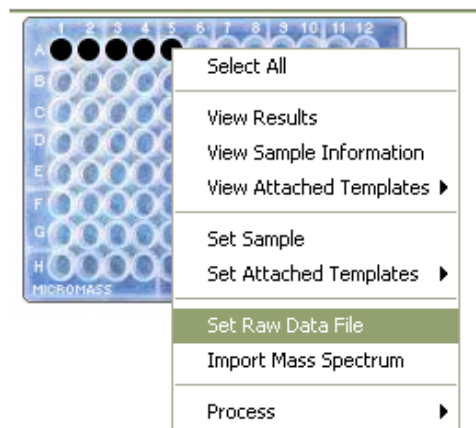
Figure 101 - Initiating raw data file

30. In the Select A Sample dialog box, click **Identity** (Figure 102).
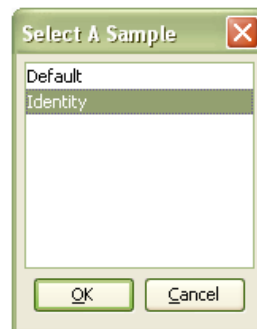
Figure 102 - Select a sample display

> **NOTE:**   *If you do not have the CD/DVD, click **Default**.*

31. Click **OK**.

32. Browse for the data files to be processed.

33. Select the last five runs **Identity_004** to **Identity_008** and click **Add** (Figure 103).

Figure 103 - Select files display

34. Click **Advanced>>** to access the templates (Figure 104).
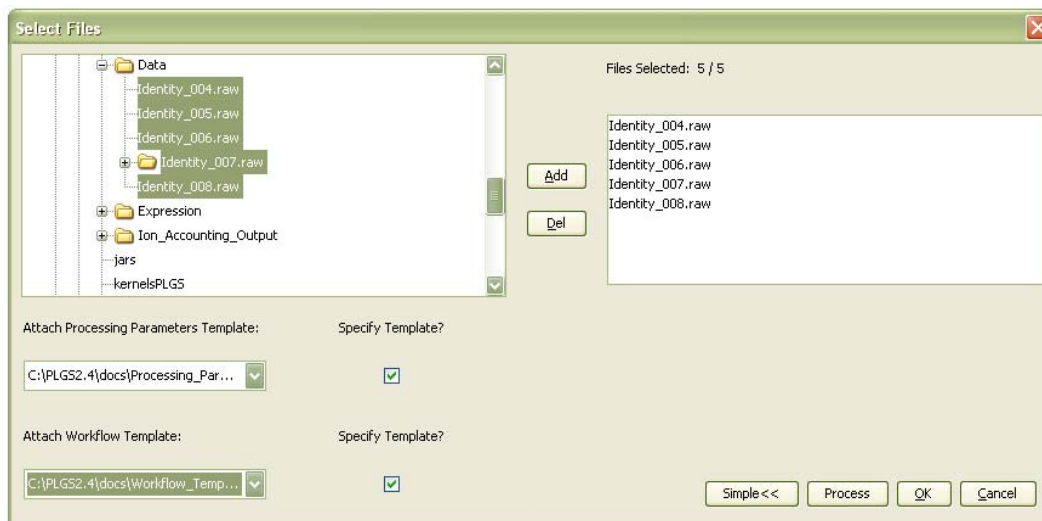
Figure 104 - Attaching processing parameters and workflow templates

35. Select the **Specify Template** check box for Attach Processing Parameters Template.

36. Click the drop-down list and select the template created in step 21 (Figure 104).

37. Select the **Specify Template** check box for Attach Workflow Template.

38. Click the drop-down list and select the template created in step 13 (Figure 104).

39. Click **Process**. The sample spots turn blue (Figure 105) as the data is processed.

> *NOTE: As the data are processed, the icons change for the workflow and spectrum. The color of each sample spot updates according to the search results; green if a match is found, otherwise blue.*

40. Once each data file has been processed successfully (and matches found), the spots turn green (Figure 105).
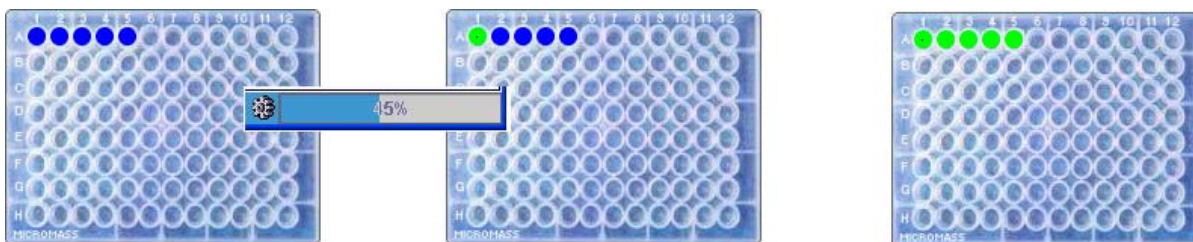
Figure 105 - Processed sample spots

41. Double-click the sample spot, or click the workflow results icon (Figure 106), to view the results.
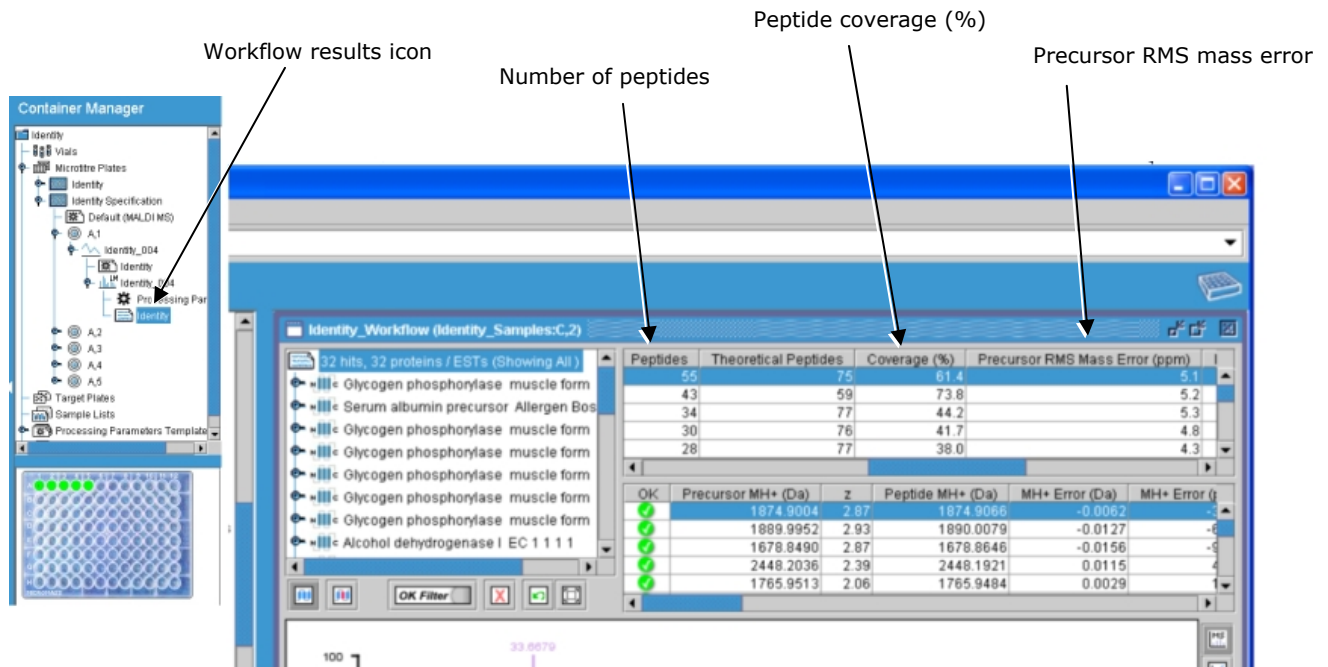
Figure 106 - Processed Mix 1 data using PLGS 2.3

42. For all data files, check that each of the four proteins has greater than 25% peptide coverage and the average mass error of the precursor mass is less than 10 ppm.

43. Record the results in the *Xevo QTof MS nanoACQUITY Installation Checklist* (p/n 715002121).

44. Use the following table to determine Accession and Entry values for the test proteins:

Table 17: Accession and corresponding Entry values

Accession	Entry
P00924	ENO1_YEAST
P02769	ALBU_BOVIN
P00489	PHS2_RABIT
P00330	ADH1_YEAST

nanoACQUITY high pressure test

> *NOTE:*   *This test is intended to exhibit high pressure operation. It is not intended to be used for the retention time reproducibility test. The MS source must be able to accommodate higher flow rates.*

1. Highlight one of the high pressure samples (10 to 12) from the sample list (see Figure 93).
2. Click **Chromatogram** to open the acquisition in the chromatogram.
3. Ensure the display is set to BPI.
4. Check that the eight peptides (refer to Table 16) are present.
5. Print the chromatogram and tick the box in the *Xevo QTof MS nanoACQUITY Installation Checklist* (p/n 715002121).

Additional source options

APCI Positive Ion mode MS specification

For all procedures in this section, ensure these conditions are met:

- Instrument is in Operate
- API and collision gases are turned on
- Tune page is set to Positive Ion mode

These settings appear as in Figure 107.

Figure 107 - Positive Ion mode icons

The procedures require the samples described in Table 18.

Table 18: APCI Positive Ion mode setup samples

Sample	Reservoir	Flow rate	Sprayer
100 pg/µL 17-α-hydroxyprogesterone	A	400 µL/min	Sample sprayer

> *NOTE:*   *The sample is infused into an LC mobile phase of 70:30 methanol/water flowing at 500 µL/min.*

The performance of the instrument is assessed using data from five repeat pseudo-loop (combined flow) injections, made into an LC flow, within a single tune page acquisition.

The average total combined intensity at m/z 331.23 from the repeat combined flow sample injections must be greater than 64,000 counts.

The maximum intensity variation between the five repeat injections must be less than 10 %RSD.

The combined spectrum intensity for the largest blank run must be less than 10% of the average total combined intensity.

Preparing test sample 100 pg/µL 17-α-hydroxyprogesterone

Preparing the mobile phase

1. Using a 1-L measuring cylinder, transfer 700 mL methanol and 300 mL water to a 1-L Duran.

2. Mix well and sonicate for two minutes.

Preparing the wash

1. Transfer 300 mL of mobile phase to a 500-mL Duran.

Preparing the test sample

1. Prepare a solution of 100 ng/µL 17-α-hydroxyprogesterone:

   NOTE: *This compound is based on Waters 17-α-hydroxyprogesterone sample kit (p/n 700002857).*

   a. Using a 1-mL pipette, add 3 mL mobile phase to the contents of the 5 mg ±0.1 mg vial of 17-α-hydroxyprogesterone supplied in the sample kit.

   b. Secure the cap, shake well, and transfer to 50-mL volumetric flask.

   c. Repeat twice more to ensure all contents are rinsed to the volumetric flask.

   d. Add a further 20-mL mobile phase to the volumetric flask.

   e. Inset the stopper, shake well and sonicate to ensure sample is fully dissolved.

   f. Make to the mark with the mobile phase.

   g. Label as "100 ng/µL 17-α-hydroxyprogesterone in 70:30 methanol/water".

2. Prepare a solution of 10 ng/µL 17-α-hydroxyprogesterone:

   a. Using a 1-mL pipette, transfer 1 mL of the 100 ng/µL solution to a 10-mL volumetric flask.

   b. Make up to the mark with the mobile phase.

   c. Secure the cap, shake well, and label as "10 ng/µL 17-α-hydroxyprogesterone in 70:30 methanol/water".

3. Prepare a solution of 1 ng/µL 17-α-hydroxyprogesterone:

   a. Using a 1-mL pipette, transfer 1 mL of the 10 ng/µL solution to a 10-mL volumetric flask.

   b. Make up to the mark with the mobile phase.

   c. Secure the cap, shake well, and label as "1 ng/µL 17-α-hydroxyprogesterone in 70:30 methanol/water".

4. Prepare the test sample:

   a. Using a 1-mL pipette transfer 2 mL of the 1 ng/µL solution to a 20-mL volumetric flask.

   b. Make up to the mark with mobile phase.

   c. Secure the cap, and shake well.

   d. Transfer the contents of the 20-mL volumetric flask to a 30-mL fluidics bottle.

   e. Label as "100 pg/µL 17-α-hydroxyprogesterone in 70:30 methanol/water".

LC/Fluidics configuration

1. Place fluidics wash lines into the wash solvent.

2. Set sample fluidics to the **Wash** reservoir, and purge lines.

3. Install the fluidics bottle containing 100 pg/µL 17-α-hydroxyprogesterone to position A.

4. Set sample fluidics to reservoir **A**, and purge.

5. Install the 1-L Duran containing the remaining mobile phase to LC, and purge as necessary.

6. Install the APCI probe and corona pin.

7. Connect the LC output to LC flow inlet on fluidics selector valve (center port).

8. Connect the LC outlet on fluidics selector valve to APCI probe.

MS configuration

1. Place the instrument in Positive Ion and MS mode.

2. Use the same system tuning parameters as those used for optimum resolution and sensitivity in ESI specifications.

3. Click **Source > APcI** from the MS Tune page menus.

4. Set the non-storage acquisition mass range from **100** to **1000**.

5. Set the initial source conditions as follows:

   • Probe temperature   **600 ºC**
   • Desolvation gas flow   **1000 L/hr**
   • Cone gas flow   **100 L/Hr**

Optimization

1. Switch the MS Tune page to System view to access the sample fluidics 'inject' function [icon].

2. Set LC (carrier flow of 70:30 methanol/water) to **500 μL/min**.

3. Click the **Fluidics** tab in MS Tune page.

4. Select reservoir **A** from the sample fluidics drop-down list.

5. Set sample fluidics flow state to **Combined**.

6. Enter a flow rate of **50 μL/min** and start infusion.

7. Optimize the source parameters to give the most intense/stable signal on the 17-α-hydroxyprogesterone peak at m/z 331.23.

   - Cone voltage   ~**30 V**
   - Corona current   ~**5 μA**
   - Probe position   ~ midway between corona pin and cone.

   NOTE: *The expected signal is approximately 500 counts/second in continuum mode after optimization.*

Test procedure

1. Set sample fluidics flow state to **LC**.

2. Select the **Wash** reservoir to purge lines with mobile phase blank.

3. Set sample fluidics flow rate to **400 μL/min**.

4. Set up a Tune page acquisition with the following parameters:

   - Source   APCI positive
   - Mass range   100 to 1000 Da
   - Scan time   0.5s
   - Data format   Centroid
   - Sensitivity   Normal
   - Duration   30 minutes

5. Start the MS Tune page acquisition.

6. Click the **Fluidics** tab in MS Tune page.

7. Click the **Inject** icon [icon] to perform three blank injections:

   - Allow ~60 seconds between injections
   - Refill the fluidics syringe between each injection.

8. After the three blank injections, set the sample fluidics to reservoir **A**.

9. Allow the purge cycle to complete.

10. Click the **Inject** icon to perform five combined flow sample injections:

   - Allow ~60 seconds between injections.
   - Refill the fluidics syringe between each injection.

Data Processing

1. Display the TIC of the blank and sample injections.

2. Click **Display > Mass** in the chromatogram window to create an extracted mass chromatogram of the observed analyte mass (331.23).

3. Combine the entire peak from the largest of the three blanks performed before the sample injections were made.

4. For each of the five sample injections, create a combined spectrum across the full injected peak width.

Results

The instrument passes the specification when the results meet the following criteria:

   - Average combined spectrum intensity for the five replicate injections must be greater than 64,000 ions
   - Maximum variation in intensity between the five replicates must be less than 10% RSD
   - The combined spectrum intensity for the largest blank must be less than 10% of the average combined spectrum intensity calculated above

   NOTE: *Record the results in the appropriate Xevo QTof MS installation checklist.*

APPI Positive Ion mode MS specification

For all procedures in this section, ensure these conditions are met:

- Instrument is in Operate
- API and collision gases are turned on
- Tune page is set to Positive Ion mode

These settings appear as in Figure 108.

Figure 108 - Positive Ion mode icons

The procedures require the samples described in Table 19.

Table 19: APPI Positive Ion mode setup samples

Sample	Reservoir	Flow rate	Sprayer
200 pg/µL cholesterol	A	400 µL/min	Sample sprayer

> *NOTE:*   *The sample is infused into an LC mobile phase of methanol flowing at 500 µL/min.*

The performance of the instrument will be assessed using data from five repeat pseudo-loop (combined flow) injections, made into an LC flow, within a single Tune page acquisition.

Preparing test sample 200 pg/µL cholesterol test solution in methanol

Preparing the mobile phase

1. Using a 1-L measuring cylinder, transfer 1000 mL methanol to a 1-L Duran.
2. Mix well and sonicate for two minutes.

Preparing the wash

1. Transfer 300 mL of mobile phase to a 500-mL Duran.

Preparing the test sample

1. Prepare a solution of 100 ng/µL cholesterol solution in methanol:

   > *NOTE:*   *This compound is based on Waters Cholesterol sample kit (p/n 700002820).*

   a. Using a 1-mL pipette, add 3 mL mobile phase (methanol) to the contents of the 5 mg ±0.1 mg vial of cholesterol supplied in the sample kit.
   b. Secure the cap, shake well, and transfer to 50-mL volumetric flask.
   c. Repeat twice more to ensure all contents are rinsed to the volumetric flask.
   d. Add a further 20-mL mobile phase to the volumetric flask.
   e. Inset the stopper, shake well, and sonicate to ensure sample is fully dissolved.

     f.   Make up to the mark with the mobile phase.

     g.   Label as "100 ng/µL cholesterol solution in methanol".

2. Prepare a solution of 2 ng/µL cholesterol solution in methanol:

     a.   Using a 1-mL pipette, transfer 1 mL of the 100 ng/µL solution to a 50-mL volumetric flask.

     b.   Make up to the mark with the mobile phase.

     c.   Secure the cap, shake well and sonicate.

     d.   Label as "2 ng/µL cholesterol solution in methanol".

3. Prepare the test sample:

     a.   Using a 1-mL pipette transfer 2 mL of the 2 ng/µL solution to a 20-mL volumetric flask.

     b.   Make up to the mark with mobile phase.

     c.   Secure the cap, and shake well.

     d.   Transfer the contents of the 20-mL volumetric flask to a 30-mL fluidics bottle.

     e.   Label as "200 pg/µL cholesterol test solution in methanol".

LC/fluidics configuration

1. Place fluidics wash lines into the wash solvent.

2. Set sample fluidics to the Wash reservoir, and purge lines.

3. Install the fluidics bottle containing 200 pg/µL cholesterol to position A.

4. Set sample fluidics to reservoir A, and purge.

5. Install the 1-L Duran containing the remaining mobile phase to LC, and purge as necessary.

6. Install the APPI source, APCI probe and corona pin.

7. Connect the LC output to LC flow inlet on fluidics selector valve (center port).

8. Connect the LC outlet on fluidics selector valve to APCI probe.

MS configuration

1. Place the instrument in Positive Ion and MS mode.

2. Use the same system tuning parameters as those used for optimum resolution and sensitivity in ESI specifications.

3. Click **Source > APPI** from the MS Tune page menus.

4. Set the non-storage acquisition mass range from 100 to 1000.

5. Set the initial source conditions as follows:

- Probe temperature   **600 ºC**
- Desolvation gas flow   **1000 L/hr**
- Cone gas flow   **100 L/Hr**

Optimization

1. Switch the MS Tune page to system view to access the sample fluidics 'inject' function ⬚.

2. Set LC (carrier flow of methanol) to 500 µL/min.

3. Click the **Fluidics** tab in MS Tune page.

4. Select reservoir **A** from the sample fluidics drop-down list.

5. Set Sample fluidics flow state to **Combined**.

6. Enter a flow rate of **100 µL/min** and start infusion.

7. Optimize the source parameters to give the most intense/stable signal on the cholesterol peak at m/z 369.35.

   - Cone voltage   ~**30 V**
   - Repeller voltage   ~**2 kV**
   - Probe position   ~ midway between corona pin and cone

   NOTE: *The expected signal is approximately 300 counts/second in continuum mode after optimization.*

Test procedure

1. Set sample fluidics flow state to **LC**.

2. Select the **Wash** reservoir to purge lines with mobile phase blank.

3. Set sample fluidics flow rate to 400 µL/min.

4. Set up a Tune page acquisition with the following parameters:

   - Source   APPI positive
   - Mass range   100 to 1000 Da
   - Scan time   0.5 s
   - Data format   Centroid
   - Sensitivity   Normal
   - Duration   30 minutes

5. Start the MS Tune page acquisition.

6. Click the **Fluidics** tab in MS Tune page.

7. Click the **Inject** icon ⬚ to perform three blank injections:

   - Allow ~60 seconds between injections.
   - Refill the fluidics syringe between each injection.

8. After the three blank injections, set the Sample fluidics to reservoir **A**.

9. Allow the purge cycle to complete.

10. Click the **Inject** icon [icon] to perform five combined flow sample injections:

- Allow ~60 seconds between injections.
- Refill the fluidics syringe between each injection.

Data Processing

1. Display the TIC of the blank and sample injections.

2. Click **Display > Mass** in the chromatogram window to create an extracted mass chromatogram of the observed analyte mass (369.35).

3. Combine the entire peak from the largest of the three blanks performed before the sample injections were made.

4. For each of the five sample injections, create a combined spectrum across the full injected peak width.

Results

The instrument passes the specification when the results meet the following criteria:

- Average combined spectrum intensity for the five replicate injections must be greater than 16,000 ions
- Maximum variation in intensity between the five replicates must be less than 10% RSD

The combined spectrum intensity for the largest blank must be less than 10% of the average combined spectrum intensity calculated above.

TRIZAIC Positive Ion mode MS specification

> **CAUTION:** *Specifications must be performed with the NanoLockSpray source prior to performing any TRIZAIC specifications.*

For all procedures in this section, ensure these conditions are met:

- Instrument is in Operate
- API and collision gases are turned on
- Tune page is set to Positive Ion mode

These settings appear as in Figure 109*.*

Figure 109 - Positive Ion mode icons

Perform the procedure in the "IdentityE specification" section.

Initial setup

1. Attach the TRIZAIC source to the MS.

   > **NOTE:** *For the correct procedure on how to achieve this, refer to TRIZAIC UPLC nanoTile: Care and User Guide (p/n 715002423).*

2. Switch the sample manager off.

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

3. Connect the TRIZAIC power cable to the QSPI connection of the nanoACQUITY UPLC sample manager.

4. Switch the sample manager on.

5. Click **Source > Trizaic**.

6. Place the instrument in Positive Ion, Sensitivity mode.

7. Use the system tuning parameters as those used for optimum resolution and sensitivity in the "Positive Ion mode MS system specification" section.

8. Connect the source injection port and the instrument inject valve, using the transfer line (p/n 430002351).

9. Connect the source trap port and the instrument trap valve, using the transfer line (p/n 430002455).

   > **NOTE:** *Refer to the "nanoACQUITY tubing connections and set pressure diagnostics" section for guidance on making the TRIZAIC connections.*

10. Insert the infusion tile and connect the capillary outlet (on the IntelliStart fluidics) to the Calibration port.

11. Infuse [Glu^1]-fibrinopeptide B at 0.5 µL/min and optimize the stage position.

12. Once the stage has been optimized for maximum sensitivity, turn off the capillary voltage and stop the [Glu^1]-fibrinopeptide B infusing.

13. Replace the infusion tile with the nanoTile.

14. Access the nanoACQUITY console and click **Binary Solvent Manager**.

15. Set the flow rate to **8 µL/min** in Trapping mode at **85% B** for 5 minutes.

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

16. Stop the flow and switch to Analytical mode.

17. Set the flow rate to **0.45 µL/min** at **85% B** and allow the column to flush for 5 minutes.

18. Stop the flow and switch to Trapping mode.

19. Set the flow rate to **8 µL/min** at **50% B** and leave to flush for 5 minutes.

20. Stop the flow and switch to Analytical mode.

21. Set the flow rate to **0.45 µL/min** at **50% B** and leave to flush for 5 minutes.

22. Stop the flow and switch to Trapping mode.

23. Set the flow rate to **8 µL/min** at **3% B** and leave to flush for 5 minutes.

24. Stop the flow and switch to Analytical mode.

25. Set the flow rate to **0.45 µL/min** at **3% B** and leave to flush for 5 minutes.

26. Record the backpressure achieved at 3% B.

27. Set the capillary voltage between **3.0 to 3.5 kV** to ensure that a beam is visible and adjust the capillary voltage as necessary to achieve a suitable signal.

> *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.***

28. Set the Tune page parameters as shown in Table 20.

Table 20: Typical Tune page parameters (TRIZAIC configuration)

Parameter	Value
Capillary voltage	3.0 to 3.5 kV
Sampling cone*	30 V
Extraction cone*	4 V
Source temperature	70 °C
Cone gas	Tune as required
Nanoflow gas	Tune as required
Purge gas	0 L/h

> *NOTE:*   ** Tune these voltages to prevent in-source fragmentation.*

nanoACQUITY UPLC method

1. Access the inlet method editor, and click [Inlet icon], to open the binary solvent manager method editor.

2. Create an inlet method using the parameters shown in Table 21.

Table 21: Binary solvent manager gradient values

	Time (mins)	Flow (µL/min)	%A	%B	Curve
1	Initial	0.45	97.0	3.0	Initial
2	1.0	0.45	97.0	3.0	6
3	30.0	0.45	60.0	40.0	6
4	35.0	0.45	15.0	85.0	6
5	40.0	0.45	15.0	85.0	6
6	45.0	0.45	97.0	3.0	6

3. Click [...] to customize a mobile phase name.

4. Click the **Trapping** tab and set the parameters in Table 22.

Table 22: Binary solvent manager trapping conditions

Parameter	Value
Run time	70 min
Flow rate	8 µL/min
%A1	100%
%B1	1%
Pressure limit (low)	0 psi
Pressure limit (high)	9000 psi
Sample loading time	2 min

5. Access the auxiliary solvent manager Lock mass flow parameters.

6. Enter a flow rate of 0.5 µL/min for pump B1.

7. Click the **Autosampler** icon to open the sample manager method editor.

>   *CAUTION:*   *If there is no ASM available, ensure that the reference fluidics are flowing at 0.5 µL/min to provide at least 150 counts per second on the precursor m/z 785.8.*

8. Create an autosampler method using the parameters in Table 23.

Table 23: 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)	1800 µL
Wash solvents (strong)	100% Acetonitrile + 0.1% Formic Acid
Wash solvent volume (strong)	200 µL
nanoTile Temperature	35 °C
Sample temperature	8 °C

9. In the Autosampler window, click **Advanced**.

10. 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.*

11. In the inlet editor, click **File > Save As**, and save the inlet method using the format CFG_ddmmyy (where ddmmyy = date).

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

MS method

Refer to the "Commission_Identity MSE method" section (Appendix H: Setting up inlet methods manually).

Performance tests

Refer to the "Acquiring sample data" section for details of how to acquire sample data and subsequent processing.

Where the system is an Identity^E system, the Identity^E specification will need to be performed. Refer to the "Processing the results" section for more details.

Appendix A: Installing the Varian external oil mist filter

1. Remove the gas ballast valve knob has shown in Figure 110.

Oil fill plug   Suction flange

Exhaust flange   Gas ballast valve knob
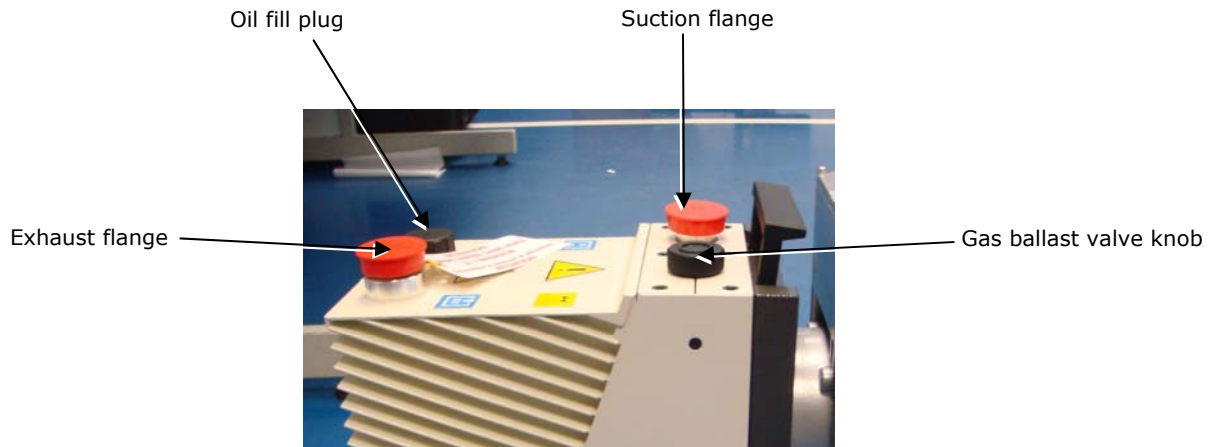
Figure 110 - Top view of the Varian rotary pump.

2. Screw in the brass oil-return fitting (Figure 111). This is provided in the pump kit.

Figure 111 - Installed oil return fitting on the Varian rotary pump

3. Turn the Varian external filter upside down.
4. Remove the bolt from the bottom of the external filter.

5. Screw in the chrome connection with 90° elbow and 6-mm push fit into the bottom of the external filter (Figure 112).
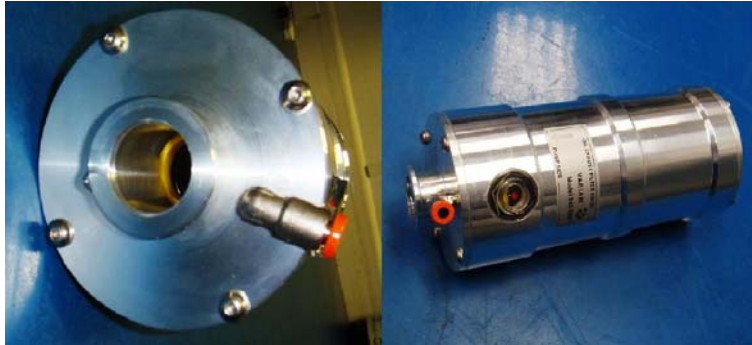
Figure 112 - The push fit connection installed to the Varian oil mist filter

6. Take the Varian external filter and install onto the exhaust flange of the rotary pump.

Figure 113 - Varian external filter fitted to Varian rotary pump

> ***NOTE:***   *The window is at the bottom when the external filter is fitted.*

7. Connect the 6-mm tubing from the external filter to the oil return fitting.

> ***NOTE:***   *Ensure that the pump location allows the glass window in the external filter, and the window for the pump oil level to be viewed.*

Appendix B: Instrument tuning

The following section provides a brief introduction to the instrument tuning parameters. The Tune page is used in two modes.

Normal view

This is the usual operational mode. When carrying out the performance specifications place the Tune page in this view wherever possible. In this mode, the tuning parameters for the source, instrument, and fluidics tabs are available.

System view

This mode is required when instrument setup is checked and allows the quad setup window to be accessed.

The tuning parameters for the Transfer lens and TOF are available on the System 1 and System 2 tabs. Additional functionality is available:

- Extra tuning sliders on the instrument tab - HM resolution, Aperture 1, Pre-filter and Ion Energy
- Access to RF Settings and Acquisition Setup dialog boxes.

The password to enable System view is **access** (case sensitive).

The tuning parameters on the Source ES window are used to optimize the ionization in the source and control the introduction of ions into the instrument. They must be tuned for maximum sensitivity for each sample and infusion conditions. These parameters do not influence TOF resolution except when the detector saturation limit has been exceeded, in which case the capillary voltage should be used to control the ion count.

The following tables provide the default values which are loaded when MassLynx is first installed. The default values apply to both Positive and Negative Ion modes.

Table 24: Source ES tab parameters

Parameter	Default value	Description
Capillary (kV)	3.0	The high voltage is applied to the nebulizing capillary Used to control the ion count to the MCP detector Maximum sensitivity usually around 3 to 3.5 kV in positive ion, lower (~2 kV) in negative ion
Sampling Cone	45	The DC voltage applied to the sampling cone Tune for maximum sensitivity depending on m/z Expected to tune in region 35 to 100 V
Extraction Cone	5	The DC voltage applied to the extraction cone Tune for maximum sensitivity depending on m/z Expected to tune in region 1 to 5 V
Source Temperature	80	The temperature to which the ion block is heated Tune for maximum sensitivity depending on infusion rate and solvent type Expected to tune 80 to 100 °C
Desolvation Temperature	150	The temperature to which the solvent and gas is heated at the nebulising capillary Tune for maximum sensitivity depending on infusion rate and solvent type Expected to tune 150 to 300 °C
Cone Gas	0	The flow rate of the gas that is applied at the sampling cone Tune for maximum sensitivity depending on infusion rate and solvent type Expected to tune 0 to 50 L/h
Desolvation Gas	500	The flow rate of the gas that is applied at the nebulising capillary Tune for maximum sensitivity depending on infusion rate and solvent type Expected to tune 300 to 1000 L/h

The tuning parameters on the Instrument window are used to optimize the passage of the ions through the rail section of the instrument.

Table 25: Instrument tab parameters (Normal view)

Parameter	Default value	Description
Collision Energy	OFF =6 ON=30	Controls the potential at which the ions enter the collision cell Fragment ions depending on charge state (less potential required for higher charge states) and molecular strength Typically set to OFF in TOF MS and Quad modes
Detector	0	The voltage applied across the MCP detectors Must be set to count all ions Set to the value determined in the detector gain test or adjust for different charge states as long as no drop in sensitivity is observed
LM Resolution	4.7	Sets the width of the quad transmission window in Quad and TOF MS/MS modes

Table 26: Instrument tab parameters (System view)

Parameter	Default value	Description
HM Resolution	15	Sets the width of the quad transmission window in Quad and TOF MS/MS modes Ensure set to 15
Aperture 1	0	Voltage applied to the aperture plate of source ion guide Expected to tune between 0 and 2
Pre-Filter	10	The DC voltage applied to the pre and post filters of the quad Set to 10 V
Ion Energy	1	The offset voltage applied to the quadrupole Set to 1 (pos) and 1.8 (neg) for TOF MS (greater than 0 V) and for good peak shape in Quad mode (less than 0 V)

CAUTION: Do not tune the pre-filter parameter.

The tuning parameters on the System 1 window are used to optimize the transmission of the ions through the transfer lens and within the TOF analyzer. They are very important parameters for tuning TOF resolution and sensitivity.

Table 27: System 1 tab parameters (System view only)

Parameter	Default value	Description
Acceleration 1*	60	The voltage applied to the first ring lens after the transfer cell exit plate Tune for sensitivity and resolution
Acceleration 2*	200	The voltage applied to the second ring lens after the transfer cell exit plate
Aperture 2*	50	The voltage applied to the differential pumping aperture between the analyzer and TOF chambers
Transport 1*	60	The voltage applied to the first split lens after Aperture 2
Transport 2*	60	The voltage applied to the second split lens after Aperture 2
Steering	0	Offsets the voltage on either side of the Transport 2 split lens Tune for maximum sensitivity and resolution. Will optimize between +1 and -1 V
Tube Lens	55	The voltage applied to the final lens before the pusher stack Tune if required
Pusher*	1900	The voltage height of the pusher pulse
Pusher Offset	0	The offset voltage applied to the pusher plate Tune for maximum resolution on the TOF Expected to tune between +3 and −3 V
Puller	1800	The voltage height of the puller pulse Tune for TOF resolution Will tune within 50 V of default; always tune lower than the pusher voltage

CAUTION: *Do not tune these parameters.*

Table 28: System 2 tab parameters (System view only)

Parameter	Default value	Notes
Collector	50	Do not adjust these values
Collector Pulse	10.0	
Stopper	10	
Stopper Pulse	20.0	
Entrance	50	
CC Static Offset	120	
Puller Offset	0.00	
Reflectron Grid	1.460	Tune for best TOF resolution using 0.001 increments
Flight Tube	7.90	Do not adjust these values
Reflectron	1.60	

Appendix C: Tuning for TOF resolution

The following flow chart describes the process used to tune the TOF for best resolution. The chart applies to both positive and negative modes. The process assumes that the Tune page is set up with the default settings or with the working tune file. Use the guidance given in "Appendix B: Instrument tuning" to tune the lenses if necessary.

```
                    ┌─────────────────┐
                    │  Infuse Sample  │
                    └────────┬────────┘
                             │
                    ┌────────▼─────────────┐
                    │ Optimize source ES   │
                    │ parameters for       │
                    │ sample ionization    │
                    └────────┬─────────────┘
                             │
              ┌──────────────▼───────────────┐
              │ Optimize the following for   │
         ┌───▶│ best peak shape, resolution  │◀───┐
         │    │ and sensitivity:             │    │
         │    │                              │    │
         │    │     Reflectron grid          │    │
         │    │     Pusher offset            │    │
         │    │     Puller                   │    │
         │    │     Tube lens                │    │
         │    │     Steering                 │    │
         │    │                              │    │
         │    │ Several iterations may be    │    │
         │    │ required                     │    │
         │    └──────────────┬───────────────┘    │
         │                   │                     │
         │              ┌────▼────┐     NO   ┌──────────────┐
         │              │Ion count│─────────▶│   Reduce     │
         │              │<200 cps?│          │  capillary   │
         │              └────┬────┘          │   voltage    │
         │                   │ YES           └──────────────┘
         │              ┌────▼────┐
         │      NO       │Resolution│
         └───────────────│achieved? │
                         └────┬────┘
                              │ YES
                    ┌─────────▼─────────┐
                    │      Finish       │
                    └───────────────────┘
```
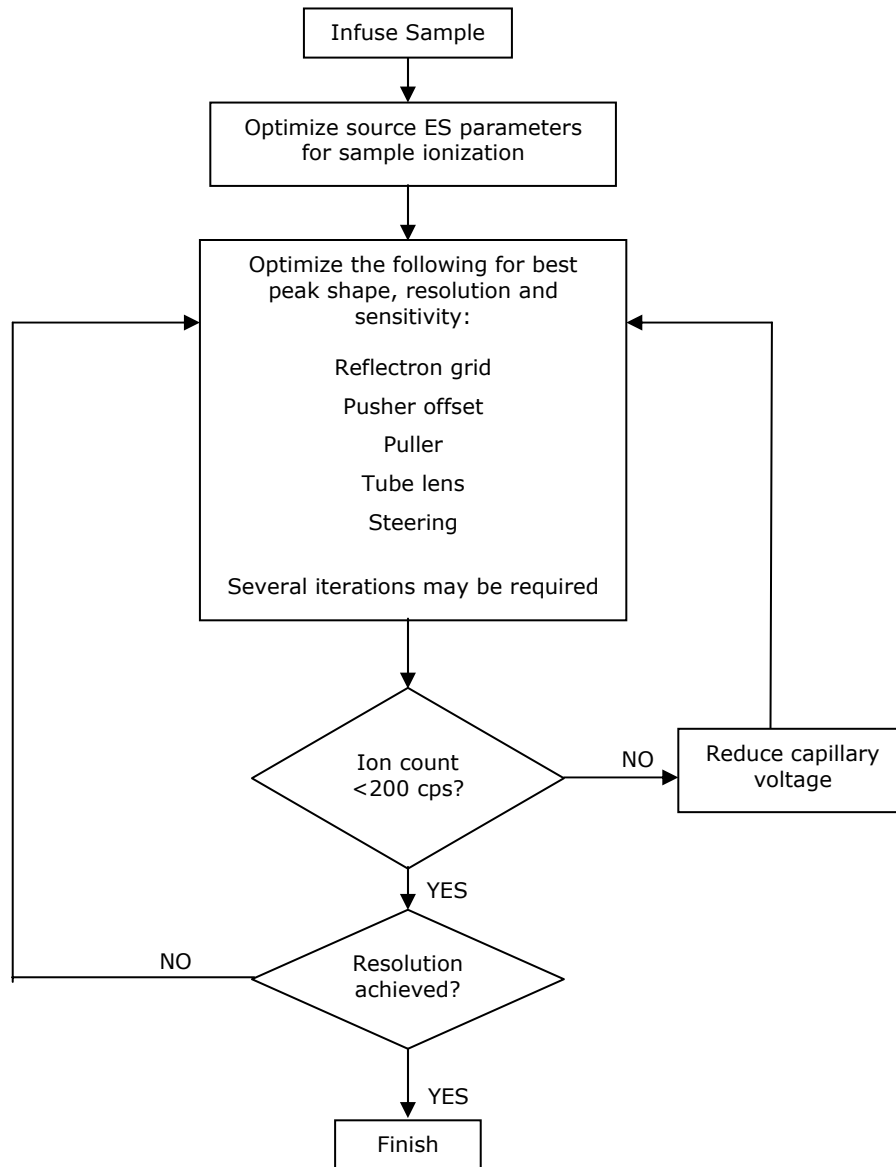
Appendix D: LockSpray source setup

> *NOTE:*   *The LockSpray sample ionization can be automatically or manually optimized using the IntelliStart software. The following procedure sets up the LockSpray automatically.*

Configure LockMass profile

The LockMass profile must first be configured so the software can identify what is to be optimized.

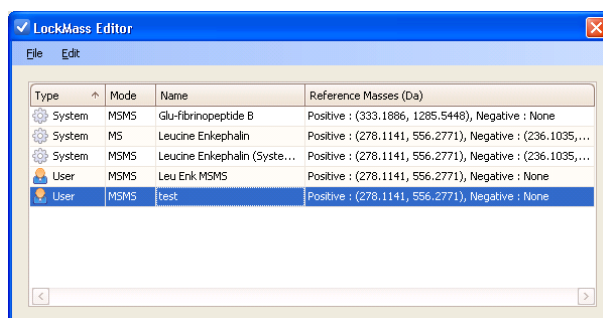8. From the MS Console, click **Configure > LockMass Editor**.

Figure 114 - LockMass Editor display

9. To create a new LockMass, click **File > New**.

10. In the new LockMass window, enter a name for the LockMass profile being created.

11. Choose the reference compound on which the profile is to be based (recommended), or specify a LockMass manually (advanced).

12. Select the acquisition mode (MS or MS/MS) in which the LockMass profile is intended to be used.

13. Select which peak (or peaks, for multipoint LockMass), is to be used as a reference.

14. If multipoint LockMass is in use, set collision energy to a suitable value to create fragments if required.

15. The LockMass dialog box is similar to Figure 115.
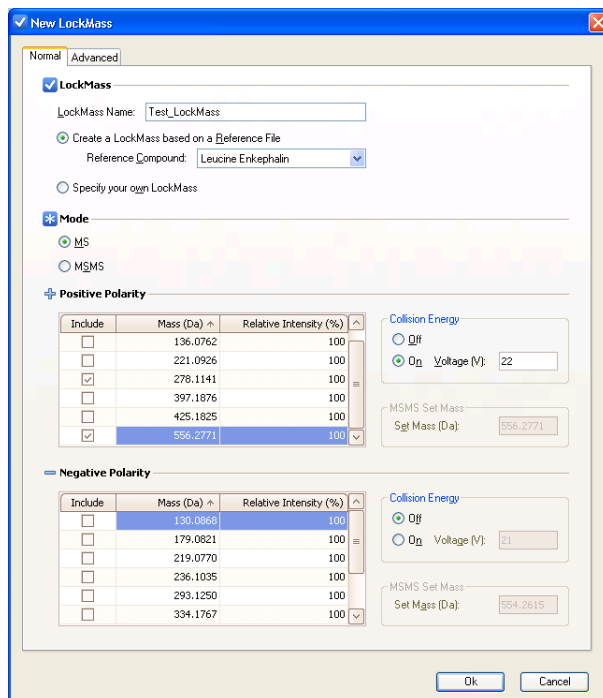
Figure 115 - LockMass dialog box

16. Click **OK**.

> *NOTE:*   *The LockMass profile created is available to select within the LockSpray source setup.*

Automatically optimizing lockmass

Once the LockMass has been configured, it can be automatically optimized by performing the following procedure.

1. Open the MS Console, and select the IntelliStart page.

2. Click **Configure > Configuration Mode**.

3. Click **LockSpray Source Setup**, then **Start**, to open the IntelliStart window.

4. Click **Next** to continue to the first part of the optimization process.

5. From the drop-down list, select the LockMass compound to set up, and click **Next**.

> *NOTE:*   *The Setup Type window offers three options:*

- Automatic – adjusts the source conditions automatically to optimize the LockSpray intensity
- Manual – allows manual values to be entered for the source conditions
- Tune Page – uses the source conditions currently entered in the Tune Page.

6. Select the **Automatic** check box.

7. Click **Next**.

> *NOTE:*   *The display allows a selection of how the reference fluidics are controlled. The options are:*

- Automatic – sets reference fluidics to flow at 50 µL/min (ESI) or 0.5 µL/min (NanoFlow)
- Custom – allows a manual value to be entered for the reference fluidics flow rate
- Manual – uses the reference fluidics flow rate currently set in the Tune page

8. Select the **Automatic** checkbox.

9. Click **Next**.

> *NOTE:*   *The system is now ready to optimize the source conditions for the LockMass profile specified.*

10. Ensure vial B contains the correct lockmass sample.

11. Click **Start**.

The IntelliStart software automatically sets up the source conditions. The values chosen are written automatically into the LockMass profile. This means that whenever the profile is used in an acquisition, these parameters are set on the LockMass sprayer.

> *NOTE:*   *The values saved in the LockMass profile can only be overwritten by repeating the whole of this procedure.*

When successfully completed, the MS Console displays the green check mark (Figure 116). The completed LockMass profile is available for selection in the LockMass window when creating new MS methods.

Figure 116 - Confirmation that source conditions are set up

Appendix E: ESI method parameters

The following methods are part of the MassLynx software and do not usually require any alteration when being used to perform the MS System Specifications on installation. The methods are initially located at: C:\MassLynx\Commission.pro\Acqudb and are:

- Commission_Pos
- Commission_Neg

Commission_Pos

TOF MS/MS Function 1

- **Acquisition**
  Duration   0 to 5 minutes
  Select   **ES Source**, **Positive Mode**
  Select   **Normal Dynamic Range & Sensitivity**.
- **TOF MS/MS**
  Parameters   150 to 1500 Da, Set Mass=785.8, Scan Time=1 sec
  Select   **Continuum**
  Deselect   **Override & Ramp Sample Cone**.
- **CE Control**
  Deselect   **Use Collision Energy**

TOF MS/MS Function 2

- **Acquisition**
  Duration   0 to 5 minutes
  Select   **ES Source**, **Positive Mode**
  Select   **Normal Dynamic Range & Sensitivity**.
- **TOF MS/MS**
  Parameters   150 to 1500, Set Mass=785.8, Scan Time=1 sec
  Select   **Continuum**
  Deselect   **Override & Ramp Sample Cone**.
- **CE Control**
  Select   **Use Collision Energy**
  Set   **Fixed Collision Energy Value** = 30.

LockSpray

- Select **Acquire LockSpray data with reference compound**
- From **LockSpray Reference Compound**
  select **Leucine Enkephalin (system test) lock mass profile**.
- Set **Acquisition Settings** - 1 sec scan, 10 sec interval and 3 scans to average.
- **Source settings** are already optimized and recorded when the LockSpray source setup is run in the MS Console.

  NOTE: *These can only be edited by running the LockSpray source setup again.*
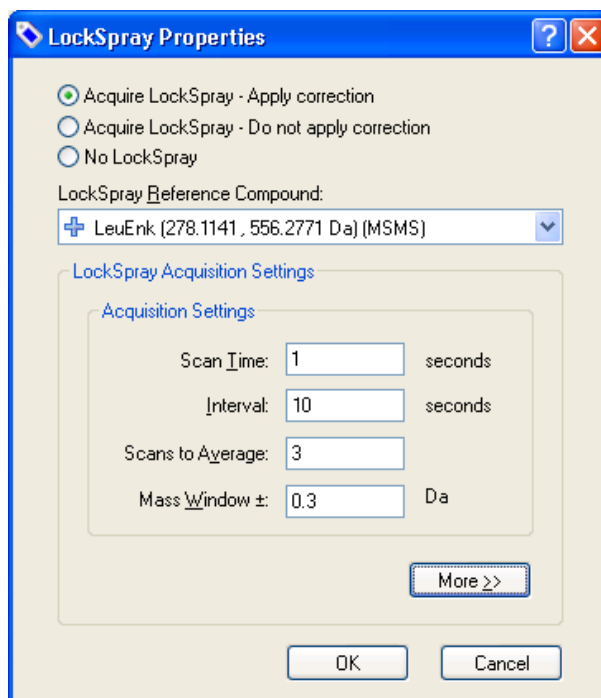
Figure 117 - LockSpray properties dialog box

Method Events

Check that **Method Events** are disabled.

Commission_Neg

TOF MS/MS Function 1

- **Acquisition**
  Duration   0 to 5 minutes
  Select   **ES Source**, **Negative Mode**
  Select   **Normal Dynamic Range & Sensitivity**.
- **TOF MS/MS**
  Parameters   150 to 1000, mass=503.2, scan=1 sec
  Select   **Continuum**
  Deselect   **Override & Ramp Sample Cone**.
- **CE Control**
  Select   Use Collision Energy
  Set   Fixed Collision Energy Value = 22

LockSpray

Select **No LockSpray.**

Method Events

Check that **Method Events** are disabled.

Appendix F: Using the Windows scientific 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 118, referring to the other calculator for the average and standard deviation.

$$\% RSD = \frac{(StdDev \times 100)}{Average}$$

Figure 118 - %RSD equation

Calculating the RMS mass error

1. Use the following formula to calculate RMS mass error

$$RMS = \sqrt{\frac{\sum (MassError)^2}{n}}$$

Figure 119 - RMS equation

Appendix G: nanoACQUITY UPLC system test resources

Illustrations of displays which appear during the nanoACQUITY verification test:

	File Name	File Text	MS Method	Inlet File	Vial	Vol
1	blank_001	Blank run	blank	LP_Identity	1:2	2.0
2	Identity_001	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.0
3	Identity_002	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.0
4	Identity_003	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.0
5	Identity_004	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.0
6	Identity_005	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.0
7	Identity_006	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.0
8	Identity_007	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.0
9	Identity_008	Commission Identity Test	Commission_Identity	LP_Identity	1:1	2.0
10	HP_Mix1_001	Commission High Pressure Test	Commission_HP	HP_Identity	1:1	2.0
11	HP_Mix1_002	Commission High Pressure Test	Commission_HP	HP_Identity	1:1	2.0
12	HP_Mix1_003	Commission High Pressure Test	Commission_HP	HP_Identity	1:1	2.0
13	blank_002	Blank run	blank	LP_Identity	1:2	2.0
14	blank_003	Blank run	blank	LP_Identity	1:2	2.0

Figure 120 - Illustration of a MassLynx sample list
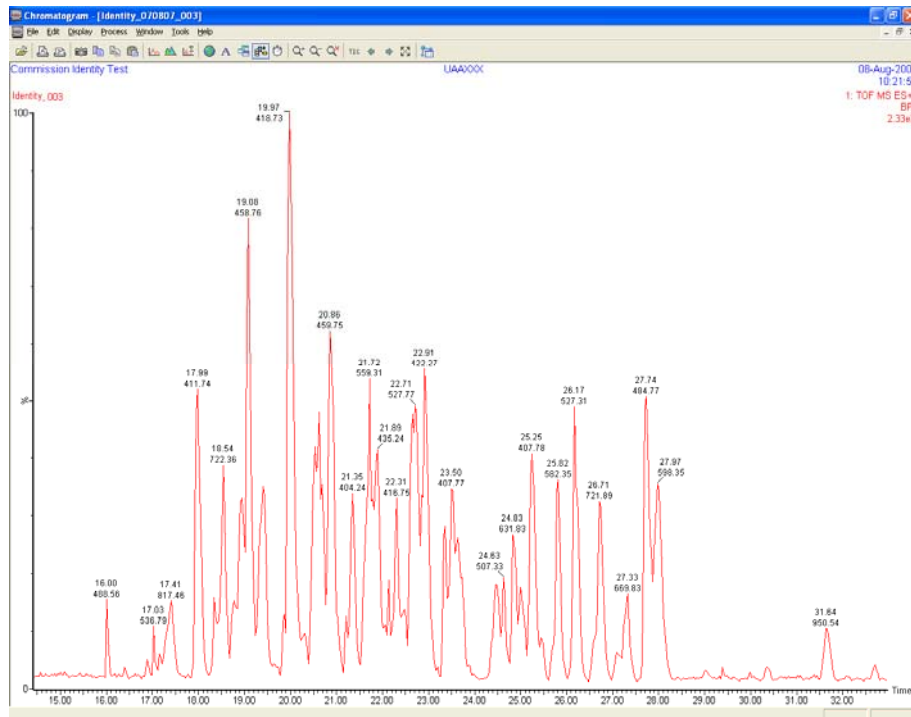
Figure 121 - Example of a chromatogram at low pressure
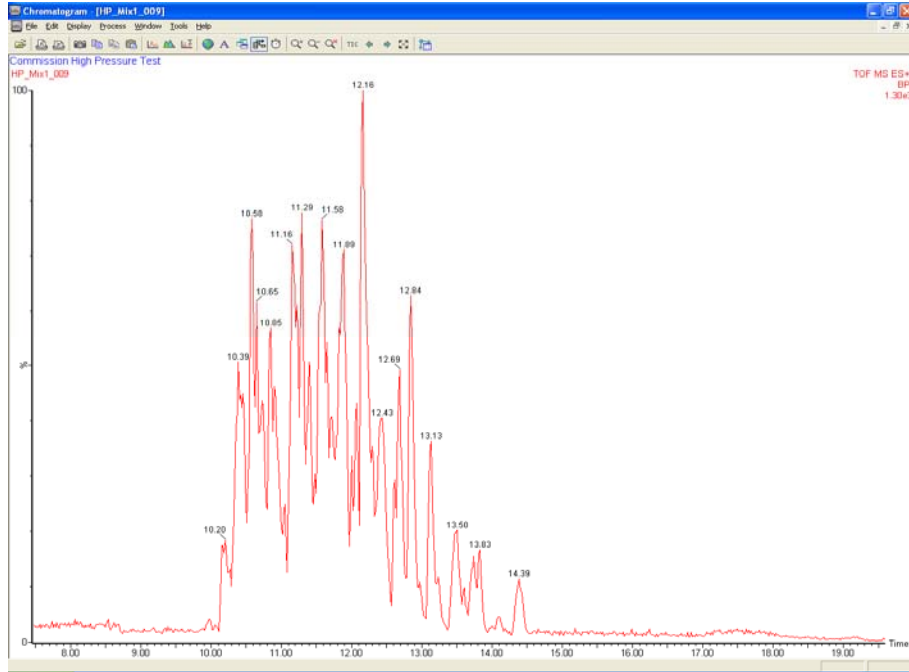
Figure 122 - Example of a chromatogram at high pressure

Appendix H: Setting up inlet methods manually

Where no predefined projects have been supplied on CD/DVD, set up the methods manually using the following displays.

Low pressure inlet method

Figure 123 - nanoACQUITY Binary Solvent Manager - General tab

Figure 124 - nanoACQUITY Binary Solvent Manager - Trapping tab
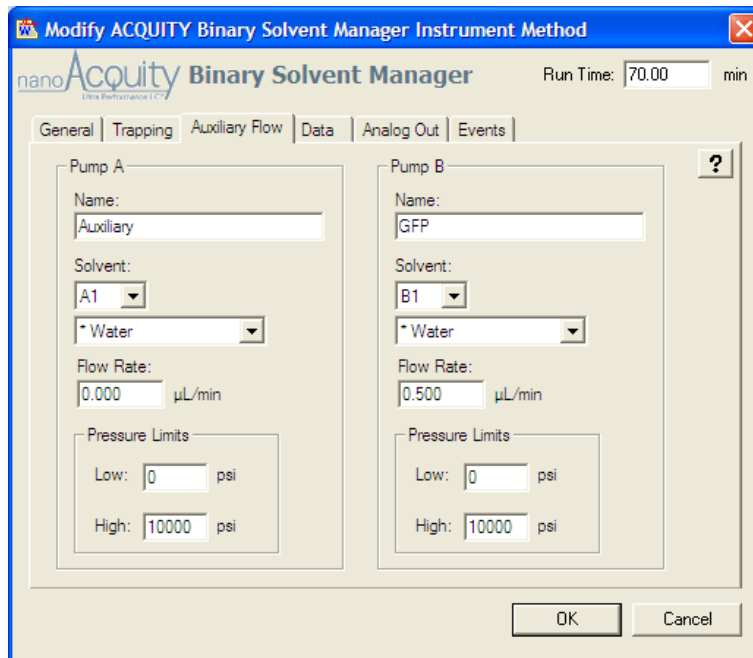
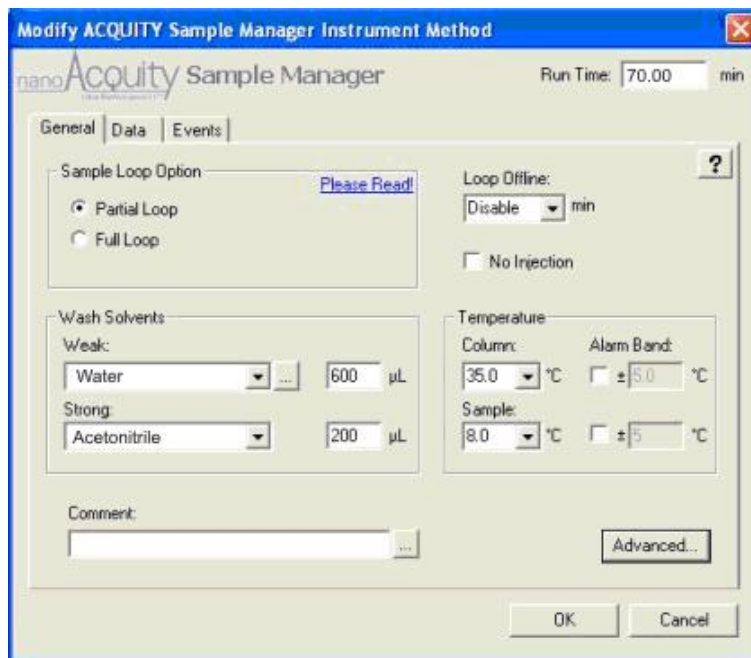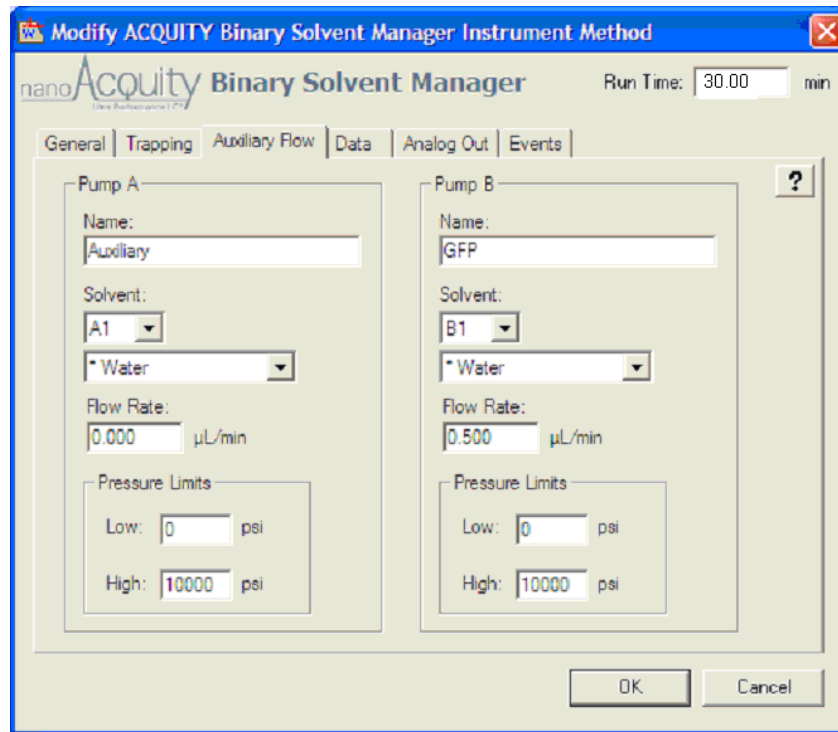*Figure 125 - nanoACQUITY Binary Solvent Manager - Auxiliary Flow tab*

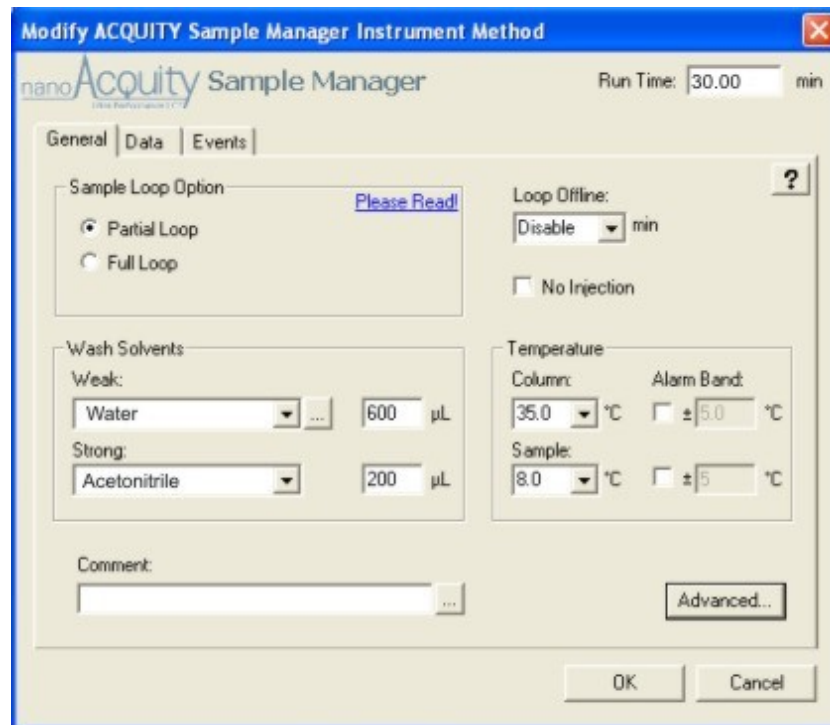*Figure 126 - nanoACQUITY Sample Manager - General tab*

High pressure inlet method

Figure 127 - nanoACQUITY Binary Solvent Manager - General tab

Figure 128 - nanoACQUITY Binary Solvent Manager - Trapping tab

Figure 129 - nanoACQUITY Binary Solvent Manager - Auxiliary Flow tab

Figure 130 - nanoACQUITY Sample Manager - General tab

Commission_Identity MS^E method

1. Accesss the Experiment Setup window.
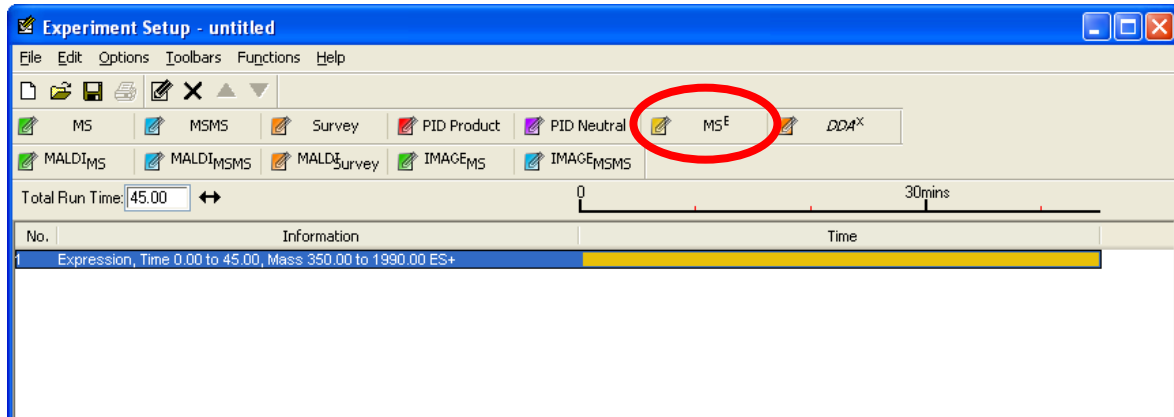

Figure 131 - Experiment Setup window

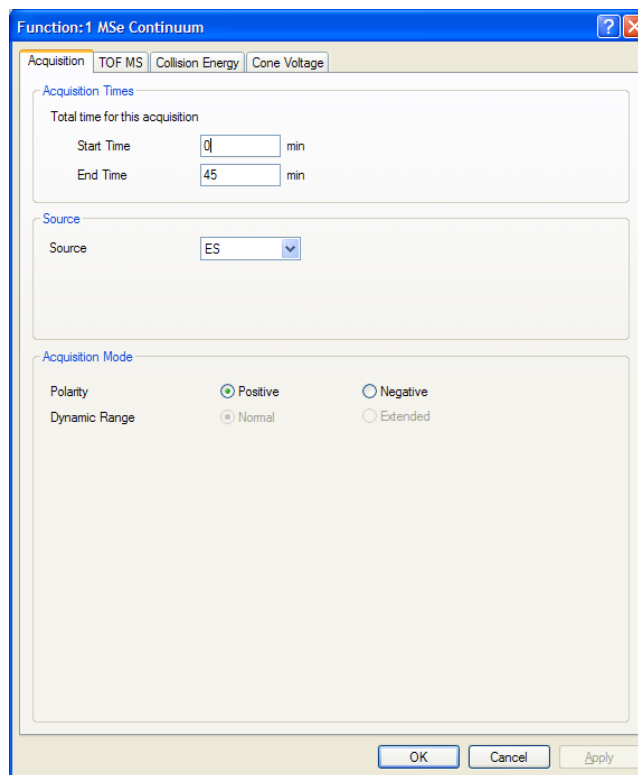2. Click **MS^E** to access the MS^E method.

Figure 132 - MS^E method

3. For each tab, complete the following information:

Table 29: Entry data for MS^E method

Tab	Values
Acquisition	Start time = 0 End time = 45 Source = ES Positive polarity
TOF MS	Start = 50 Da End = 1990 Da Scan time = 0.5 sec
Collision Energy	Low energy = 6 V Ramp high energy from 15 to 35 V
Cone Voltage	Override clear

NOTE: *Alternatively, the Lock Mass parameters can be derived from the in-source fragmentation test.*

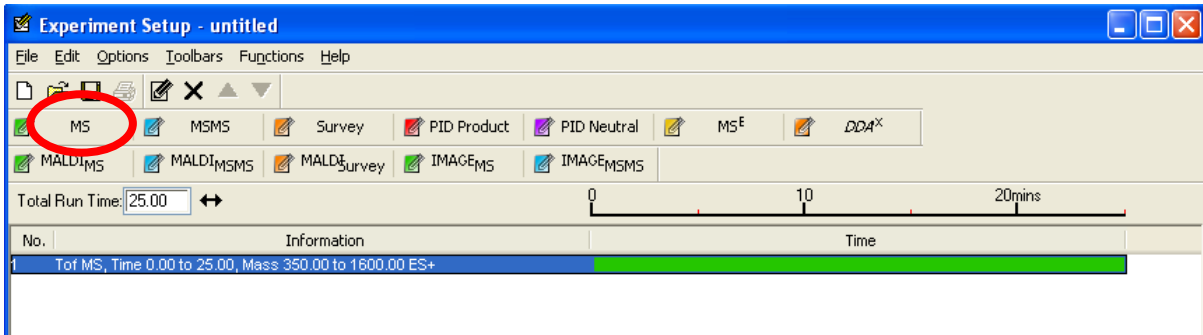## Commission_HP MS method

1. Access the Experiment Setup window.

Figure 133 - Experiment Setup window

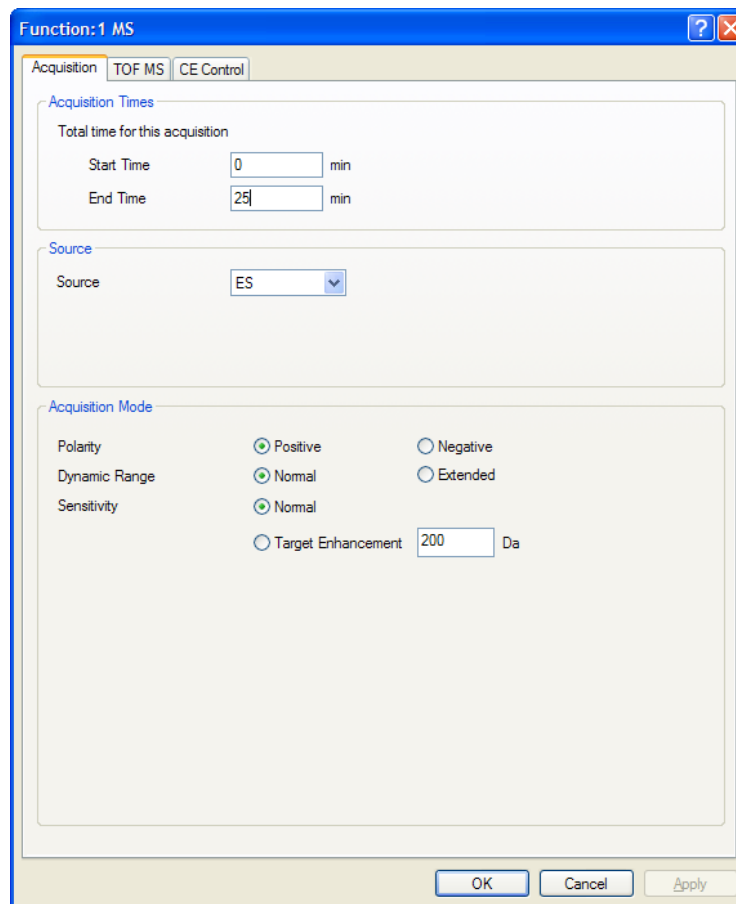2. Click **MS** to access the Function dialog box.

Figure 134 - MS experimental setup

3. For each tab, complete the following information:

Table 30: Entry data for Function dialog box

Tab	Parameter	Setting
Acquisition	Start time	0
	End time	25
	Source	ES
	Polarity	Positive
	Dynamic range	Normal
	Sensitivity	Normal
TOF MS	Low mass	100 Da
	High mass	1600 Da
	Scan time	0.6 sec
	Data format	Continuum
	Override and Ramp conditions	Unchecked
CE Control	Fixed collision energy value	6 V

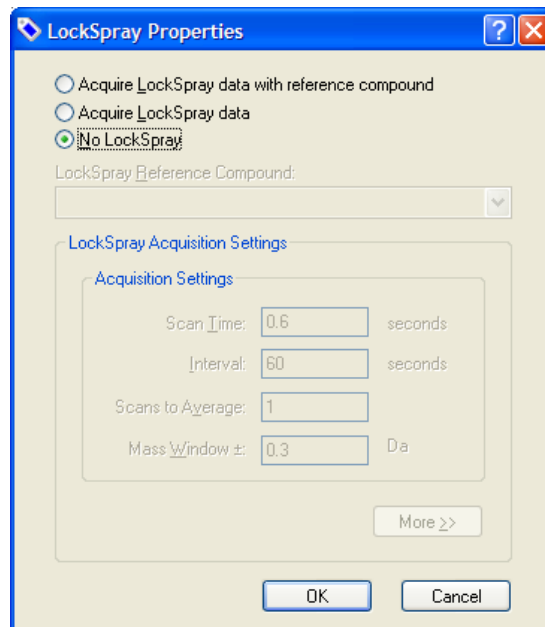4. Click **LockSpray** on the MS Method screen.

Figure 135 - LockSpray acquisition settings

5. Select the **No LockSpray** check box.