

# TN3601: Waters HPLC System Connection Guide for Wyatt Instrumentation

## Summary

This document provides the basic guidelines for interfacing a Waters™ HPLC system with Wyatt Technology™ instruments. If you are planning to connect a Waters ACQUITY UPLC™ system to Wyatt instruments, please refer to Technical Note TN3610.

The first section details the electrical connections necessary to transfer the signal of a Waters UV or RI concentration detector and the contact closure signal from an autosampler (to synchronize sample injection and data collection) into the ASTRA™ software.

The second section describes the necessary software settings in ASTRA software and Empower™ software.

## Related References

M1000/M1006/M1111 ASTRA software User's Guides  
 M3200/M3220 DAWN MALS detector User's Guides  
 M3000/M3010/M3020 miniDAWN MALS detector User's Guides  
 M1510/M1520 Optilab detector User's Guides  
 M1600/M1610/M1620 ViscoStar viscometer User's Guide  
 TN1007 ASTRA software Quick Guide  
 TN3610 ACQUITY UPLC System Connection Guide  
 Any relevant Waters User's Guides

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## Introduction

This guide describes the connections necessary to interface Wyatt detectors and a Waters HPLC system such as the Alliance™ HPLC system. During installation, place the optical detectors of the HPLC system as close as possible to the Wyatt instruments: short tubing reduces delay volumes and maintains the chromatographic resolution. Note the arrangement of the components in Figure 1, with the chromatography system located on the left of and under the Wyatt Technology instrumentation to minimize inter detector tubing lengths. If necessary, adjust the placement of the detectors within the Waters instrument stack to ensure the minimum tubing length.



*Figure 1. Example of a representative Waters ACQUITY™ UHPLC system with a Wyatt DAWN™ (NEON)™ light scattering detector and a Wyatt Optilab™ (NEON) refractive index detector. Note that the Waters components, column oven, optical detectors, and Wyatt detectors are stacked to help minimize the tubing length. The image shows a Waters ACQUITY UHPLC system, but the placement example also applies to a HPLC system. No tubing connections are present in this image.*

## Establishing the Connection

Transferring signals from Waters instruments to ASTRA software requires establishing a physical connection to a Wyatt instrument. Waters uses a screw terminal I/O signal connector on the rear panel of their equipment (Figure 2) for signal output. A RJ-12 cable with flying leads (Wyatt p/n P4045-19; Figure 2) transfers the signal to an in-port on the rear panel of a Wyatt instrument (Figure 3). The principle is the same for all Waters equipment, but the identity of the ports of the screw terminal vary between instruments.

After describing the general procedure in this section, some representative port assignments will be shown for different Waters detectors in [Transferring Signals from Waters UV/Vis and RI Detectors](#). Sending a contact closure signal from a Waters autosampler to ASTRA software is covered in [Connecting to a Waters 2695 Autosampler](#).

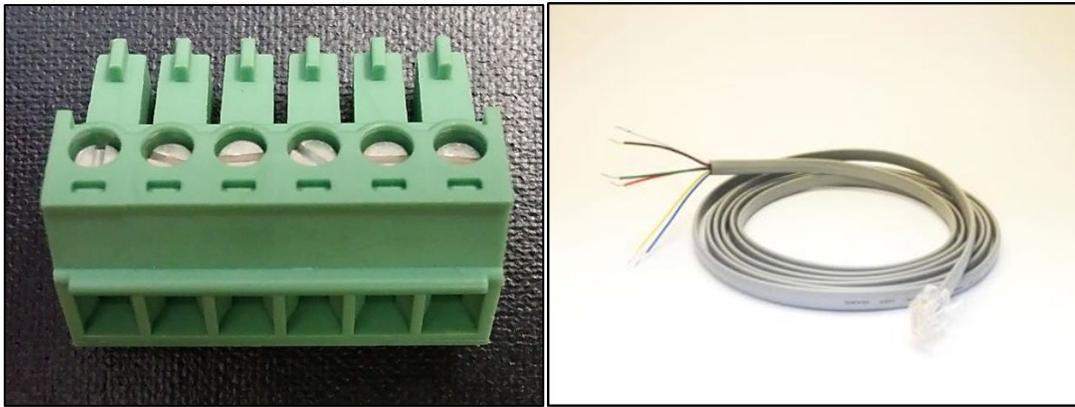
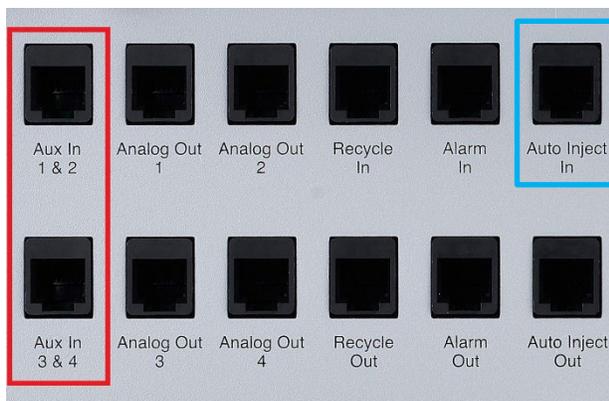
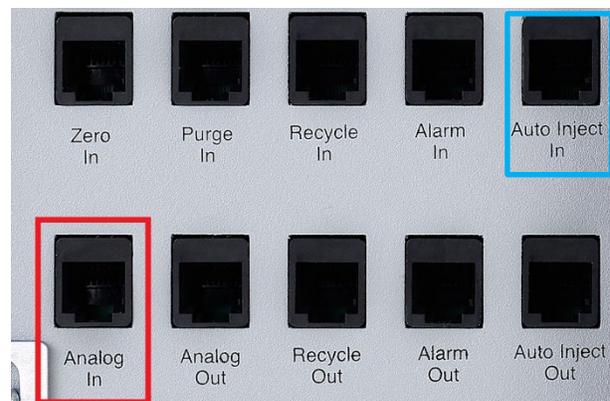


Figure 2: Screw terminal connector used on the rear panel of Waters instruments (left) and RJ-12 cable (right).

On the Wyatt instrument side, the RJ-12 cable has to be connected to an in-port. For analog detector signals, the receiving ports of the light scattering detectors are labelled **Aux In 1 & 2** and **Aux In 3 & 4** (Figure 3a). The ports of Optilab detector and ViscoStar™ viscometer instruments are labelled **Analog In** (Figure 3b). Contact closure signals (auto inject signals) that denote sample injection are received through the **Auto Inject In** port of all Wyatt instruments (Figure 3).



a) Auxiliary signal ports (red) and auto inject in signal port (blue) on the rear panel of the DAWN (NEON) MALS detector. Auxiliary port locations are similar in other DAWN MALS detectors, but they are positioned side by side along the first row (DAWN HELEOS™ I/II MALS detector, and miniDAWN™ TREOS™ I MALS detector). The miniDAWN (NEON) MALS detector and miniDAWN TREOS II MALS detector only have one Aux In port (1&2). Auto-Inject In port location is the same for all the DAWN™ MALS detectors and miniDAWN detectors.



b) Auxiliary signal port (red) and auto inject in signal port (blue) on the rear panel of the Optilab (NEON) detector. These port locations are the same for the other Optilab detectors (Optilab T-rEX™ detector, Optilab UT-rEX™ detector), and ViscoStar II viscometer. The ViscoStar III viscometer and ViscoStar NEON viscometer locations are similar, but their rear panels have fewer ports.

Figure 3. In-ports of Wyatt Technology instruments.

The RJ-12 cable has six available leads to establish connections. The input channel assignment of an optical signal in ASTRA software depends on which lead is connected to the analog out port of the Waters instrument. The possible combinations are detailed in Table 1.

		BNC		
		red	black	
DAWN MALS detector and miniDAWN MALS detector	AUX IN 1 & 2	channel 1	White RJ-12	Black RJ-12
		channel 2	Red RJ-12	Green RJ-12
	AUX IN 3 & 4	channel 3	White RJ-12	Black RJ-12
		channel 4	Red RJ-12	Green RJ-12
Optilab detector, ViscoStar viscometer	Analog In		Red RJ-12 White RJ-12	Green RJ-12 Black RJ-12

Table 1. Wire color conventions for using an RJ-12 cable.

Crimping the white and black wires to the BNC adaptor and using **Aux In 1 & 2** as the signal in-port transmits the signal to Aux channel 1. If **Aux In 3 & 4** is used as the in-port, the transmission is to Aux channel 3. Alternatively, connecting the red and green wires to the BNC adaptor transmits the signal to Aux channel 2 or 4, depending on the Wyatt Aux In port the RJ-12 is plugged in to.

\*The Wyatt Optilab T-rEX detector, Optilab UT-rEX detector and Optilab (NEON) detector or Wyatt ViscoStar II viscometer instruments have only one Analog Input port, labeled "**Analog IN**". This port has only one active channel, which uses the green (-) and red (+) wires of the RJ-12 cable. When wiring to connect to either of these ports, one must connect the green wire from the RJ-12 cable to the black binding post of the BNC connector, and the red wire from the RJ-12 cable to the red binding post of the BNC connector. In contrary to miniDAWN TREOS I MALS detector and the DAWN HELOS I/II MALS detectors and DAWN (NEON) MALS detectors with two Aux In ports (Aux In (1&2) and Aux In (3&4)), the miniDAWN TREOS II MALS detector/ miniDAWN (NEON) MALS detector have one **Aux In (1&2)** port where the active channel depends on the wiring in the above table. The ViscoStar III viscometer/ViscoStar (NEON) viscometer have one **Analog In** port where the active channel depends on the wiring and is different than the above table (2 channels with either white-black (Analog In 2) or red-green (Analog In 1) wiring pairs).

Note: The blue and yellow wires of the RJ-12 cable are ground wires and, therefore, are not necessary for any connections. We recommend taping off any unused wires on the RJ-12 cable with electrical tape unless you observe excessive noise pickup.

## Transferring Signals from Waters UV/Vis and RI Detectors

This section provides connection information for common Waters optical detectors. In case of any questions about your instrument's port assignment, please refer to the respective Waters manual.

### Connecting to a Waters 2487 Dual $\lambda$ Absorbance Detector

1. Locate a RJ-12 cable with flying leads.
2. Pull out the appropriate screw terminal connector on the back of the Waters detector, in this case connector B. (See Figure 4.)
3. This detector can be used to record absorbance at two wavelengths simultaneously. To output channel A, attach two of the wires of the RJ-12 cable (see Table 2 for wire colors) to screw terminals B1 and B3 of connector B on the back panel of the UV detector. For channel B, connect two wires to B5 and B7. Figure 4 shows an example of an RJ-12 connected to the terminal connector.

Table 2. Wire colors for outputting UV channels from Waters ports.

Waters port	Description	RJ-12 lead
B1	ChA 2V	white or red
B3	ChA Ground	black or green
B5	ChB 2V	white or red
B7	ChB Ground	black or green

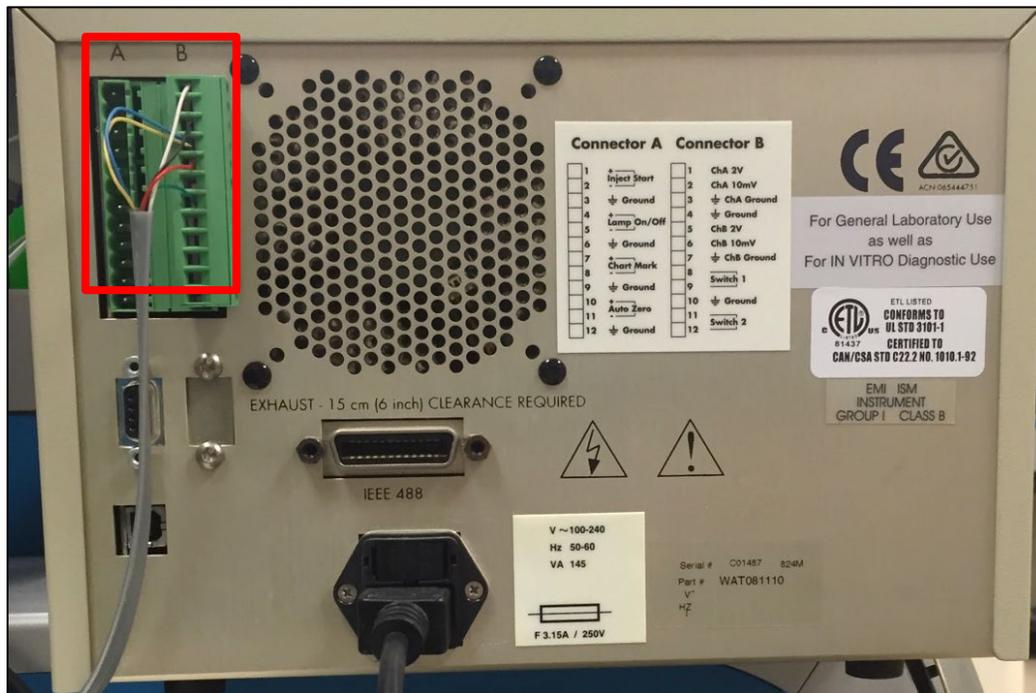


Figure 4. Back panel of a Waters 2487 Dual  $\lambda$  Absorbance Detector. In this case, the RJ-12 white and black leads are connected to screw connector ports B1 and B3 and transmit channel A; and the red and green leads are connected to ports B5 and B7 to transmit channel B.

4. Plug the connector back into the rear panel of the UV detector.
5. Plug the RJ-12 cable into your Wyatt instrument. See Figure 3 for port locations and [Table 1](#) for channel assignments.

### Connecting to a Waters 2489 UV/Vis Detector

Follow the steps detailed for the Waters 2487 Dual  $\lambda$  Absorbance Detector above. Use connector I and screw terminal ports 1 and 2 (labelled “Analog 1”) to output the data of channel A. Use ports 4 and 5 (labelled “Analog 2”) to output data of channel B.

### Connecting to a Waters 2996 PDA Detector

Follow the steps detailed for the Waters 2487 Dual  $\lambda$  Absorbance Detector above. Use the right connector and screw terminal ports 5 and 6 (“Analog Out 1”) to output data.

## Connecting to a Waters 2414 Refractive Index Detector

Follow the steps detailed for the Waters 2487 Dual  $\lambda$  Absorbance Detector above. Use connector B and screw terminal ports 9 and 10 (labelled “Detector out”) to output data.

The Waters 2414 Refractive Index Detector can be run in standalone mode (410 or RIU mode) to generate an analog output voltage.

In RUI mode, the RIU Full Scale (RIU-FS) setting is used to generate the output. In 410 mode, the sensitivity setting is used to generate the output. The RI calibration constant in the ASTRA software corresponding to the Waters setting is given in the middle column. See [Configuring the ASTRA Software to Accept Auxiliary RI Signals](#) below for details.

*Table 3. Waters RI detector settings and the ASTRA software calibration constant.*

Sensitivity	ASTRA software RI calibration constant (RIU/V)	Equivalent $\mu$ RIU-FS
1	$5.000 \times 10^{-3}$	10000
2	$2.500 \times 10^{-3}$	5000
4	$1.250 \times 10^{-3}$	2500
8	$6.25 \times 10^{-4}$	1250
16	$3.125 \times 10^{-4}$	625
32	$1.5625 \times 10^{-4}$	313
64	$7.8125 \times 10^{-5}$	156
128	$3.9063 \times 10^{-5}$	78
256	$1.9531 \times 10^{-5}$	39
512	$9.766 \times 10^{-6}$	20
1024	$4.883 \times 10^{-6}$	10

## Connecting to a Waters 2695 Autosampler

To synchronize sample injection and data collection in the ASTRA software, the contact closure signal (autoinject signal) has to be transferred to the Wyatt detector.

1. Locate a RJ-12 cable with flying leads.
2. Pull out screw terminal connector B on the back of the Waters Autosampler. (See Figure 5)
3. Using a flat head screwdriver, connect the green and red wires to screw terminals 1 and 2 of I/O signals connector B on the back panel of the autosampler. Make sure the wires are secure and contact with the connector is achieved.

Table 4. Wire colors for outputting the autoinject signal from a Waters device.

Waters port	Description	RJ-12 lead
port B1	Inject Start (Out)	green
port B2	Inject Start (Out)	red



Figure 5. Back panel of a Waters 2695 autosampler. The I/O screw terminal connector B is boxed in red.

- Place the screw terminal connector back into the Waters Autosampler.
- Plug the RJ-12 cable into the [Auto Inject In](#) port located on the rear panel of the Wyatt instrument (see Figure 3).
- The autosampler is probably already configured to send an adequate electrical pulse (contact closure) to the Wyatt detector upon injection; this pulse can be used to initiate the ASTRA software data collection if ASTRA software is configured as described in [Configuring the ASTRA Software to Accept Autoinject Signals](#).

**Note:** The ASTRA software cannot control the Waters HPLC system. There is no formal communication between the Waters HPLC system and ASTRA software beyond this auto-injection contact closure signal.

## Synchronization of the Data Traces

To optimize synchronization of the data traces recorded with two Wyatt detectors, we recommend sending the HPLC autoinject signal to both detectors. This is done by connecting the two instruments through an autoinject crossover cable (Wyatt p/n 164250; Figure 18). Plug the end of the cable labeled [AUTO INJECT OUT](#) into the [Auto Inject Out](#) port on the rear panel of the detector that received the signal from the HPLC system. Plug the other end

labeled **AUTO INJECT IN** into the **Auto Inject In** port on the rear panel of the second detector. See Figure 3 for the location of the ports and Figure 6 for an illustration of the connection.



Figure 6. An autoinject crossover cable connection between a Wyatt light scattering detector (top) and a Wyatt RI detector (bottom). The crossover cable transmits the autoinject signal received by the light scattering detector to the RI detector for optimized synchronization.

## Configuring the ASTRA Software to Accept Auxiliary UV Signals

In order for the ASTRA software to process analog signals from external instruments connected through the auxiliary inputs of Wyatt instruments, the experiment **Configuration** has to contain a definition of the *instrument* that is connected and the *channel* used to transmit the signal. These steps for connecting a third-party UV detector are detailed below.

1. Select the appropriate ASTRA software method and instrument configuration. Refer to the *ASTRA software Quick Guide* and the *ASTRA software User's Guide* for assistance with your particular detector configuration.
2. In **Configuration**, select **Generic UV Instrument** to define the parameters of the instrument that has been connected (see Figure 7).
  - **Cell Length (cm)** – The length of the UV cell in cm. (This value is often 1.0 cm.)
  - **Channels** – Enable the auxiliary channels that have been physically connected in the procedure detailed above.

- **Wavelength** – The wavelength monitored in this channel (typically 280 nm for proteins).
- **UV response** – The UV calibration constant for the Aux channel which is used to convert the volts of the analog signal to absorbance units. Often, this value is 1 AU/V.
- **Active Channel** – Select the channel that will be used to calculate sample concentration from UV absorption.

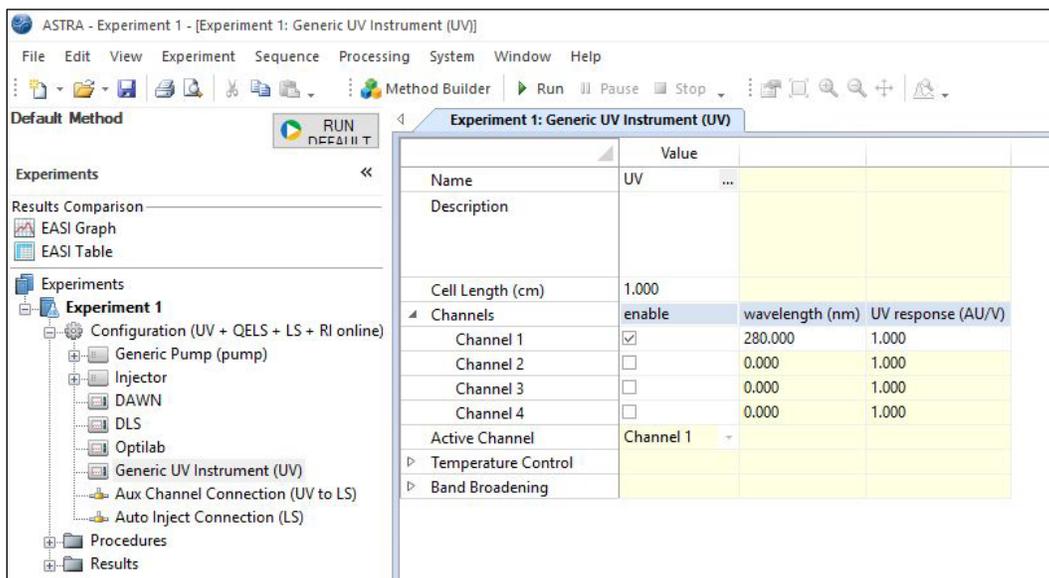


Figure 7. A screenshot of ASTRA software with the **Configuration** node expanded and **Generic UV Instrument** selected.

3. Select **OK** to save changes and finish defining the UV instrument parameters.
  4. Double-click on **Aux Channel Connection (UV to LS)** to see the settings for this configuration in the right-hand pane (see Figure 8). One **Aux Channel Connection** has to be defined in the experiment configuration for every physical analog input connection that has been established to enable ASTRA software to display and use the analog signal. In case multiple auxiliary input channels have been connected, multiple **Aux Channel Connections (UV to LS)** need to be defined. (Calculating the concentration of a sample from an auxiliary input is only possible for an **Active Channel** defined in step 2 above.)
- The parameters are:
    - **Source Device** – The instrument that sends analog data over this connection. The default setting here is ‘Generic UV Instrument’.
    - **Source AUX Channel** – If multiple channels are enabled under the **Generic UV Instrument** configuration (step 2 above), the drop down menu will list all available channels. Select the channel for this connection.
    - **Destination Device** – The instrument that receives analog data over this connection. The default setting is the light scattering detector (e.g. ‘DAWN’).
    - **Destination AUX Channel** – The destination channel is defined by the physical connection to the Wyatt instrument **AUX IN** port from the UV detector (refer to Table 1). For example, a RJ-12 cable with its white and black cables connected to the analog out of a UV detector and plugged into the **AUX IN 1 & 2** port of the DAWN MALS detector instrument will be input AUX channel number 1.

**Note:** For the Wyatt Optilab T-rEX detector, Optilab UT-rEX detector and Optilab (NEON) detector or Wyatt ViscoStar II viscometer detectors, the input AUX channel is always 2. For the DAWN HELEOS I/II MALS detector, DAWN (NEON) MALS detector, miniDAWN TREOS I/II MALS detector, miniDAWN (NEON) MALS detector, and ViscoStar III viscometer/ViscoStar (NEON) viscometer the input AUX channel depends on the wiring as described in [Table 1](#).

- **Calibration Constant** – This number is a correcting constant that can be used to adjust the value by which the AUX signal should be scaled. Under most circumstances, this value should be left at 1. If the calibration constant is changed to 2, then the calculated molar mass will be doubled. If this value is changed to 0.5, then the calculated molar mass will be halved, if the concentration source is chosen to be UV signal.

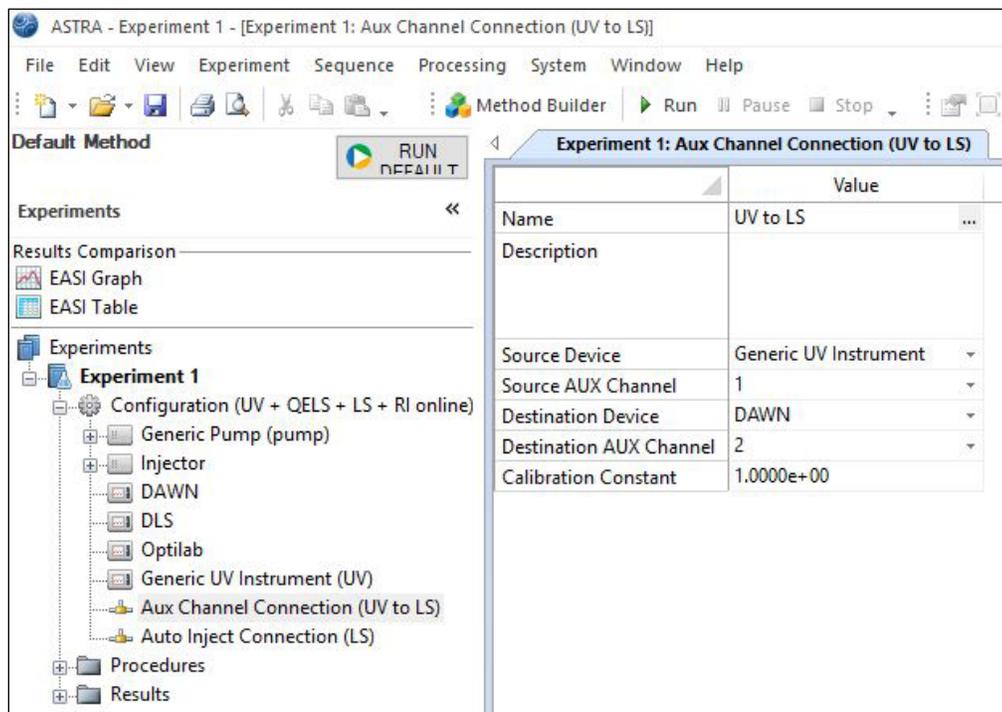


Figure 8. A screenshot of the ASTRA software with the **Configuration** node expanded and the **Aux Channel Connection (UV to LS)** selected.

5. Select **OK** to save changes and to exit this window.

This completes the ASTRA software setup necessary for the UV detector.

## Configuring the ASTRA Software to Accept Auxiliary RI Signals

1. Select the appropriate ASTRA software method and instrument configuration. Refer to the *ASTRA software User's Guide* for assistance with your particular detector configuration.
2. Once the correct ASTRA software method and instrument configuration have been selected, expand the **Configuration** node and double-click on **Generic RI Instrument (Generic RI)** to select this instrument profile.
3. Enter the wavelength of your detector.

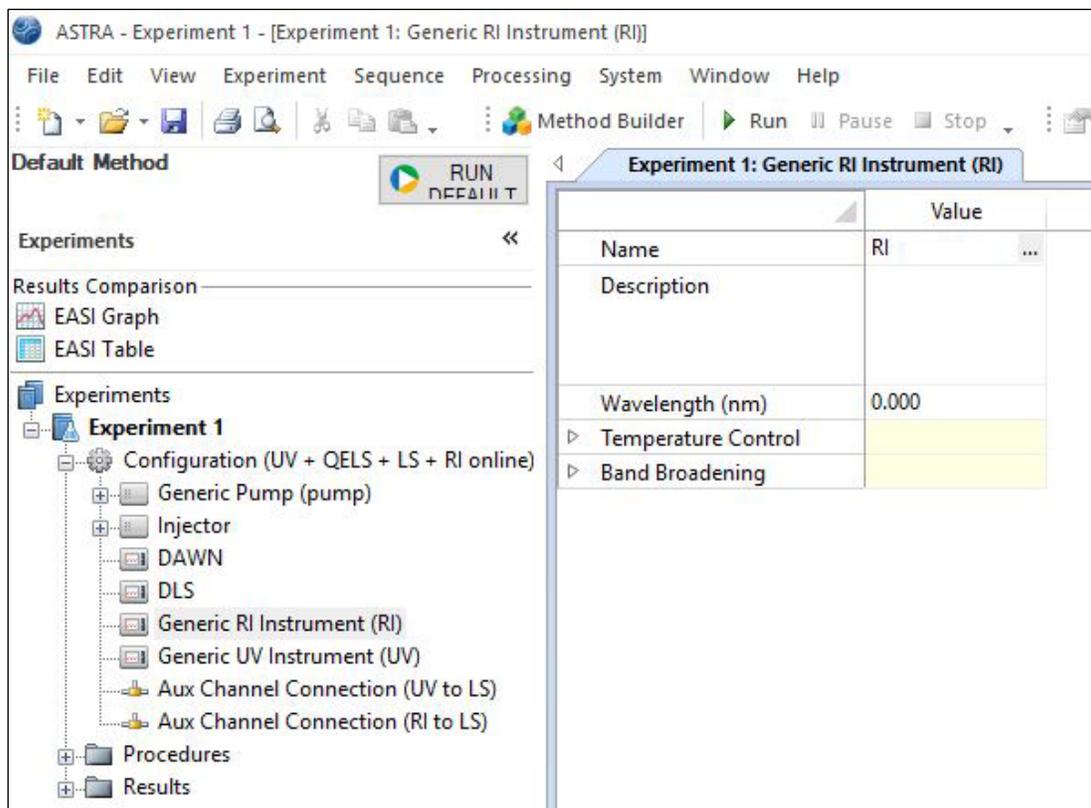


Figure 9. A screenshot of the ASTRA software with the **Configuration** node expanded and **Generic RI Instrument (Generic RI)** selected.

4. Select **OK** to save these changes and close the window.
5. The **Aux Channel Connection (RI to LS)** configuration defines the physical analog input connection that has been established between the RI detector and the Wyatt instrument as described above. This definition is necessary for ASTRA software to be able to use the analog RI signal. Expand the **Aux Channel Connection (RI to LS)** configuration by double-clicking on the heading (see Figure 10). The parameters are:
  - **Source Device** – The instrument that sends analog data over this connection. The default setting is Generic RI Instrument.
  - **Destination Device** – The instrument that receives analog data over this connection. The default setting is the light scattering detector (e.g., 'DAWN').
  - **Destination AUX Channel** – The destination channel is defined by the physical connection to the Wyatt instrument **AUX IN** port from the RI detector (refer to Table 1). For example, a RJ-12 cable with its white and black cables connected to the analog out of a RI detector and plugged into the **AUX IN 1 & 2** port of the DAWN MALS detector instrument will be **Destination AUX Channel 1**.
  - **Calibration Constant** – Unlike with the UV detector, in this case, this value is the calibration constant used to convert from volts to refractive index units, entered in units of RIU/V (refractive index units per volt). Refer to Table 3 for Waters RI detector settings and corresponding Calibration Constants. These are starting points. The calibration constant may need to be adjusted using standard. See the Waters detector hardware manual for more information on how to configure this constant.

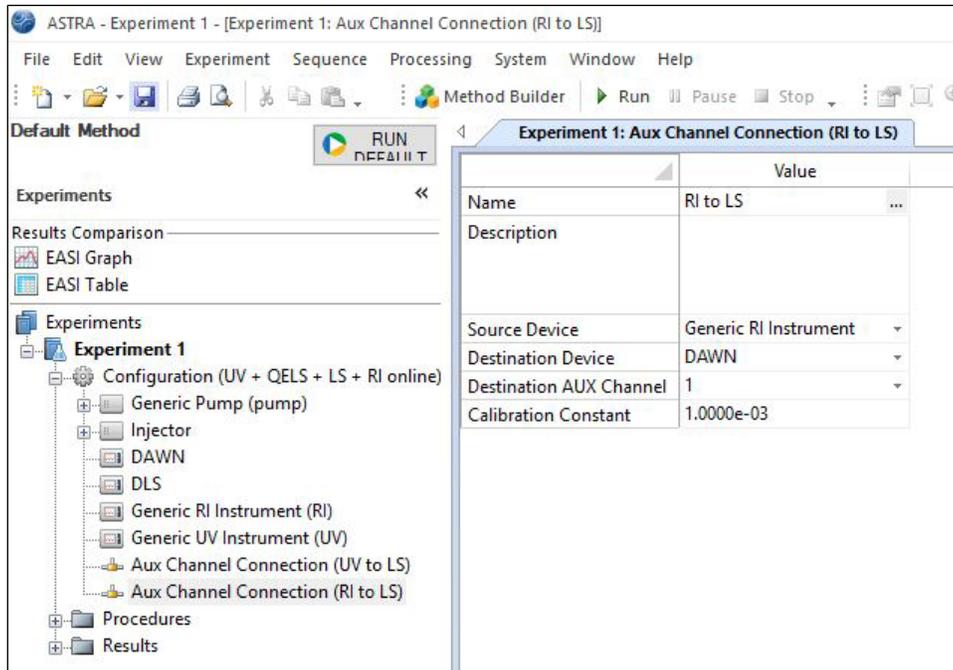


Figure 10. A screenshot of ASTRA software with the Configuration node expanded and Aux Channel Connection (RI to LS) selected.

6. Select **OK** to save changes and exit this window. This completes the ASTRA software setup necessary to connect to a Waters RI detector.

## Configuring the ASTRA Software to Accept Autoinject Signals

Follow the steps below to enable the ASTRA software to accept an autoinject signal from your HPLC system:

1. Expand the experiment **Configuration** and double-click on **Injector** to define the **Injected Volume** ( $\mu\text{L}$ ) (see Figure 11):

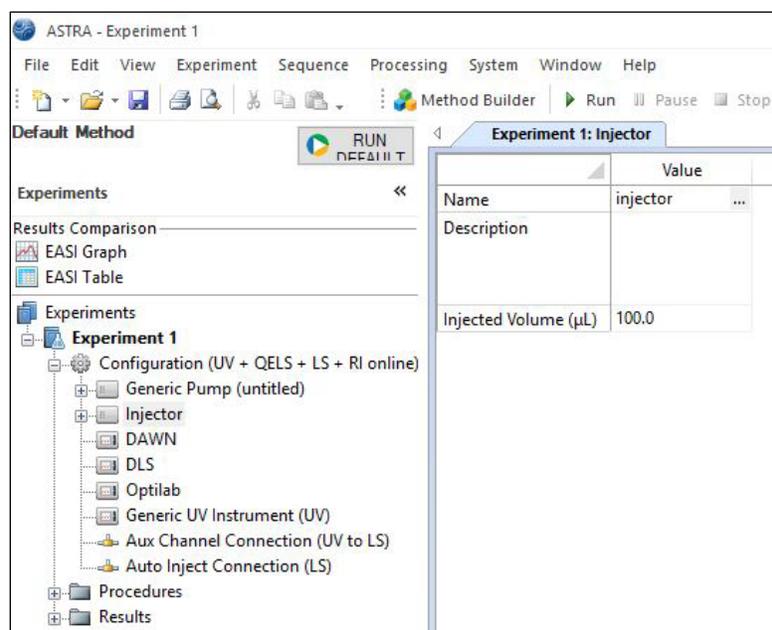


Figure 11. A screenshot of the ASTRA software with the **Configuration** node expanded and **Injector** selected.

2. Select **OK** to exit the window.

**Note:** ASTRA software uses the injection volume to calculate the injected mass. The injected mass is the product of the injection volume and the sample concentration entered in the **Sample** profile (sub-category of the **Injector** node). Using these values, the ASTRA software calculates the recovered masses, as well as associated mass recovery percentages. Entering the injection volume in ASTRA software does not control the injection volume that the autosampler will draw from the sample vial.

3. Double-click on **Auto Inject Connection (LS)** to open that profile. The parameters are:
  - **Source Device** – The instrument that sends the inject signal. The default setting is **Injector**.
  - **Destination Device** – The instrument that receives the inject signal. The default setting is the light scattering detector (e.g. 'DAWN').

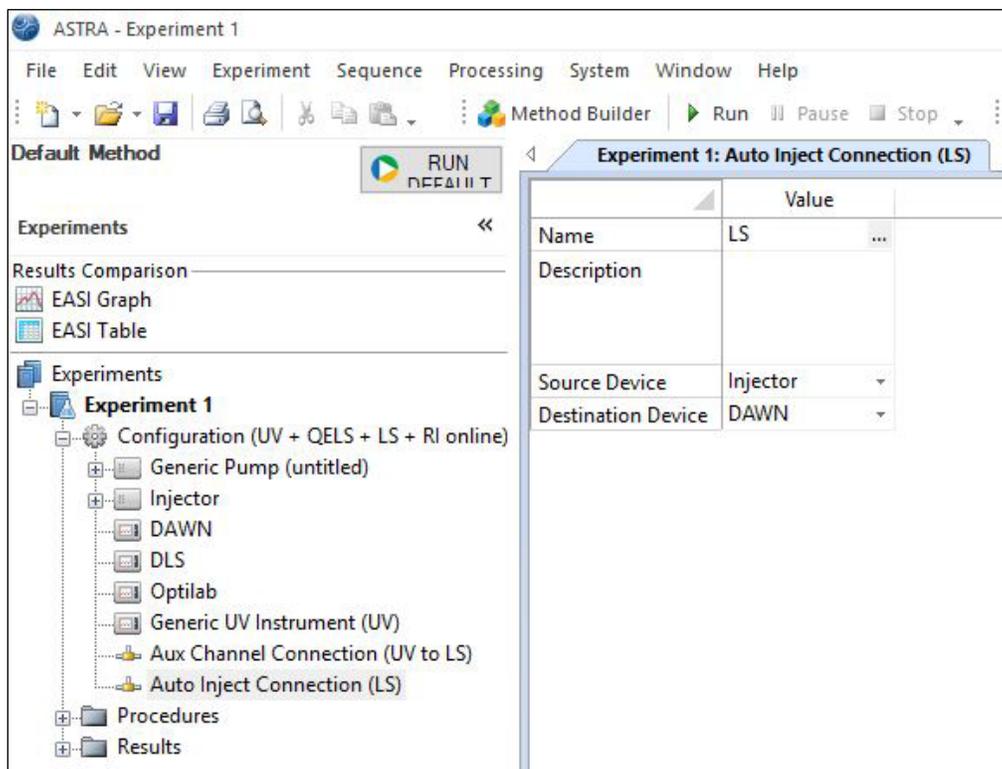


Figure 12. A screenshot of ASTRA software with the **Configuration** node expanded and **Auto-Inject Connection (LS)** selected.

4. Select **OK** to save and close this window.
5. Expand the **Procedures** node in the ASTRA software workspace and double-click on **Basic Collection** (see Figure 13).

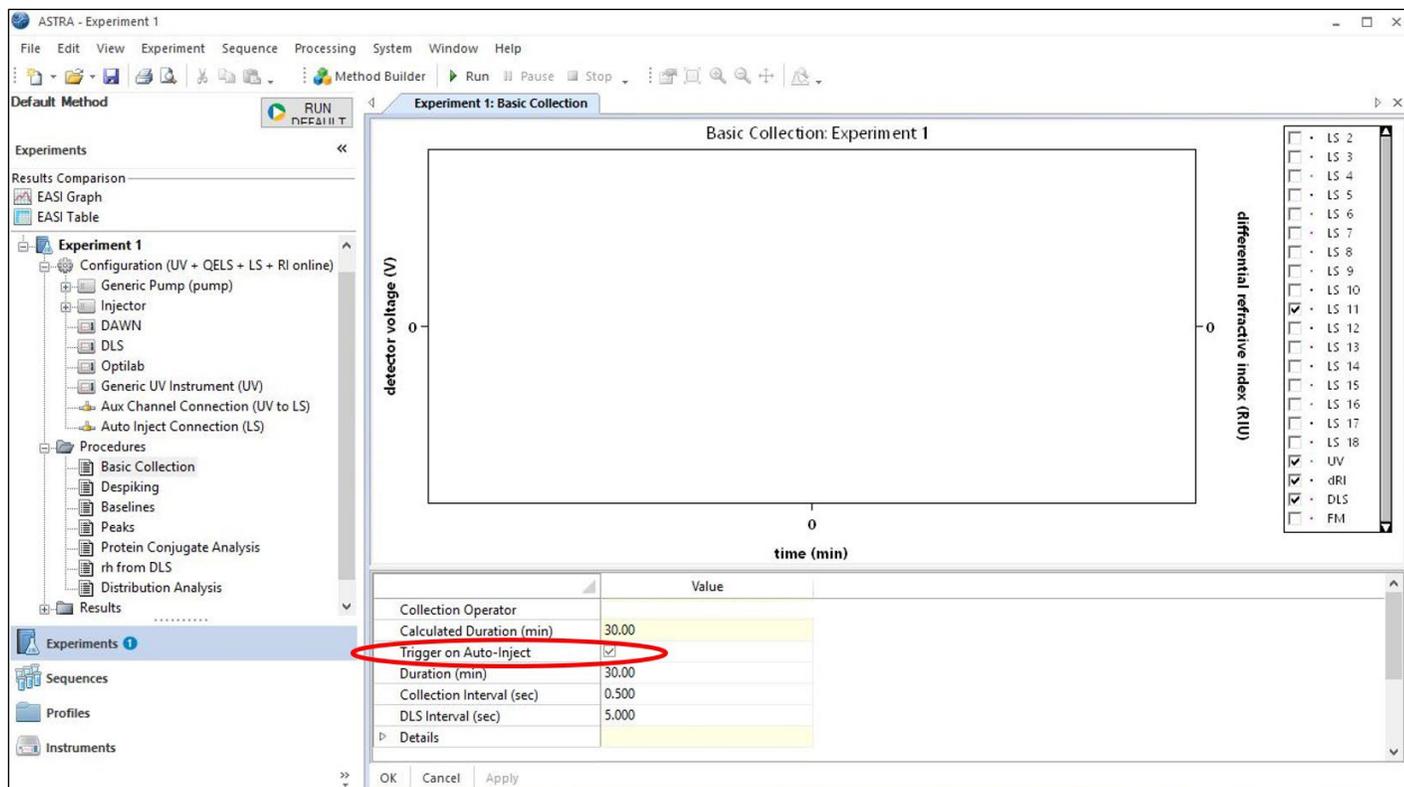


Figure 13. A screenshot of the ASTRA software screen with the *Procedures* node expanded and *Basic Collection* selected.

6. Check the **Trigger on Auto-Inject** box to have the Waters injection signal initiate the start of the ASTRA software data collection. Select **Apply** to save your settings. Data collection can begin now. This experiment can also be saved as a method for use in a future experiment or a sequence.

## Configuring the Empower 3 Software to Export the UV Signal

The Empower 3 software must be configured to export the UV signal so that a Wyatt detector can import it and send it to the ASTRA software.

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**Note:** The following screenshots and procedures are specific to Empower 3 software. If additional assistance is needed to configure different versions of Empower software, please contact Wyatt Technology Support.

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1. Launch the Waters Empower 3 software.
2. In the main workspace, select the method you wish to run and click **Edit** (see Figure 14).

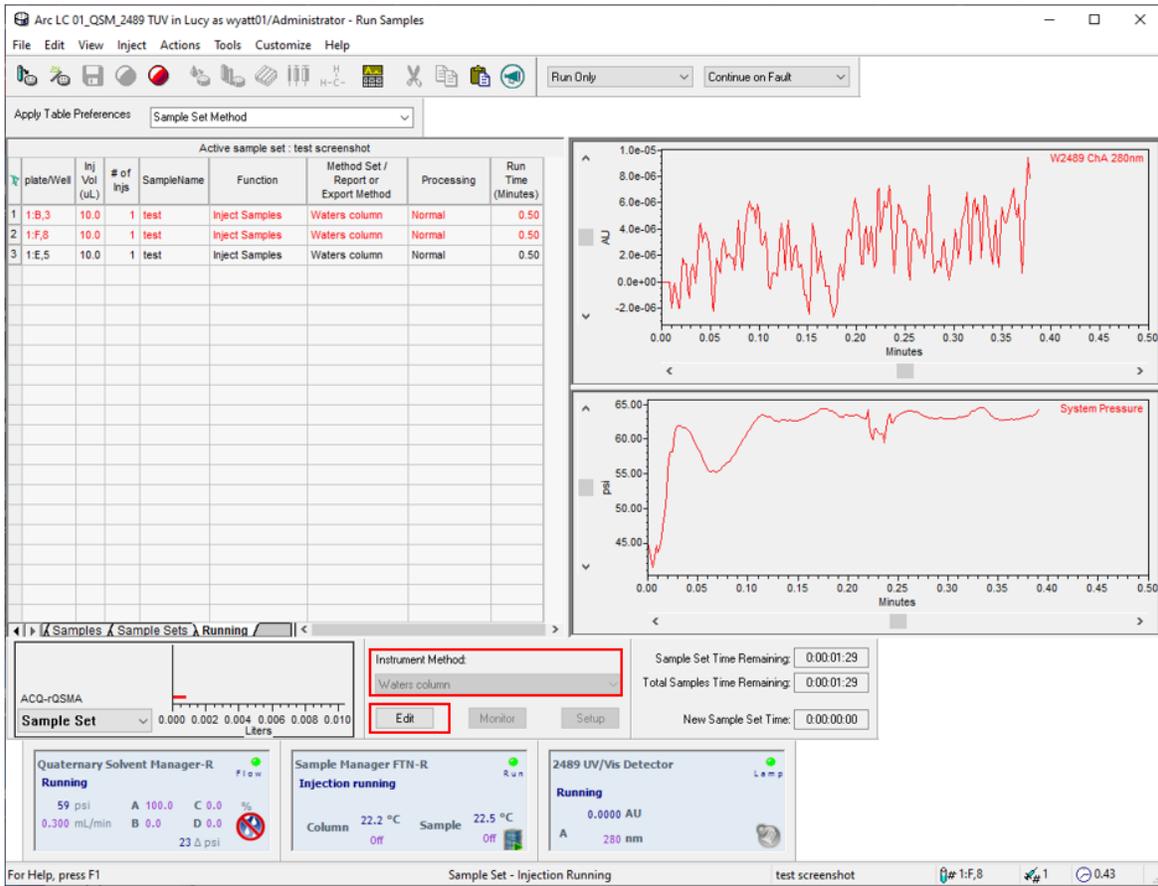


Figure 14. Screenshot of the Empower 3 software main screen.

3. Select the UV-detector module from the top of the method window. Refer to Figure 15.

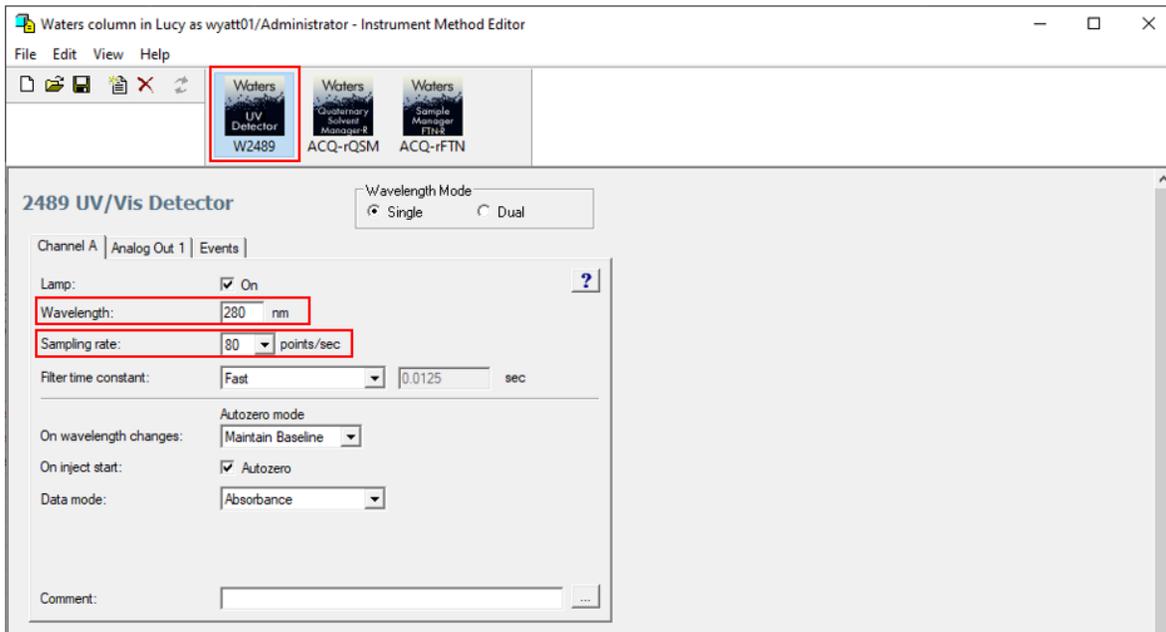


Figure 15. Screenshot of the Empower 3 software showing the method editing menu. The location of UV-detector module is highlighted by the red square as well as the field for Wavelength and Sampling Rate.

5. Under the **Channel A** tab, enter your wavelength (typically 280 nm) and set the sampling rate to **80 points/sec**.
6. Click on the **Analog Out** tab. This will open the menu shown in Figure 16. Verify that the **Sensitivity** is set to 1.00 AUFS and the **Voltage Offset** is set to 0 mV.

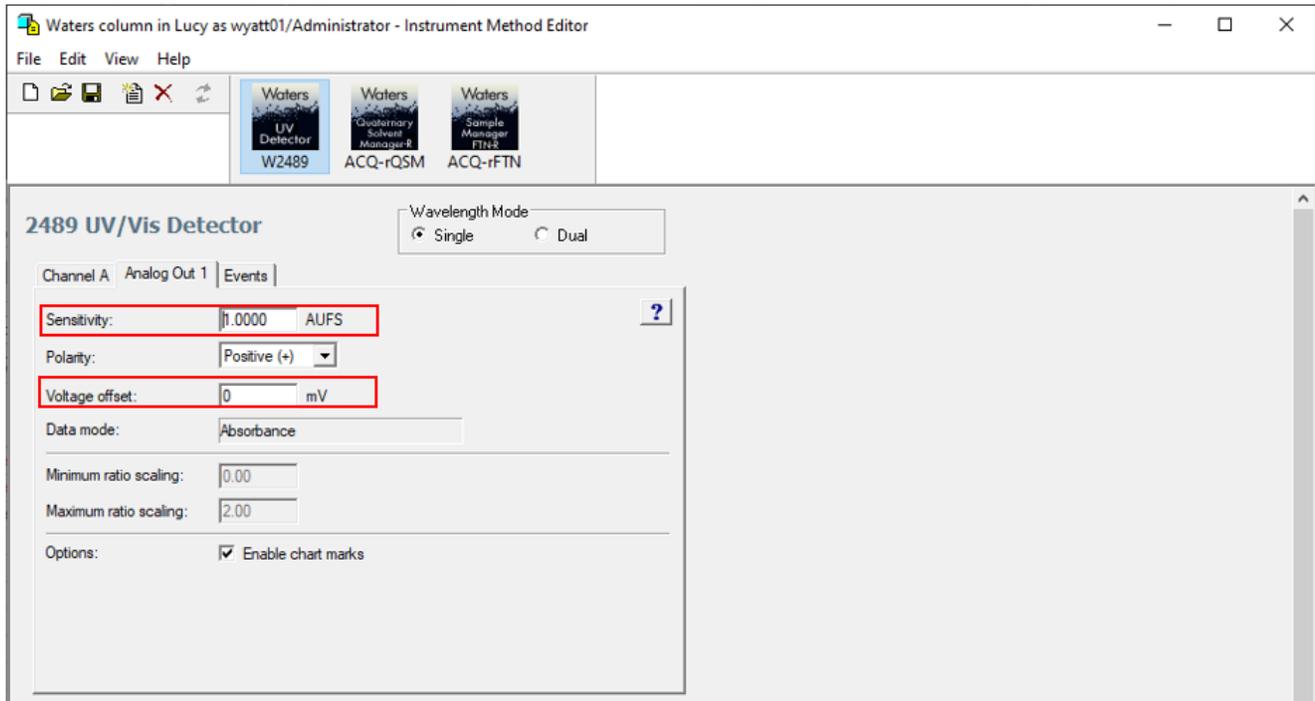


Figure 16. Screenshot of the Empower 3 software showing the **Analog Out** UV detector settings menu. The location of **Sensitivity**, and **Voltage Offset** are highlighted by the red squares.

If you have any questions, please email [support@wyatt.com](mailto:support@wyatt.com) or call Wyatt's support team at 805-681-9009, option 4 (within the US and Canada).

If you are one of our international customers, feel free to contact your local representative directly. You can find contact information for our global offices at [www.wyatt.com/Distributors](http://www.wyatt.com/Distributors).

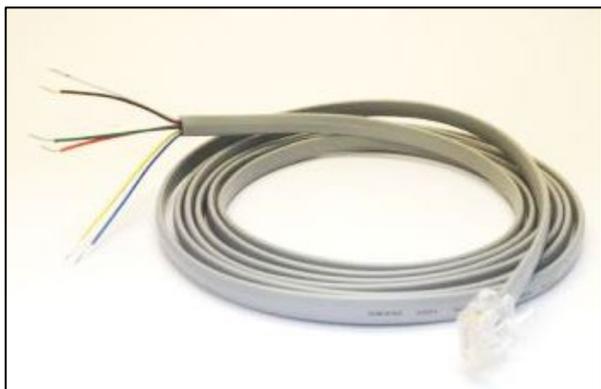
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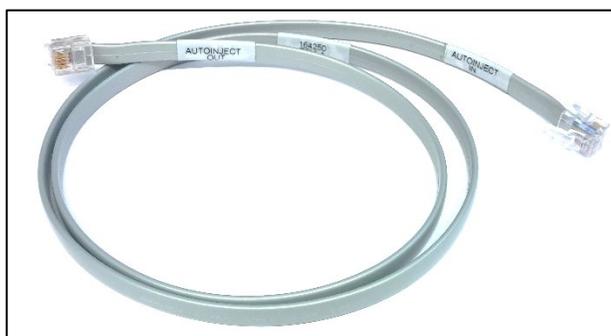
## Appendix

### Tools and Hardware

The following tools and hardware are necessary for a successful installation:



*Figure 17: RJ-12 Cable with Flying Leads (Wyatt p/n P4045-19).*



*Figure 18: Autoinject crossover cable (Wyatt p/n 164250).*



*Figure 19: Six (6) foot Ethernet Cable (Wyatt p/n P4045-14).*



*Figure 20: Slotted Screwdriver 3/16" (Wyatt p/n P9016-A).*



*Figure 21: Instrument Power Cable. 120V120VAC US plug (Wyatt p/n P4100-120-A) or a universal power cord. International customers will be sent a power cord that meets their country's specifications.*

## HPLC System Tubing Connection Overview

The following figure summarizes the relevant fluid connections and recommended tubing sizes:

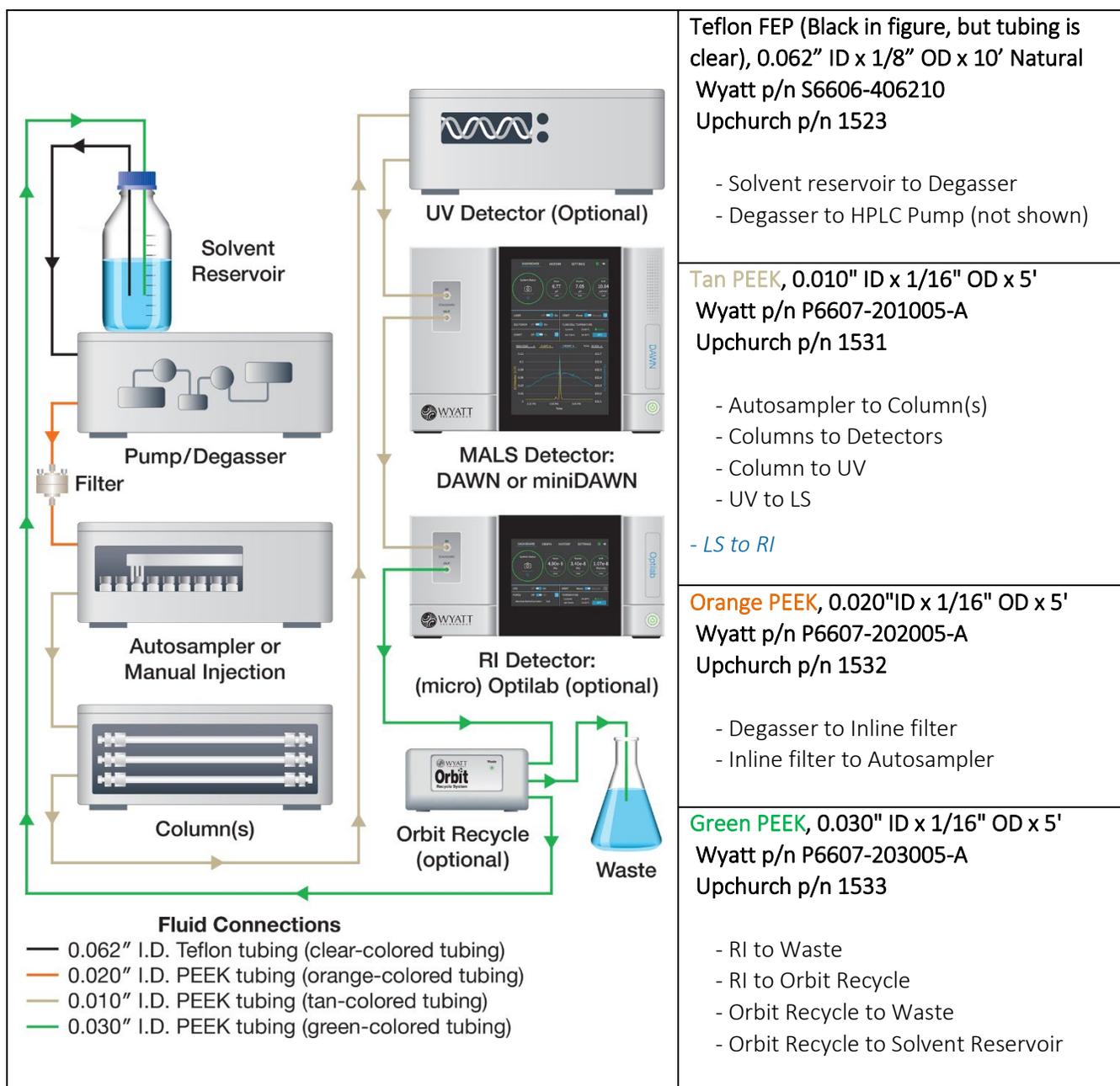


Figure 22. Overview of the recommended tubing connections between the HPLC and instrumentation. Tubing distances are not draw to scale, tubing lengths should be minimized to reduce delay volumes and maintain the chromatographic resolution. Refer to the tubing reference table above for tubing descriptions and relevant part numbers. Use standard Upchurch Scientific® PEEK finger-tight fittings (Wyatt p/n 6412-610; Upchurch p/n 55067-U).