

Agilent 1100 (DAD) LC with Thermo Fisher TSQ Quantum Ultra MS/MS

120523MB03

For urgent problems please report to Matt Burleson, AP 346, mburleson@wcu.edu, x2239!*

Scope

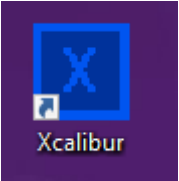
This SOP details the operation of the Agilent 1100 LC and Thermo Quantum MS as a coupled system. The LC can be operated without the MS which is detailed as a separate document.

Gases and Startup

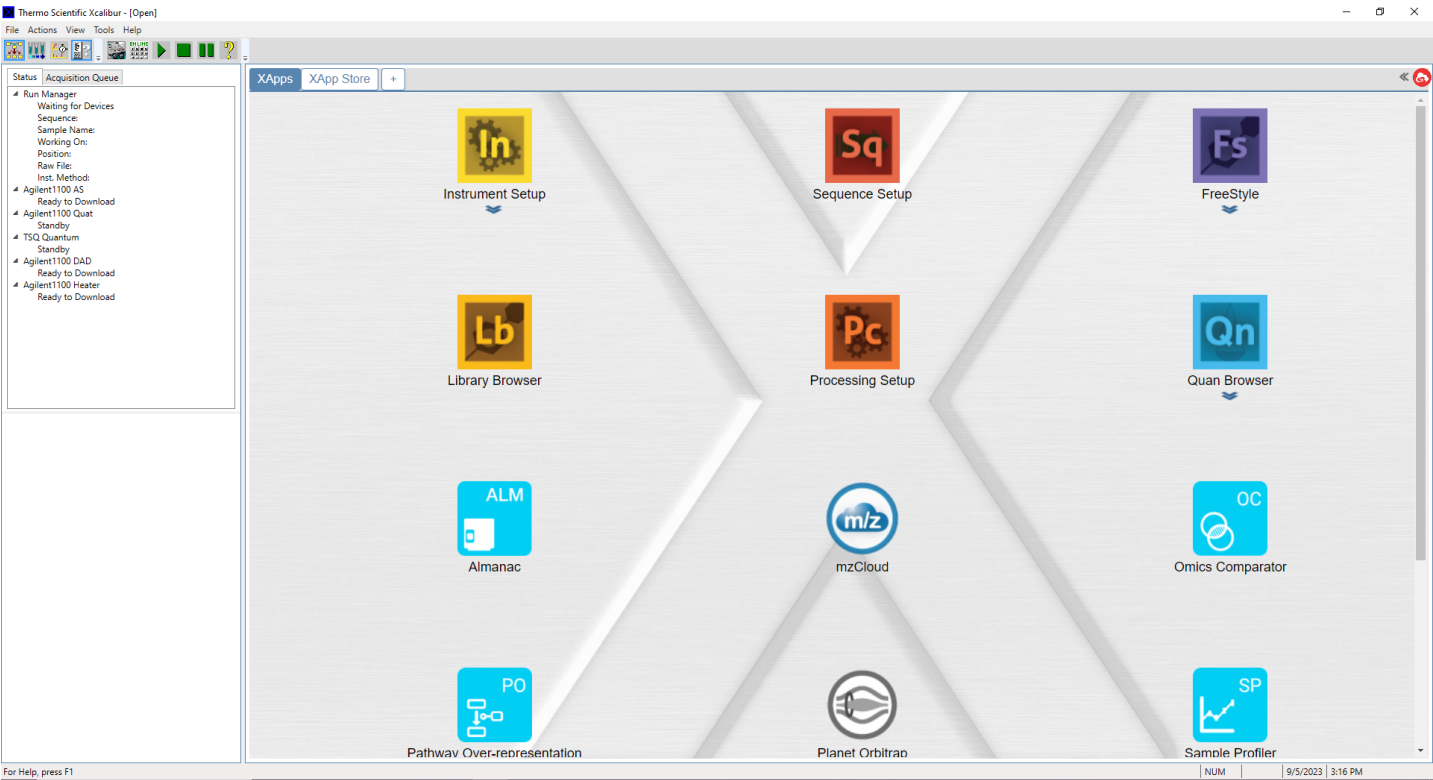
1. The LC-MS uses both argon and nitrogen gases. The argon should only be on if performing MS/MS experiments, and can be turned on using the main valve of the cylinder and the quick connect valve towards the back of the mass spec. The nitrogen should already be flowing in the lab.
2. The power switch to each module of the LC is located on the lower-left front of each module. Press these if the modules are not already on.

Login

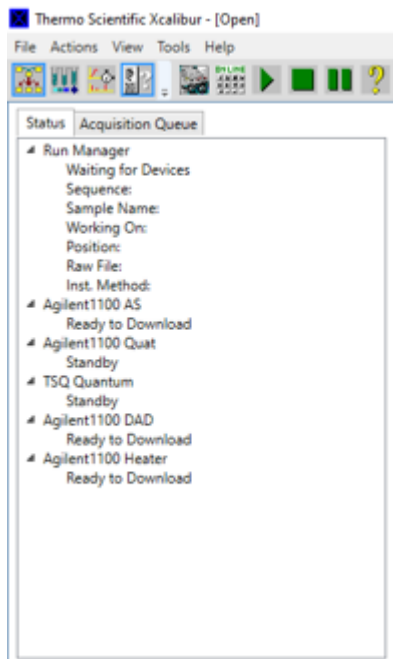
1. If you have not already done so, reserve the instrument and log your time using it. Please check the calendar first to make sure no one else has reserved it.
2. Login to Windows using the username **labuser** and the password **labuser**.
3. The software to control the unit is **Xcalibur**. Open the **Xcalibur** software by clicking on the shortcut on the desktop that is shown below.



4. The main window of Xcalibur (roadmap) is shown below. **Instrument Setup**, **Sequence Setup**, and **FreeStyle** will primarily be used.



5. Ensure all modules of the LC, and the TSQ Quantum, appear in the "Status" window to the top-left of the Xcalibur window as shown below. It is okay for modules to be off or in standby for now.

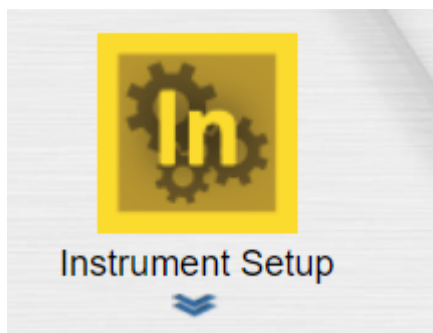


Preparing the Instrument

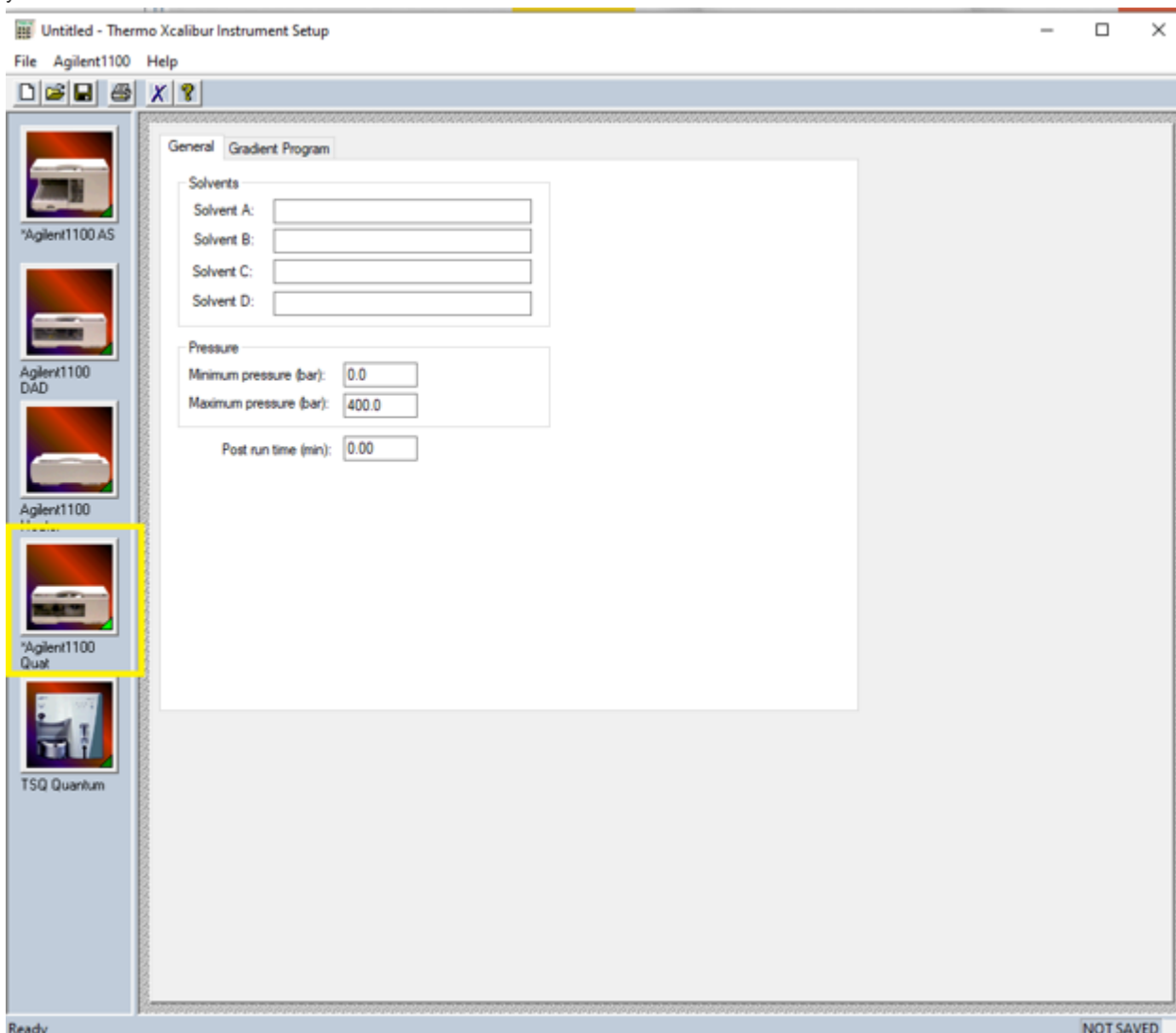
1. Ensure there is enough mobile phase for your analysis in the mobile phase bottles on top of the instrument.

NEVER let the mobile phase level get below the level of the filter in the bottle! NEVER let air get into the LC plumbing!

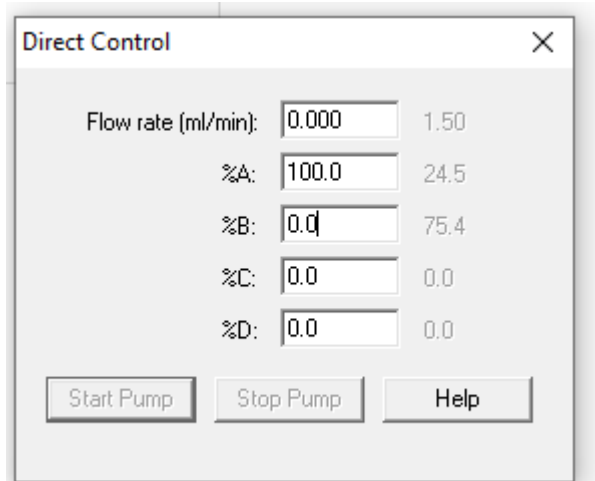
2. The LC lines must be purged with the necessary mobile phase prior to use. Open the purge valve of the LC pump by turning the purge valve counterclockwise.
3. In the main window of Xcalibur, click **Instrument Setup** shown below. The Instrument Setup popup will default to the autosampler selected since it is the module that communicates to the mass spectrometer.



4. In the Instrument Setup popup, click the **Agilent1100 Quat** icon in the left-hand toolbar to select the pump's setup window as shown below in yellow.

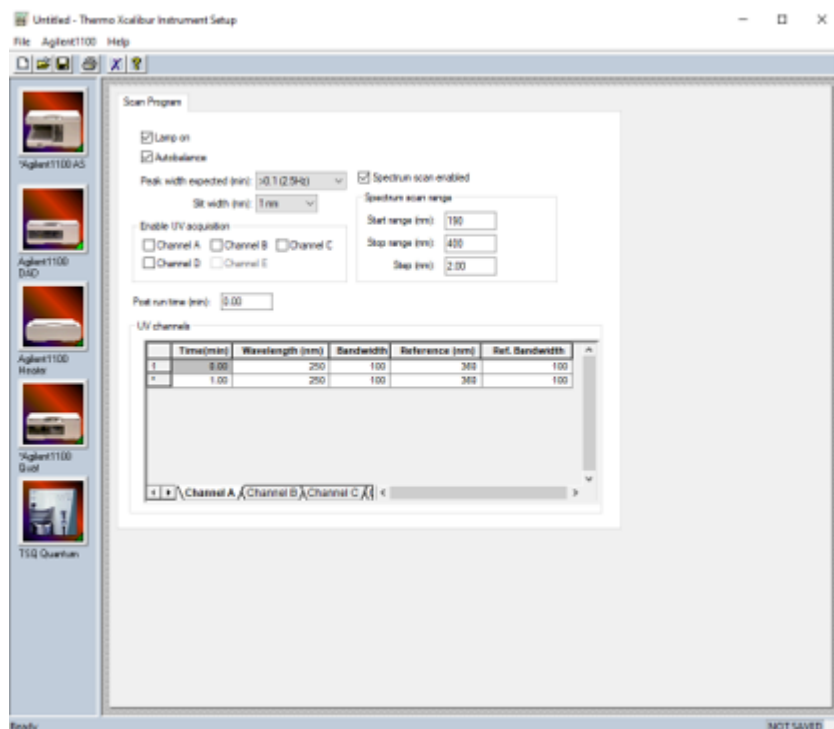


5. Click **Agilent1100** in the top toolbar followed by **Direct Control...** to open the Direct Control window shown below.

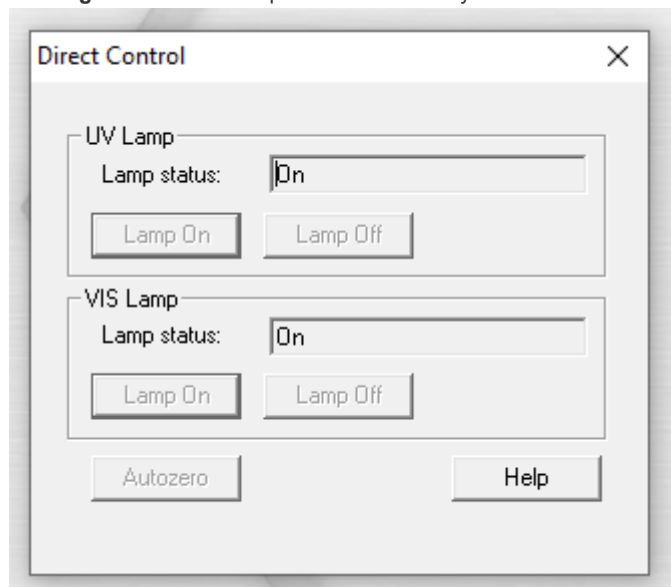


6. Change the "Flow rate" to 5.00 mL/min, and the composition to be an even split of the lines you are using. For example, if using lines A & B, they would be set to 50:50. If using all four lines, they would be 25% each.
7. Click **Start Pump** to begin purging the mobile phase. The pump may take a moment to start pumping.
8. Allow the pump to purge for ~5 mins.
9. Once the lines have been purged, change the composition and flow rate to that of the starting conditions of your method.
10. Close the purge valve to begin equilibrating the column to the initial conditions.
11. Close the Direct Control window by clicking the "X" in its top-right corner.

12. Back in the Instrument Setup popup, click the icon labelled **Agilent1100 DAD** in the left-hand toolbar to access the DAD's setup window shown below.



13. Click **Agilent1100** in the top toolbar followed by **Direct Control...** to open the Direct Control window shown below.



14. Use this window to turn on the UV Lamp by selecting **Lamp On**. Wait until the "Lamp status" reads "On" before proceeding.
15. Close the Direct Control window for the DAD.

Creating/Loading A Method

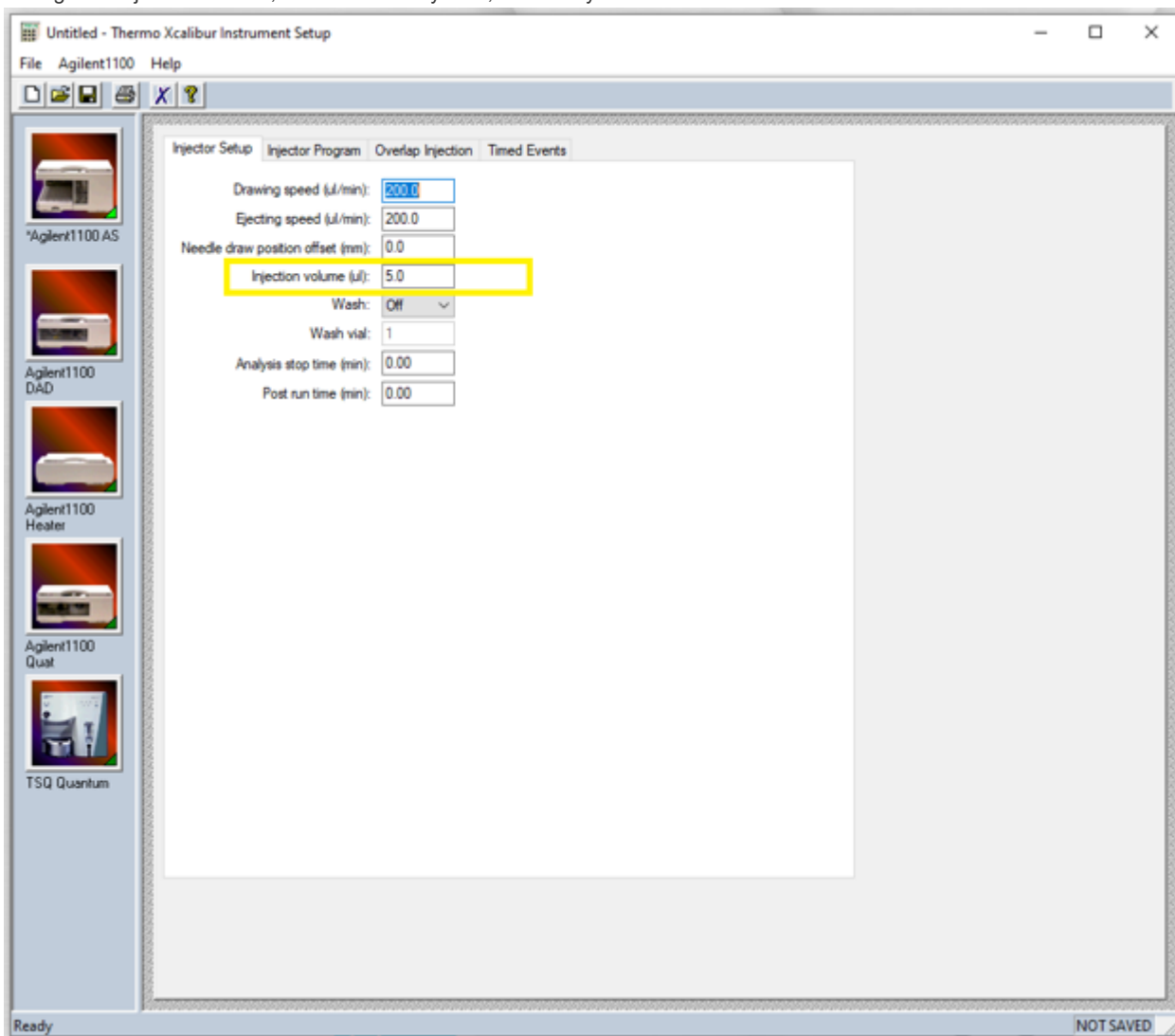
Loading A Method

1. Within the roadmap window, load your method by clicking on **File** then **Open**. Locate your method and select it.

Creating A method (Advanced)

Autosampler

1. If you need to create a new method, click **Instrument Setup** within the roadmap window. The Instrument Setup window defaults to the autosampler module.
2. Change the Injection Volume, shown below in yellow, to that of your method.



3. Click the **Timed Events** tab.

4. The autosampler needs to be set as the "Start instrument" which is responsible for telling the rest of the system the injection has been made. Complete the table so that it matches that shown below.

MS TIC

Untitled - Thermo Xcalibur Instrument Setup

File Agilent1100 Help

Injector Setup Injector Program Overlap Injection Timed Events

	Time(min)	Contact A	Contact B	Contact C	Contact D
1	0.00	Closed	Open	Open	Open
2	0.10	Open	Open	Open	Open
*	1.10	Open	Open	Open	Open

Relative Abundance

*Agilent1100 AS

Agilent1100 DAD

Agilent1100 Heater

Agilent1100 Quat

TSQ Quantum

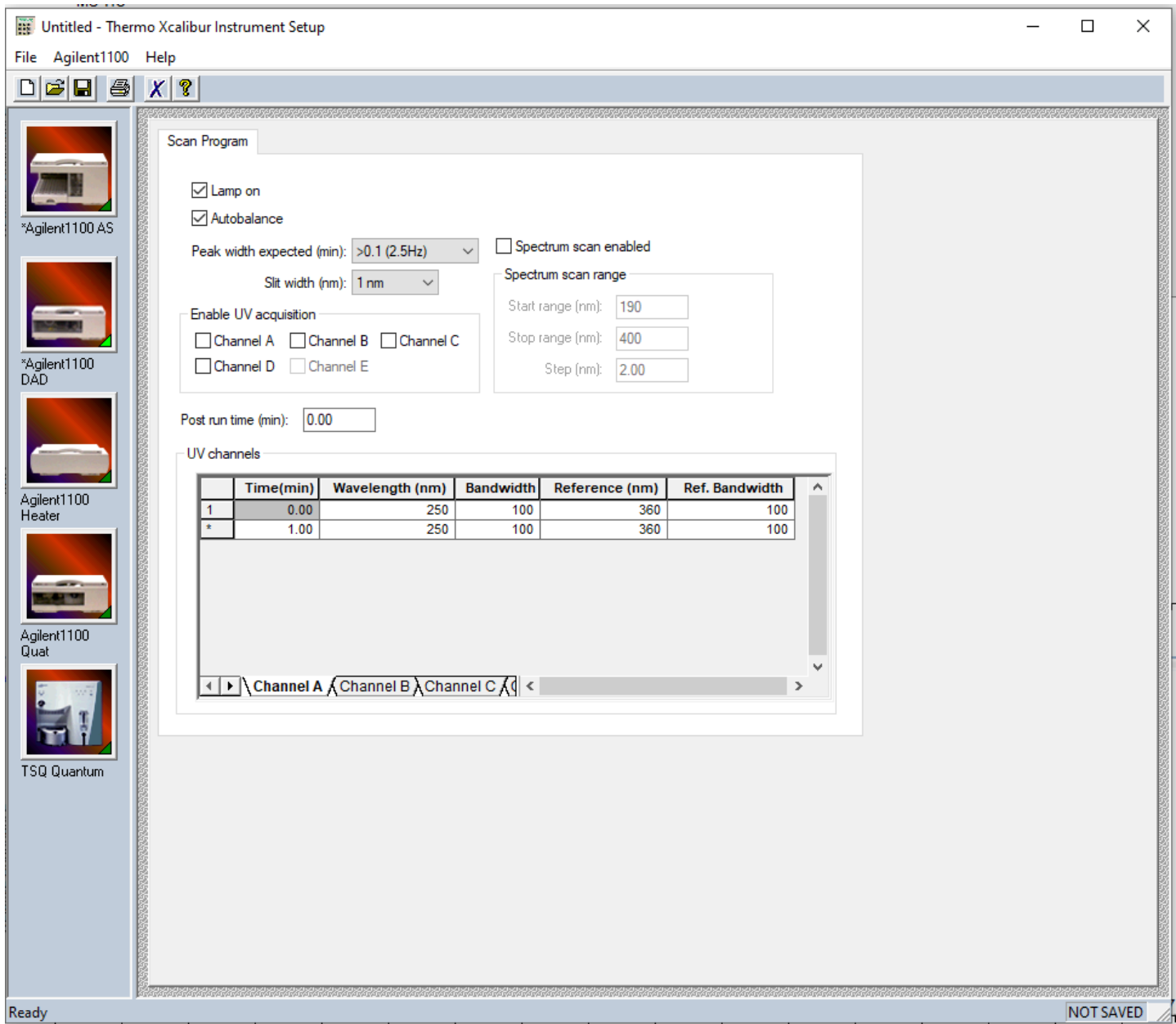
Ready

NOT SAVED

5. Save your method.

Detector

1. Click the icon for the DAD in the left-hand toolbar to open the DAD's setup page as shown below.



- 2. Ensure that "Lamp On" and "Autobalance" are both checked.
- 3. "Peak width expected" and "Slid Width" default values will load automatically, but can be changed by clicking their respective dropdown arrows.
- 4. Under "Enable UV Acquisition", click a check beside the channel that will be used for analysis. For example, if only using one wavelength use Channel A. If using two wavelengths, use Channels A & B, etc.
- 5. Change the wavelength to that of your method under the Wavelength tab in the UV Channels table. The reference wavelength and bandwidths can also be changed as necessary.

6. Add a row for the ending time of your method and be sure the parameters match that of the previous step. Repeat this for each wavelength being analyzed.

***Advanced** A spectrum scan (locating lambda max) can be enabled by checking its respective checkbox. The starting wavelength, ending wavelength and step can then be entered.

7. The final result should be similar to that shown below.

Untitled - Thermo Xcalibur Instrument Setup

File Agilent1100 Help

Scan Program

☒ Lamp on
☒ Autobalance

Peak width expected (min): >0.1 (2.5Hz) ☐ Spectrum scan enabled

Slit width (nm): 1 nm

Spectrum scan range

Start range (nm): 190
Stop range (nm): 400
Step (nm): 2.00

Enable UV acquisition

☒ Channel A ☐ Channel B ☐ Channel C
☐ Channel D ☐ Channel E

Post run time (min): 0.00

UV channels

	Time(min)	Wavelength (nm)	Bandwidth	Reference (nm)	Ref. Bandwidth
1	0.00	274	100	360	100
2	40.00	274	100	360	100
*	41.00	274	100	360	100

Channel A Channel B Channel C

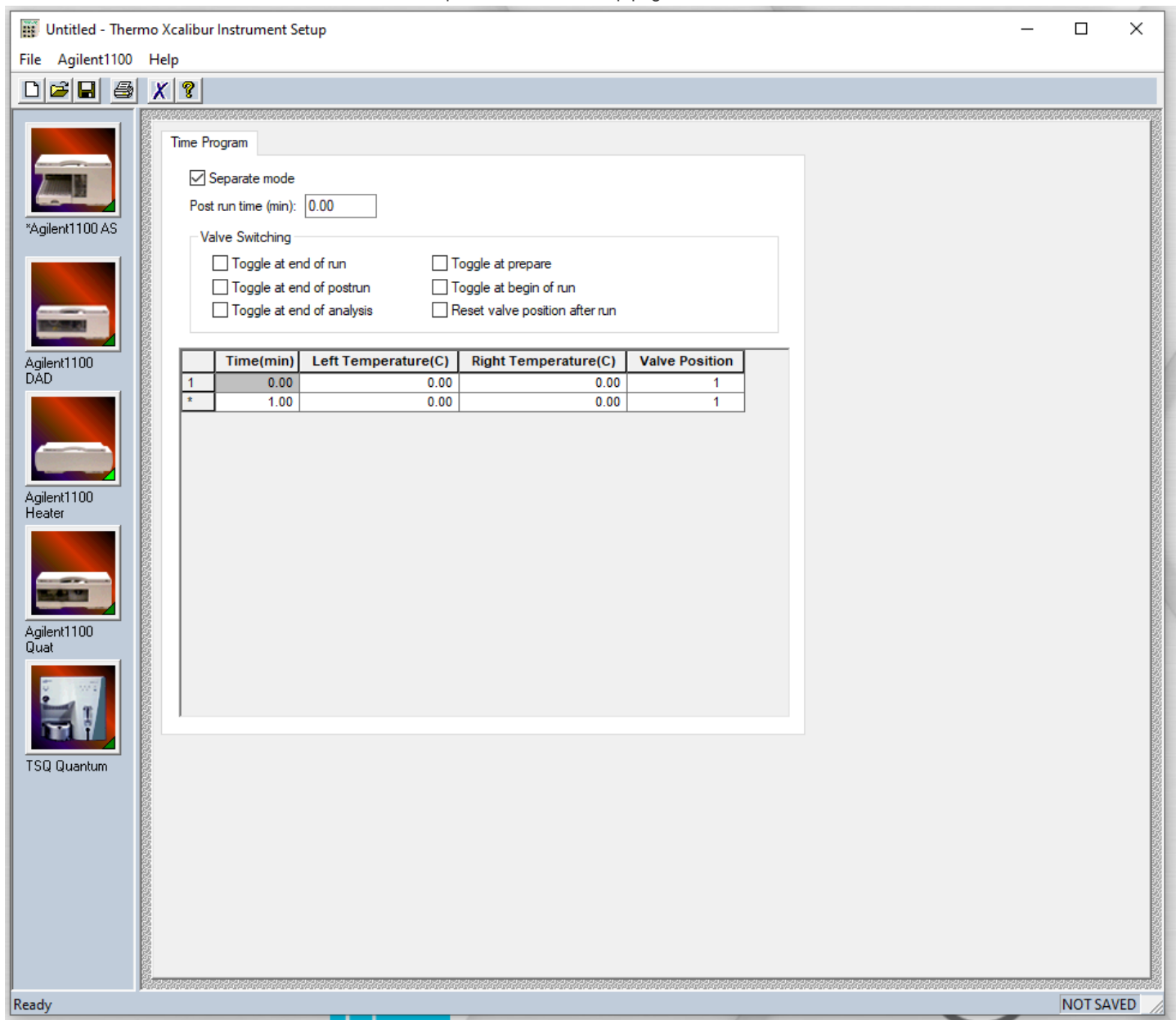
Ready NOT SAVED

*For the DAD, and remaining modules of the LC, a new row will automatically show up with an asterisk for a time you did not enter. That is fine, proceed to the next step.

8. Save your method.

Heater

1. Click the icon for the Heater in the left-hand toolbar to open the Heater's setup page as shown below.



- 2. Ensure that "Separate mode" is enabled.
- 3. No boxes should be checked under "Valve Switching."
- 4. Under both "Left/Right Temperature", enter the temperature of your method.

5. Add a row for the ending time of your method and be sure the parameters match that of the previous step. The final result should be similar to that shown below.

Untitled - Thermo Xcalibur Instrument Setup

File Agilent1100 Help

Time Program

☒ Separate mode

Post run time (min): 0.00

Valve Switching

☐ Toggle at end of run ☐ Toggle at prepare

☐ Toggle at end of postrun ☐ Toggle at begin of run

☐ Toggle at end of analysis ☐ Reset valve position after run

	Time(min)	Left Temperature(C)	Right Temperature(C)	Valve Position
1	0.00	30.00	30.00	1
2	40.00	30.00	30.00	1
*	41.00	0.00	0.00	1

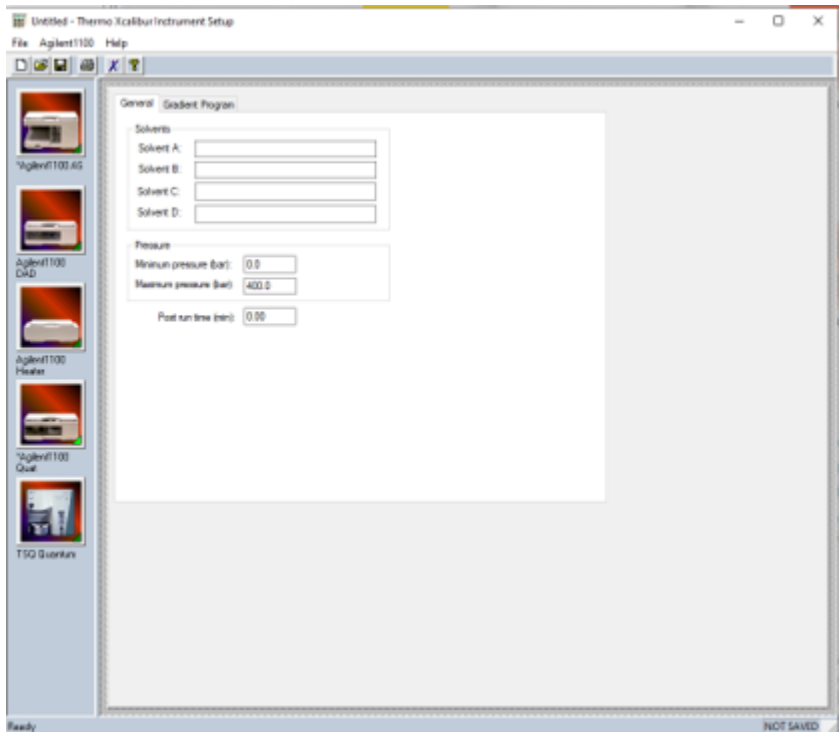
Ready

NOT SAVED

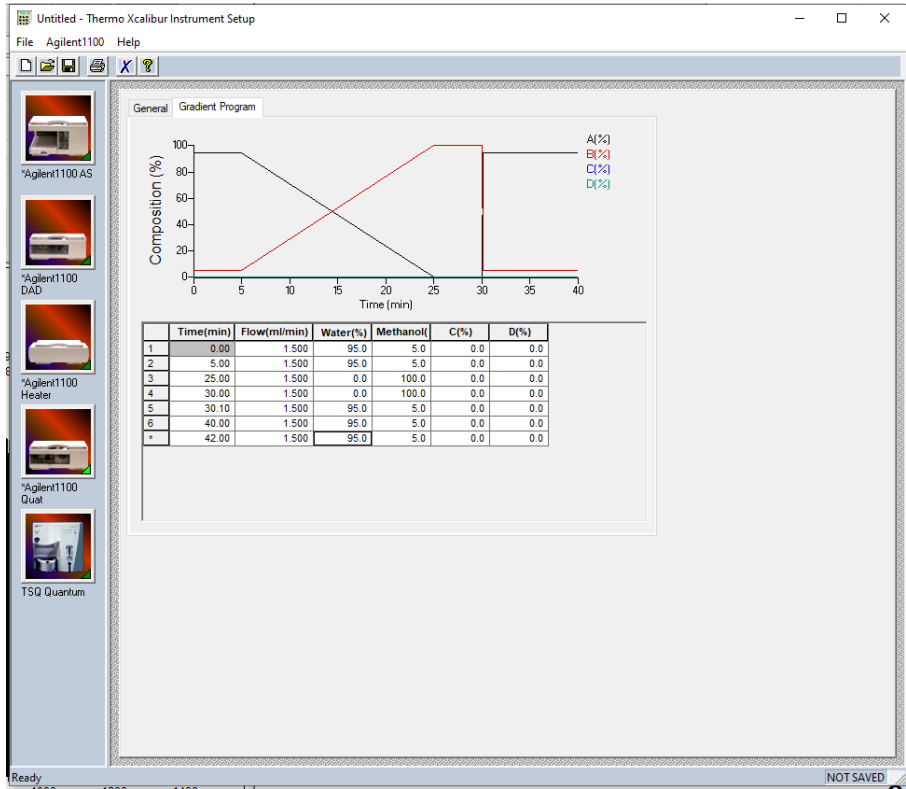
6. Save your method.

Quat Pump

- 1. Click the icon for the Quat in the left-hand toolbar to open the Quat's setup page as shown below.



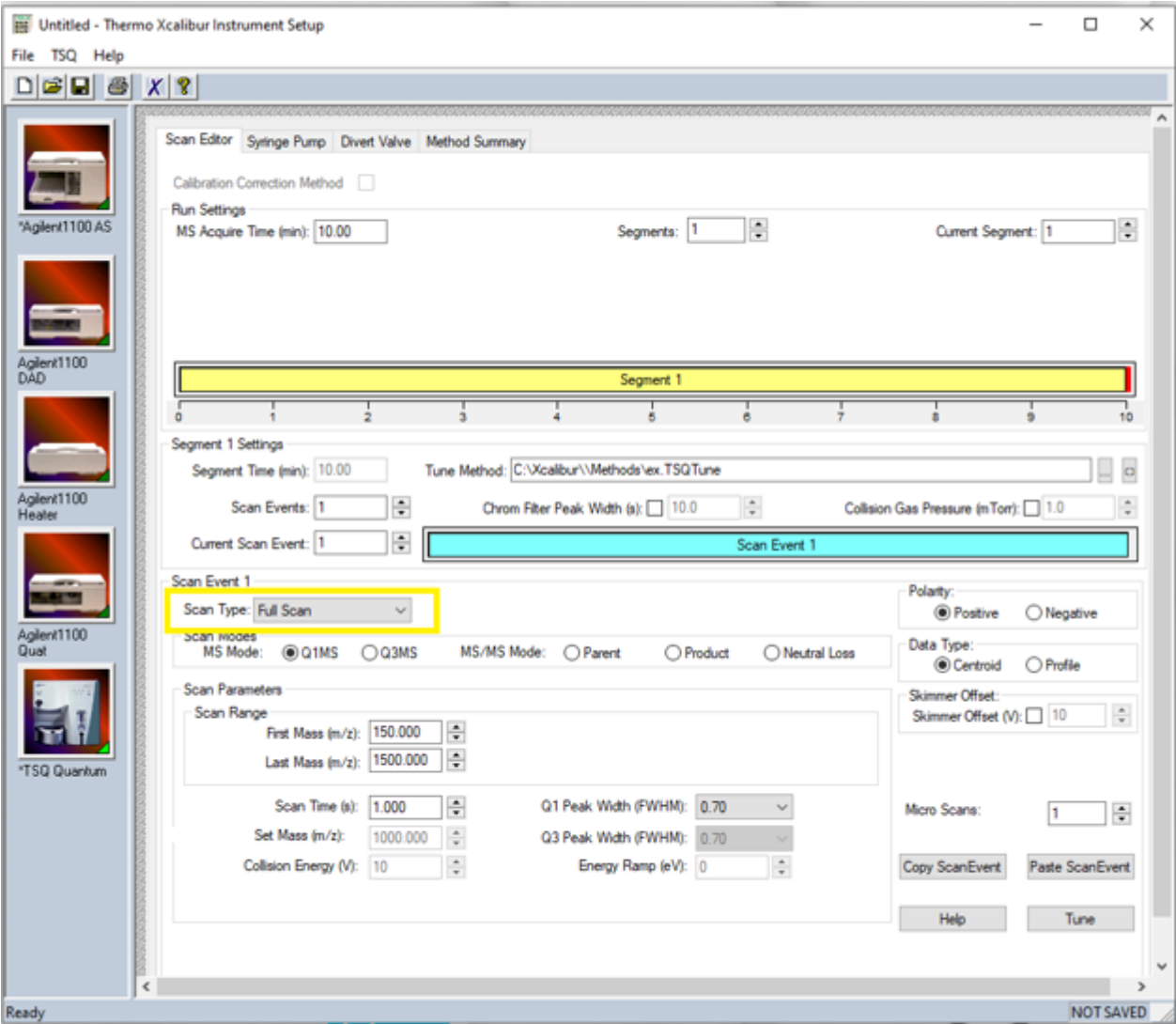
- 2. Add the information of your mobile phases to each respective line.
- 3. Click the **Gradient Program** tab to open the gradient setup window.
- 4. Under the "Flow" column, enter the flow rate value of your method.
- 5. Click the value under the column headers to change the composition to that of the method starting conditions for time 0.
- 6. In the next row, change the time and percentages of each line as necessary to match the method. Repeat this step as necessary. The final result should be similar to that below if using a gradient.



- 7. Save your method.

Mass Spectrometer

- 1. Click the icon for the TSQ Quantum in the left-hand toolbar to open the mass spec's setup page.
- 2. The Scan Type can be selected based on the needs of the user by clicking the dropdown arrow beside "Scan Type" shown below in yellow. The rest of this procedure is for a full scan analysis using Q1. Q3 and MS/MS mode can be enabled here.



3. The image below shows where to locate the Polarity. Change the Polarity of the MS to either positive or negative by clicking the respective button beside the option.

Untitled - Thermo Xcalibur Instrument Setup

File TSQ Help

Scan Editor Syringe Pump Divert Valve Method Summary

Calibration Correction Method ☐

Run Settings

MS Acquire Time (min): 10.00 Segments: 1 Current Segment: 1

Segment 1

Segment 1 Settings

Segment Time (min): 10.00 Tune Method: C:\Xcalibur\Methods\ex.TSQTune

Scan Events: 1 Chrom Filter Peak Width (s): 10.0 Collision Gas Pressure (mTorr): 1.0

Current Scan Event: 1

Scan Event 1

Scan Type: Full Scan

Scan Modes

MS Mode: ☒ Q1MS ☐ Q3MS MS/MS Mode: ☐ Parent ☐ Product ☐ Neutral Loss

Scan Parameters

Scan Range

First Mass (m/z): 150.000 Last Mass (m/z): 1500.000

Scan Time (s): 1.000 Q1 Peak Width (FWHM): 0.70

Set Mass (m/z): 1000.000 Q3 Peak Width (FWHM): 0.70

Collision Energy (V): 10 Energy Ramp (eV): 0

Polarity: ☒ Positive ☐ Negative

Data Type: ☒ Centroid ☐ Profile

Skimmer Offset: Skimmer Offset (V): 10

Micro Scans: 1

Copy ScanEvent Paste ScanEvent

Help Tune

Ready NOT SAVED

4. Change the **MS Acquire Time** to match that of your LC method.

5. Change the **Scan Ranges** (below in yellow) to scan for the desired masses, and scan time to be 0.2 for the fastest scan speed for the desired mass range. It's okay if the software changes that value accordingly.

Untitled - Thermo Xcalibur Instrument Setup

File TSQ Help

Scan Editor Syringe Pump Divert Valve Method Summary

Calibration Correction Method ☐

Run Settings

MS Acquire Time (min): 10.00 Segments: 1 Current Segment: 1

To display a chromatogram here, use Quantum/Open Raw File...

Segment 1

Segment 1 Settings

Segment Time (min): 10.00 Tune Method: C:\Xcalibur\Methods\ex.TSQ.Tune

Scan Events: 1 Chrom Filter Peak Width (s): 10.0 Collision Gas Pressure (mTorr): 1.0

Current Scan Event: 1 Scan Event 1

Scan Event 1

Scan Type: Full Scan

Scan Modes

MS Mode: ☒ Q1MS ☐ Q3MS MS/MS Mode: ☐ Parent ☐ Product ☐ Neutral Loss

Scan Parameters

Scan Range

First Mass (m/z): 150.000

Last Mass (m/z): 1500.000

Scan Time (s): 1.000

Set Mass (m/z): 1000.000

Collision Energy (V): 10

Q1 Peak Width (FWHM): 0.70

Q3 Peak Width (FWHM): 0.70

Energy Ramp (eV): 0

Polarity: ☒ Positive ☐ Negative

Data Type: ☒ Centroid ☐ Profile

Skimmer Offset: Skimmer Offset (V): 10

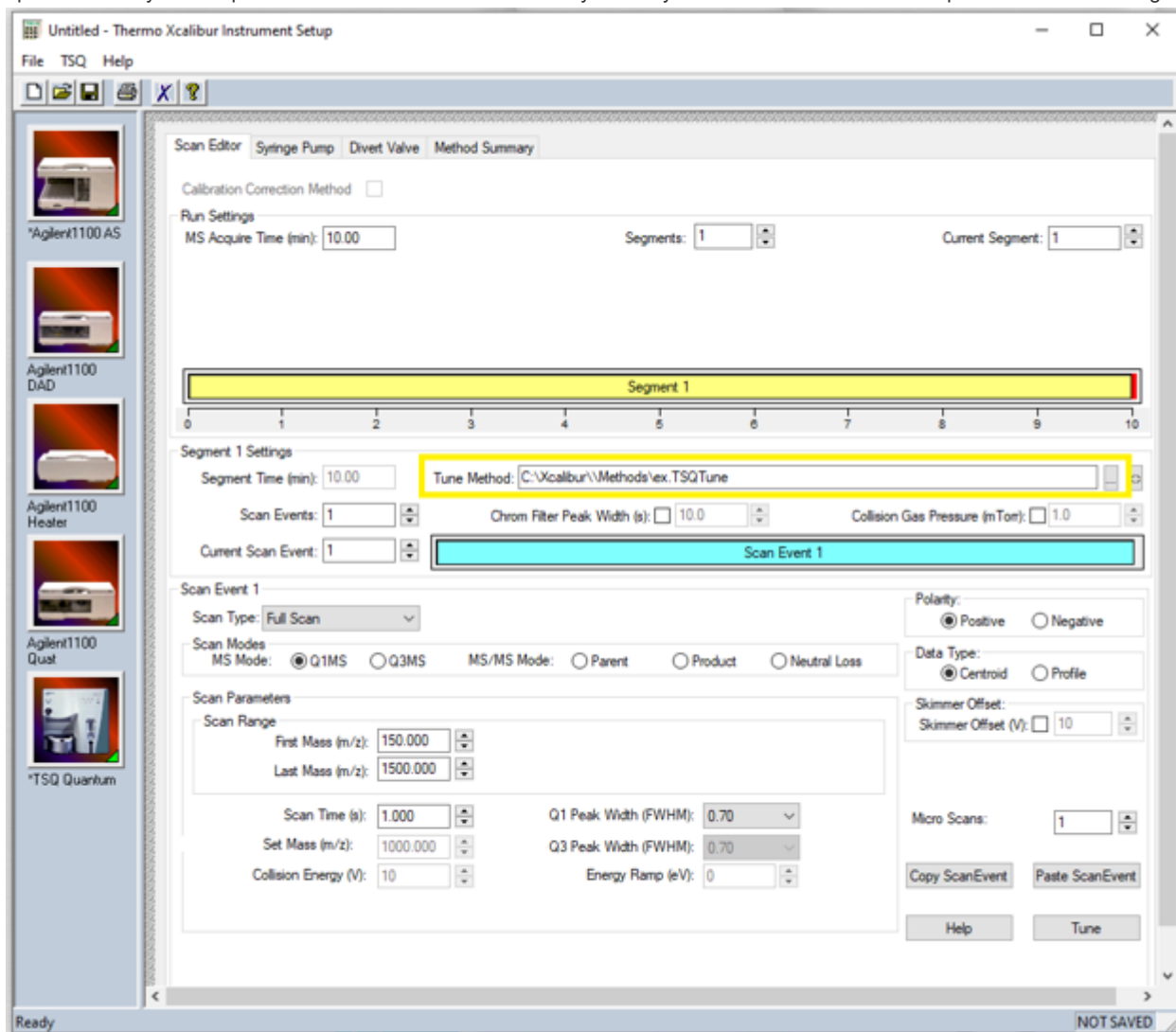
Micro Scans: 1

Copy ScanEvent Paste ScanEvent

Help Tune

Ready NOT SAVED

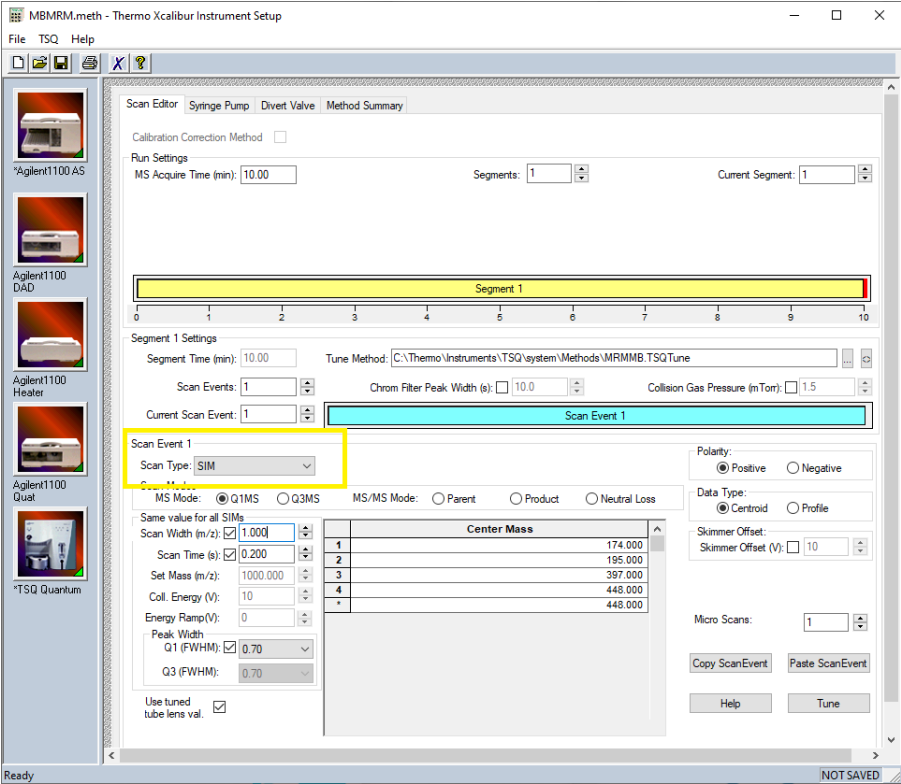
6. The image below shows where to change the number of microscans performed. This is the number of times to scan the current mass range. the scans are averaged together to produce a single scan of data that is less affected by ion source conditions. This is only used in full scan mode.
7. The image below shows where to locate the Tune Method. Change the Tune Method to the appropriate tune file that was created from the optimization of your sample. You have to first clear the directory listed by "Tune Method" before the ellipsis will allow browsing.



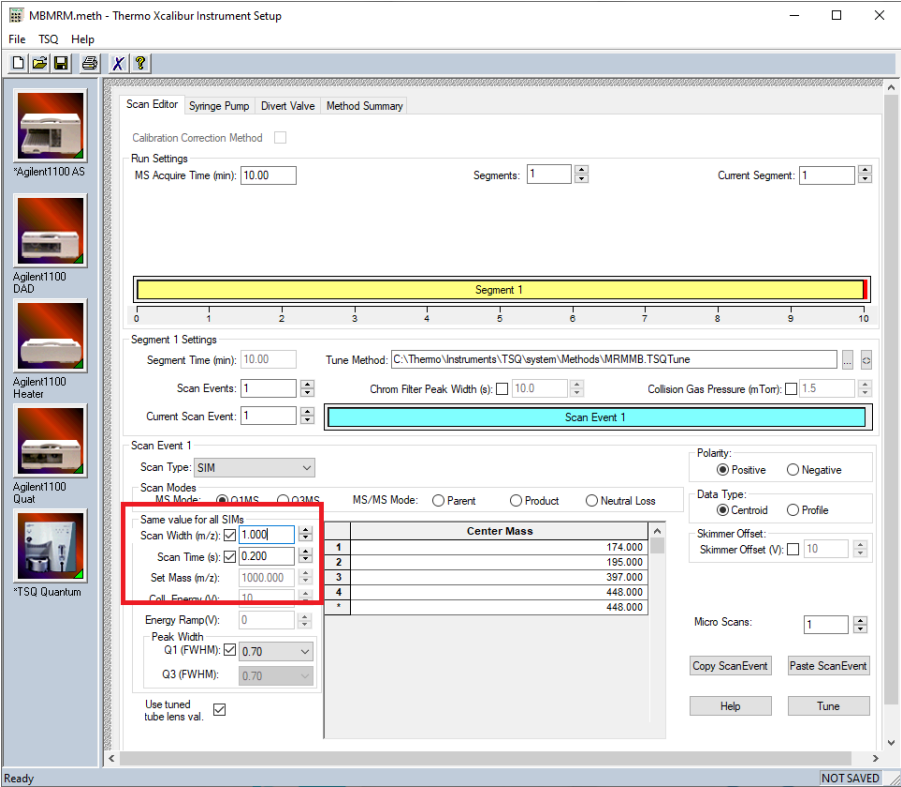
8. Once the MS parameters have been adjusted, save the method and close the Instrument Setup window.

Creating A SIM Method

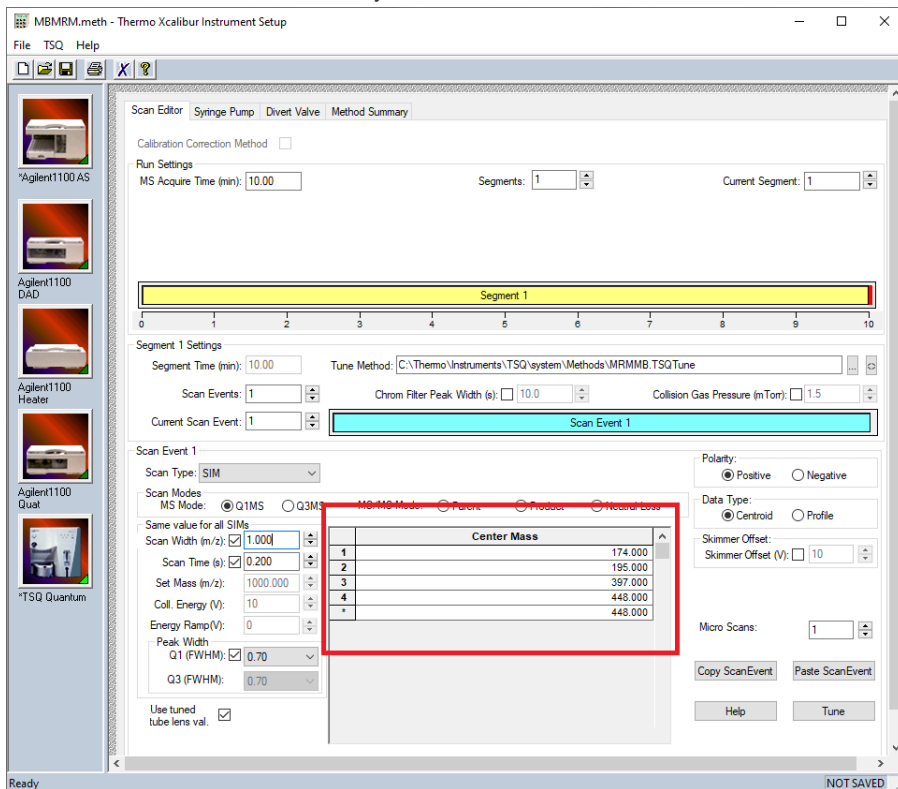
- 1. The creation of a SIM method is similar to a full scan detailed above. The following steps detail the different setup required.
- 2. Click the icon for the TSQ Quantum in the left-hand toolbar to open the mass spec's setup page.
- 3. The SIM Scan Type can be selected by clicking the dropdown arrow beside "Scan Type" and selecting "SIM" shown below in yellow.



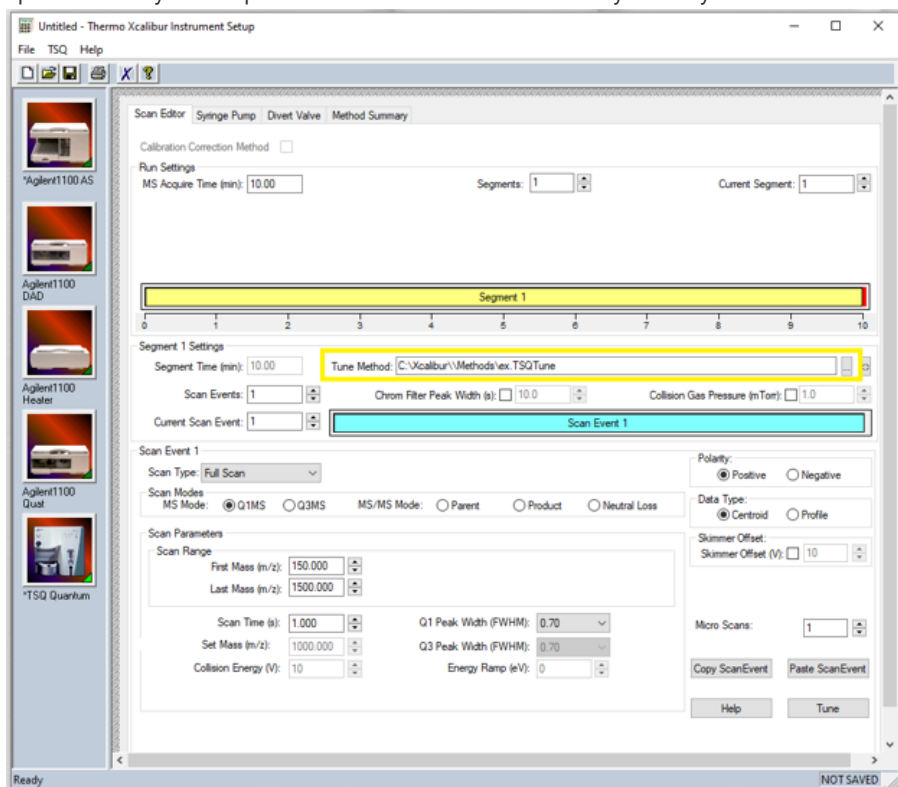
- 4. Ensure the check boxes beside "Scan Width" and "Scan Time" are checked and set to 1.0 and 0.2, respectively. This is shown below.



5. In the table labeled as "Center Mass" you will enter the ions of interest to monitor such as those shown below.



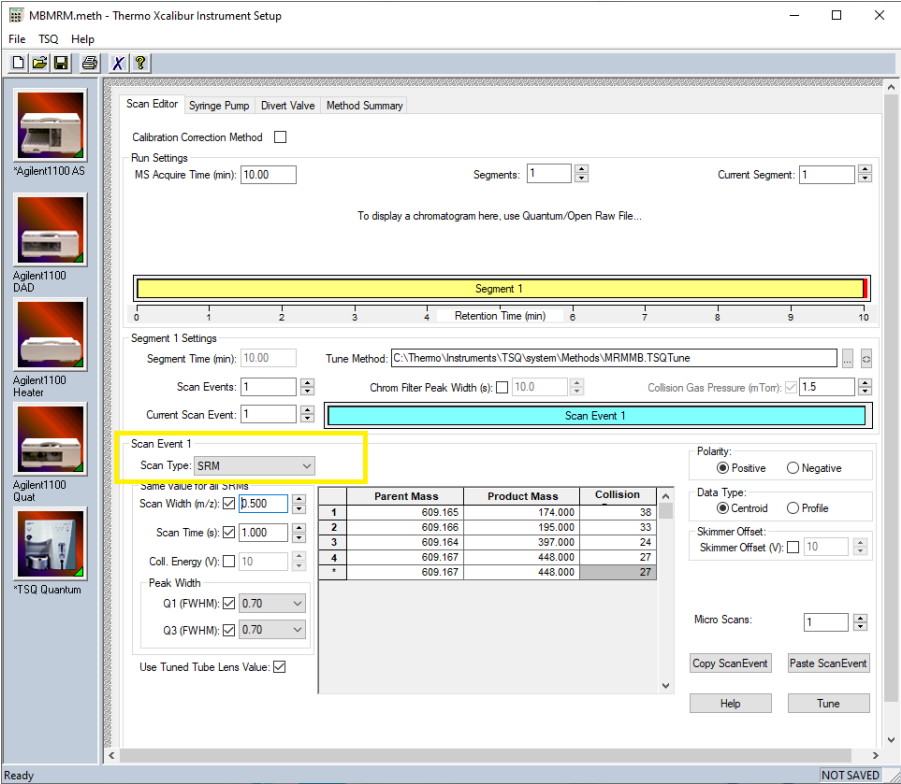
6. The image below shows where to locate the Tune Method. Change the Tune Method to the appropriate tune file that was created from the optimization of your sample. You have to first clear the directory listed by "Tune Method" before the ellipsis will allow browsing.



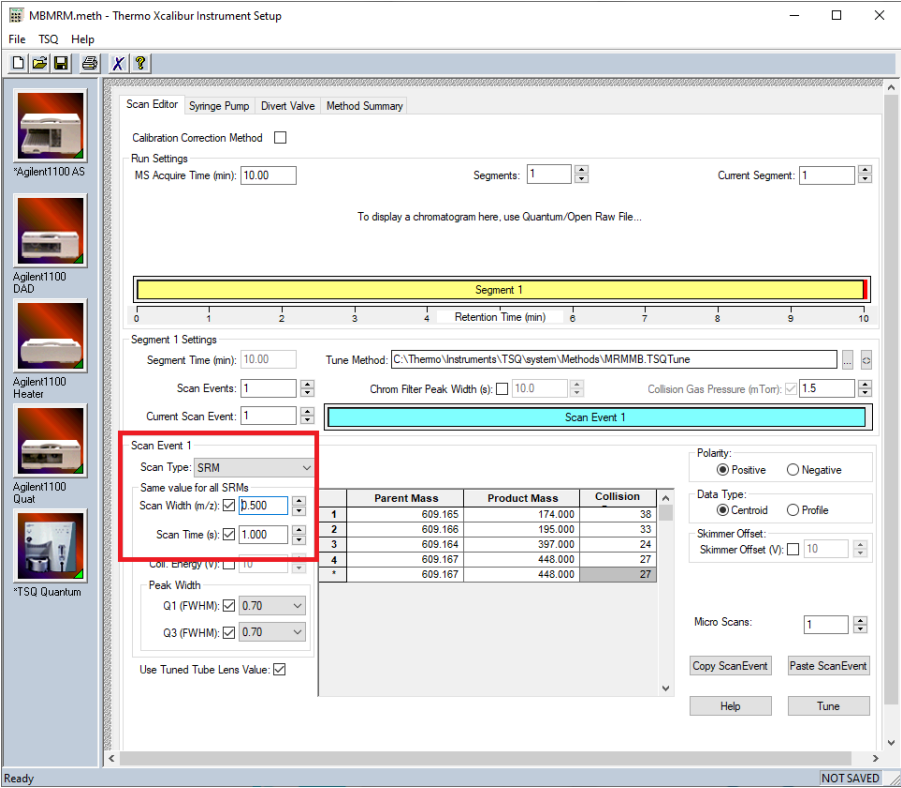
7. Once the MS parameters have been adjusted, save the method and close the Instrument Setup window.

Creating A MRM/SRM Method (ADVANCED)

- 1. The use of MRM requires optimization with the Instrumentation Specialist prior to any method creation.
- 2. Click the icon for the TSQ Quantum in the left-hand toolbar to open the mass spec's setup page.
- 3. The SRM Scan Type can be selected by clicking the dropdown arrow beside "Scan Type" and selecting "SRM" shown below in yellow.



- 4. Ensure the check boxes beside "Scan Width" and "Scan Time" are checked and that the scan width is set to 0.5. This is shown below. **NOTE: The scan time will vary from experiment to experiment.**



5. The SRM table contains a column for the parent mass, product mass, and collision energy and is shown below.

The screenshot shows the 'Scan Editor' window in Thermo Xcalibur. The 'Scan Type' is set to 'SRM'. A table is highlighted with a red border, containing the following data:

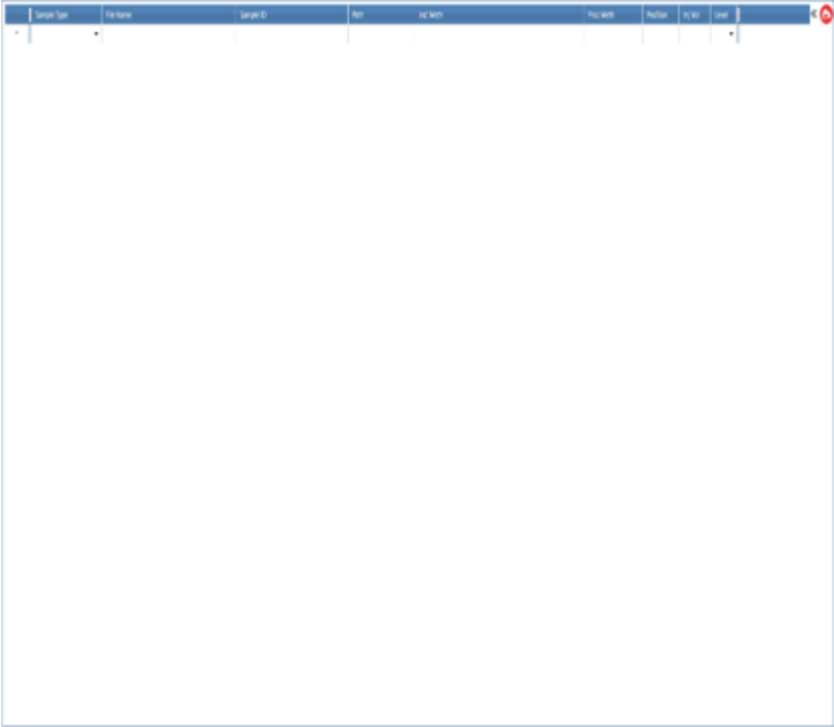
	Parent Mass	Product Mass	Collision
1	609.165	174.000	38
2	609.166	195.000	33
3	609.164	397.000	24
4	609.167	448.000	27
*	609.167	448.000	27

Other visible settings include: MS Acquire Time (min): 10.00, Segments: 1, Current Segment: 1, Scan Events: 1, Chrom Filter Peak Width (s): 10.0, Collision Gas Pressure (mTorr): 1.5, Scan Time (s): 1.000, Coll. Energy (V): 10, Peak Width Q1 (FWHM): 0.70, Q3 (FWHM): 0.70, Use Tuned Tube Lens Value: ☒.

- Enter the parent mass for each SRM of interest using slightly different values for the parent mass as shown above. The values need to differ slightly because if the same parent mass is specified for two or more SRMs, the instrument performs a SIM experiment on the product ions - that is, the product ions appear in one scan instead of in separate scans.
- Enter the product masses for the expected transitions in the "Product Mass" column.
- Enter the collision energy obtained from the curve breakdown during the optimization performed for SRM with the Instrumentation Specialist.
- Once the MS parameters have been adjusted, save the method and close the Instrument Setup window.

Prepare A Sequence

1. At the Xcalibur roadmap, click **Sequence Setup**. The default window is shown below.



2. Within the sequence table complete the following for each of your samples:

- i. Under Sample Type, click to select either a Blank or Unknown.
- ii. File Name will be a unique data file given to each injection. It cannot contain any spaces or special characters.
- iii. Sample ID can be the actual name of the sample. For example, MeOH.
- iv. The Path is where the data is stored and should be set to a folder with your name, your class or your PI's name.
- v. The Inst Method (instrument method) can be selected by right-clicking within the row and selecting Browse. Locate the method that was created previously.
- vi. Position is the location of the sample in the HPLC autosampler. Update the positions of samples accordingly.
- vii. The Injection volume should match that of the method.
- viii. Repeat this as necessary for all samples. The Processing Method and Level columns can be skipped.

3. A sequence template is shown below.

	Sample Type	File Name	Sample ID	Path	Inst Meth	Proc Meth	Position	Inj Vol	Level	
▶ 1	Blank	090523MB03	Blank	C:\Xcalibur\Data	C:\Xcalibur\methods\General Analytical MB		1	10.00	▼	
2	Blank	090523MB04	Blank	C:\Xcalibur\Data	C:\Xcalibur\methods\General Analytical MB		1	10.00	▼	
3	Unknown	090523MB05	Benzophenone post optimization	C:\Xcalibur\Data	C:\Xcalibur\methods\General Analytical MB		2	10.00	▼	
4	Blank	090523MB06	Blank	C:\Xcalibur\Data	C:\Xcalibur\methods\General Analytical MB		1	10.00	▼	
*									▼	

*NOTE: Always begin a sequence with at least TWO blanks and always end a sequence with at least ONE blank.

4. Save the sequence once you have finished.

Analyze Samples

1. To submit the sequence to the instruments, click the **Run Sequence** icon at the top toolbar. The icon is shown below.



2. A new window will open. Ensure the following are correct.

- i. The "Agilent1100 AS" is checked as the Start Instrument.
- ii. "Run Rows" matches the number of rows you created in your sequence.
- iii. "Start When Ready" is checked.
- iv. "After Sequence Set System" is set to "Standby."

3. The window should be similar to that shown below once completed.

Instrument	Start Instrument
Agilent1100 AS	<input checked="" type="checkbox"/>
Agilent1100 Quat	<input type="checkbox"/>
TSQ Quantum	<input type="checkbox"/>
Agilent1100 DAD	<input type="checkbox"/>
Agilent1100 Heater	<input type="checkbox"/>

☒ Start When Ready Change Instruments...

Instrument Method

Start Up Browse...

Shut Down Browse...

Programs

Pre Acquisition Browse...

Post Acquisition Browse...

Run Synchronously ☐ Pre Acquisition ☐ Post Acquisition

After Sequence Set System ☐ On ☒ Standby ☐ Off

User

Run Rows To

☐ Priority Sequence

Processing Actions

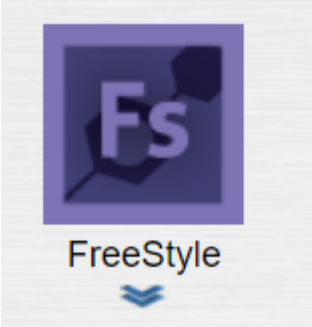
☐ Quan ☐ Qual ☐ Reports ☐ Programs ☒ Create Quan Summary

OK Cancel Help

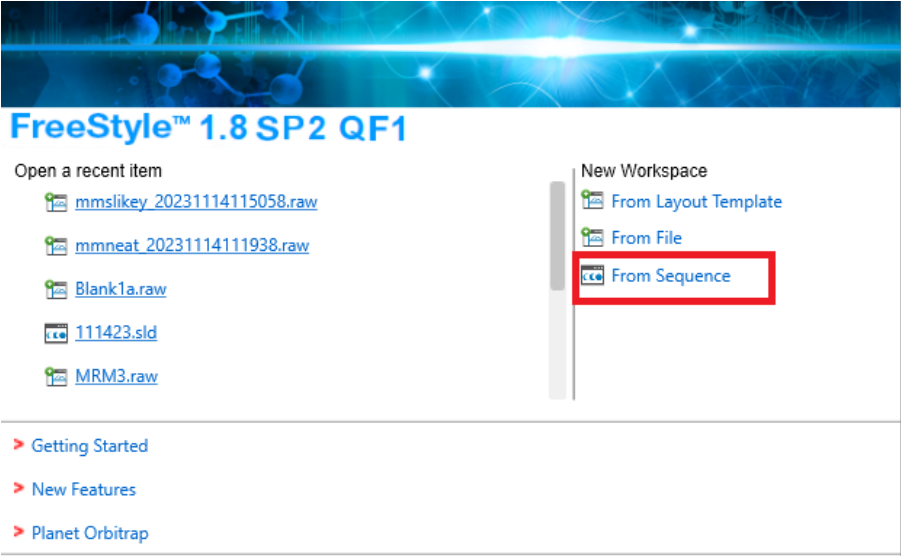
4. Click **OK** when done to submit the sequence to the instruments. In the left-hand panel, you should observe status change from **Ready to Download** to **Waiting for Contact Closure**. This means the MSD is waiting for the LC to complete the injection.

Analyzing and Exporting Data

1. Open the Freestyle software from the main page of Xcalibur. The icon is shown below.



2. Within Freestyle's startup screen shown below, click **From Sequence** under the "New Workspace" list and locate the sequence of interest.



3. Double-click on the datafile for the sample you wish to process. The TIC of that sample should display as shown below.

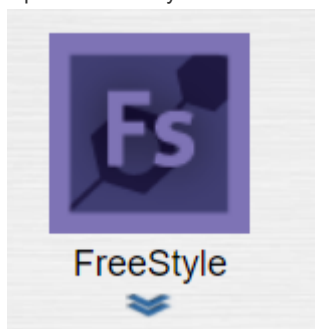


4. You can now click around on the TIC and see the resulting mass spectrum.

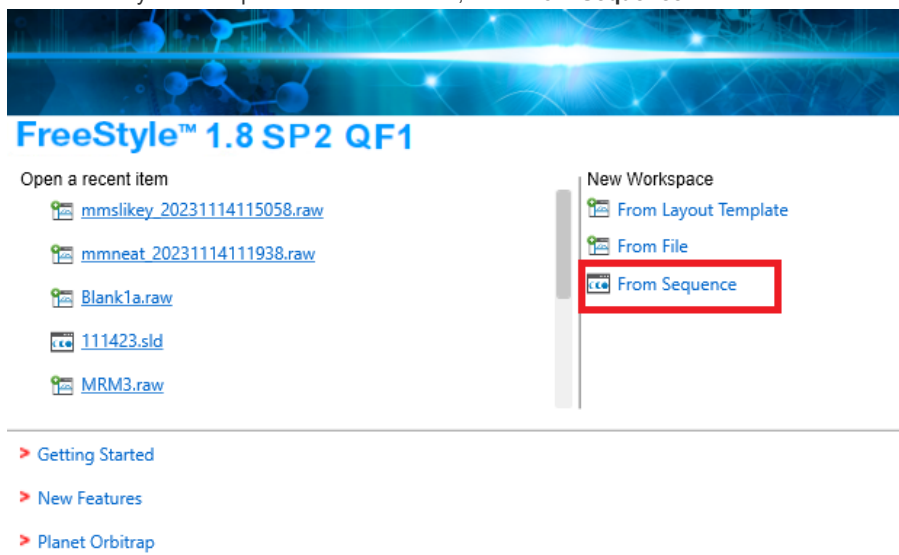
5. Data can be processed by clicking **Peak Detection** within the info bar to the left of the chromatograms and spectra.
6. First, select the plot you wish to select peaks in. It will turn gray in the background.
7. Select the peak algorithm you want to use by using the dropdown arrow under "Peak Detection Algorithm". The ICIS algorithm is used for MS data (Genesis is legacy detection and should **NOT** be used). The Avalon algorithm is used for PDA, UV, and analog data.
8. Click **Auto Cal Initial Events** (for UV data) or **Apply** (for MS data) to perform the processing. You will have the option to adjust thresholds as necessary to display all data. This process can be repeated for each plot.
9. The data can be printed using the typical File > Print option, but can also be exported as a CSV.
10. To export the data as a CSV, click **Workspace Options** followed by **Exports** and finally **Export Selection As**.
11. The next window will give you the option to select CSV as the exported format. Clicking **OK** will then launch a browser window to select where the file should be exported to.

Analysis of MRM Data (Advanced)

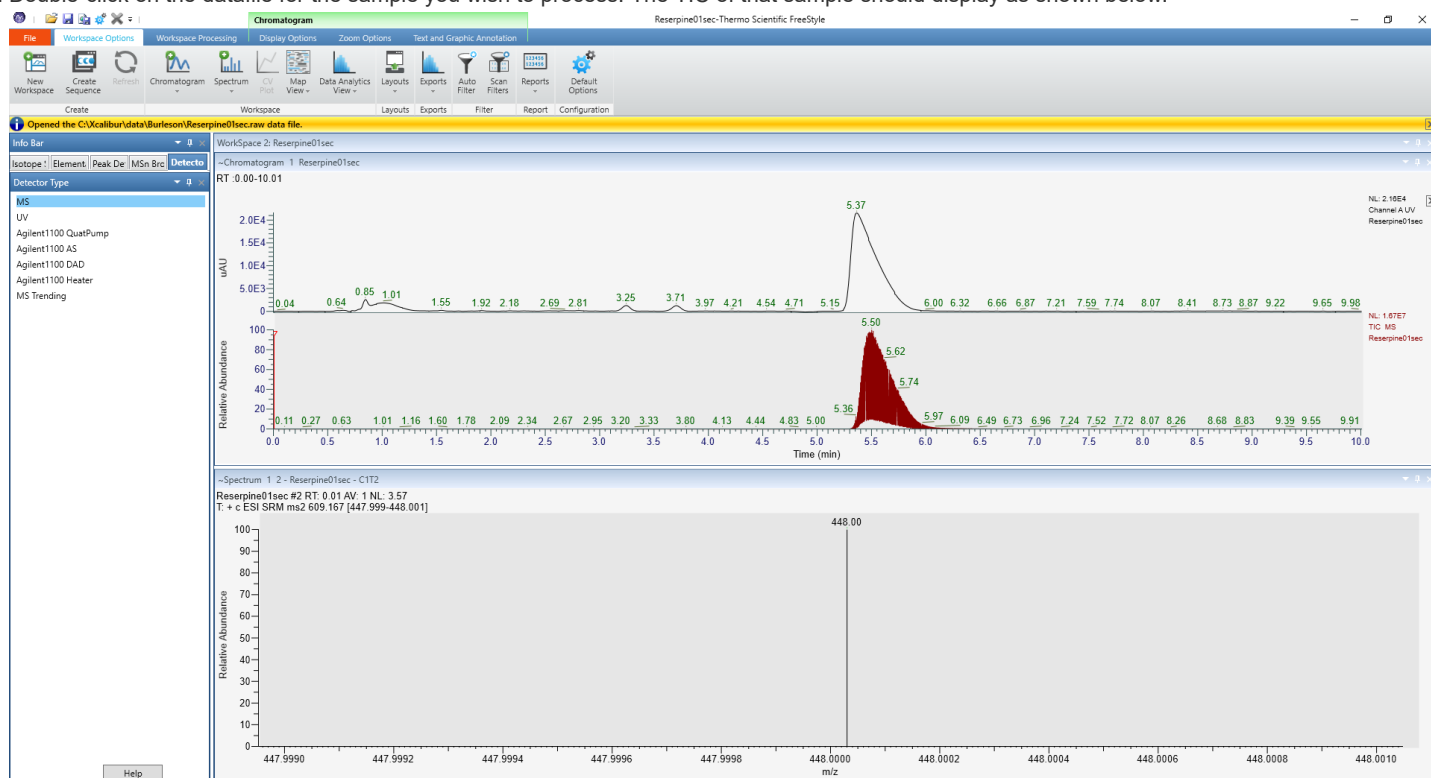
1. Open the Freestyle software from the main page of Xcalibur. The icon is shown below.



2. Within Freestyle's startup screen shown below, click **From Sequence** under the "New Workspace" list and locate the sequence of interest.



3. Double-click on the datafile for the sample you wish to process. The TIC of that sample should display as shown below.

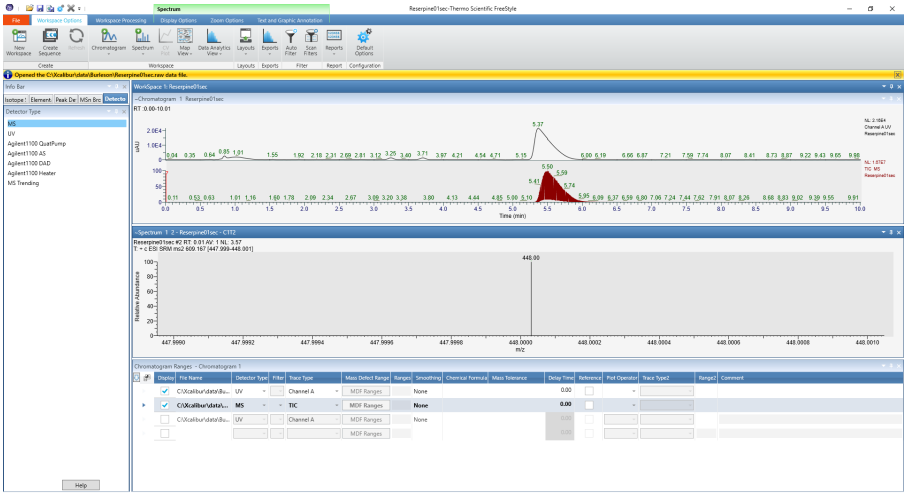


4. The next steps will detail inserting/removing plots, smoothing data and filtering the scans. **Inserting/Deleting Chromatograms and Spectra:**

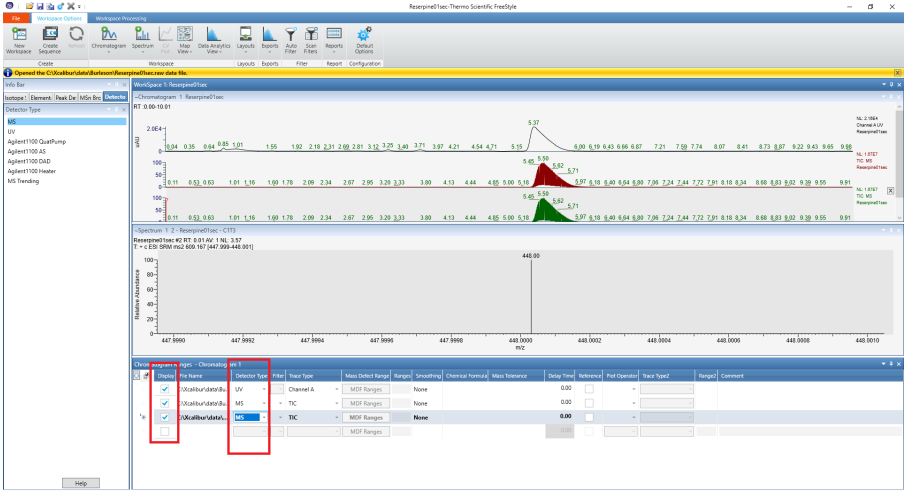
- To **insert** a chromatogram, such as an additional TIC, click in the TIC window so that the background is gray meaning it has been selected.
- Right-click and select **Insert Chromatogram** to add the new TIC spectrum.
- To **delete** a chromatogram, click the **X** to the far-right of the chromatogram.
- To **insert** an additional spectrum, under the "Workspace Options" tab, click **Spectrum** followed by clicking **Insert View**. Be sure to click within a spectrum to activate it before clicking through the TIC.
- To **delete** the additional spectrum, click the **X** in the top-right corner of the newer spectrum.

5. The next steps will detail filtering the scans.

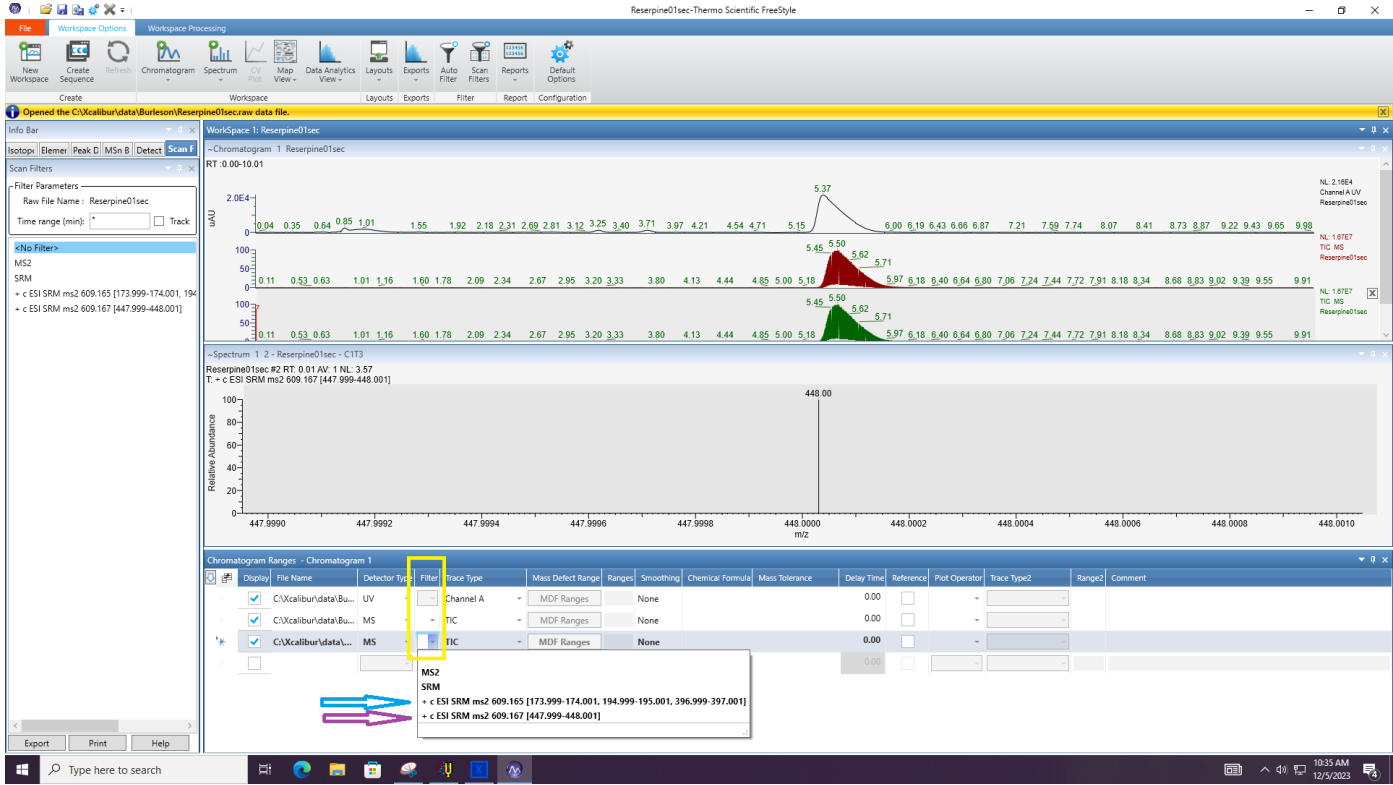
- i. To **filter a chromatogram** to examine a specific range, right-click in the TIC window and select **Ranges**. The "Chromatogram Ranges" window will open at the bottom of the screen. It is shown below.



- ii. It is recommended to open a new chromatogram of the TIC. Do this by clicking the checkbox under "Display" as shown below by the left-most red rectangular box. Then, under "Detector Type", select **MS** as shown by the right-most red rectangular box in the same image below.



iii. A specific transition can be extracted from the TIC by clicking the dropdown arrow under "Filter" (highlighted in yellow below). You will see the chromatograms that can be extracted for analysis. Clicking a specific transition will plot its TIC in the newly inserted plot.



iv. In the image above, two arrows can be observed in the mass filtering list. The blue arrow indicates data for three transitions that were collected with the SRM Parent Mass set to the same value for the transitions. The purple arrow indicates a transition where a slightly different parent mass was entered in the SRM Parent Mass. This highlights the detail in Step 6 under the **Creating A MRM/SRM Method (ADVANCED)** section.

- The data can be printed using the typical File > Print option, but can also be exported as a CSV.
- To export the data as a CSV, click **Workspace Options** followed by **Exports** and finally **Export Selection As**.
- The next window will give you the option to select CSV as the exported format. Clicking **OK** will then launch a browser window to select where the file should be exported to.

Shutdown

- When you are finished with the instrument, ensure the MSD is in standby mode with the argon gas turned off (if used).
- Make sure there is no flow from the LC and that the detector lamp(s) is off. Both of these can be adjusted from the Direct Control windows mentioned above.
- You may close all programs.