ASTRA Quick Guide

Summary

The ASTRA Quick Guide is intended to help users after their Wyatt system has been installed and interfaced with an HPLC or UHPLC. The guide is written for ASTRA 7. Consult the ASTRA 6 User's Guide for more details.

Part I: Routine Operations covers running experiments and processing data. It assumes that an ASTRA method has already been setup.

Start at **Part II: System Setup**, if you need to set up an ASTRA method, change an existing method, or set system parameters such as *normalization*, *alignment* (interdetector delay), and *band broadening*.

Related Technical Notes and References

M1000 ASTRA 6 User's Guide

M1006 ASTRA 7 User's Guide

TN1001 SEC-MALS Branching Analysis Using the Radius Method

TN1002 SEC-MALS Branching Analysis Using the Viscosity Method

TN1006 Performing Protein Conjugate Analysis in ASTRA

HPLC & UHPLC connection guides: TN3600, TN3603, TN3604, TN3605, TN3606, TN3610, TN3611

Contents

Overview

If you already have an ASTRA method defined, start at **Part I: Routine Operations**. Otherwise, go to **Part II: System Setup.** At various places in this document, instructions differ depending on whether or not HPLC control is enabled in ASTRA. If you don't know whether your system has HPLC control see the **Appendix** for instructions to determine this.

Part I: Routine Operations

If you are ready to run an experiment and analyze your data, start here. The open spaces below provide a place for you to keep track of the location of your methods and sequence templates.

Page 3 **Running a Single Injection**

Use this mode when collecting data from only one injection or recording solvent data. You need to have previously created and saved an ASTRA method, either using the Method Builder or manually.

Method name(s) and location / user profile name:

_____

_____

Page 5 **Running a Sequence**

Use this mode to collect data from multiple samples automatically. You need to have previously created and saved an ASTRA method.

Sequence template name(s) and location:

_____

_____

Page 8 **Processing SEC-MALS Data**

This section explains how to process your ASTRA SEC-MALS data after you have run an ASTRA experiment and saved it. It assumes that all procedures for setting system parameters such as normalization, alignment (interdetector delay), and band broadening have been performed and incorporated into the method.

Part II: System Setup

If you need to create a new method, edit a method, or set system parameters, start here.

Page 12 **Creating a New Method**

You will need to create a method before you can run an experiment.

Page 16 **Editing a Method**

You can edit the settings of a method you've created or change one of the system methods.

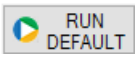
Page 17 **Setting System Parameters**

This section helps you setup system parameters in ASTRA, including normalization, alignment (interdetector delay), and the band broadening correction. It assumes that SEC-MALS data (an ASTRA experiment) suitable for setting normalization and the interdetector delay volume has been collected and processed (baselines defined and a peak region set).

Part I: Routine Operations

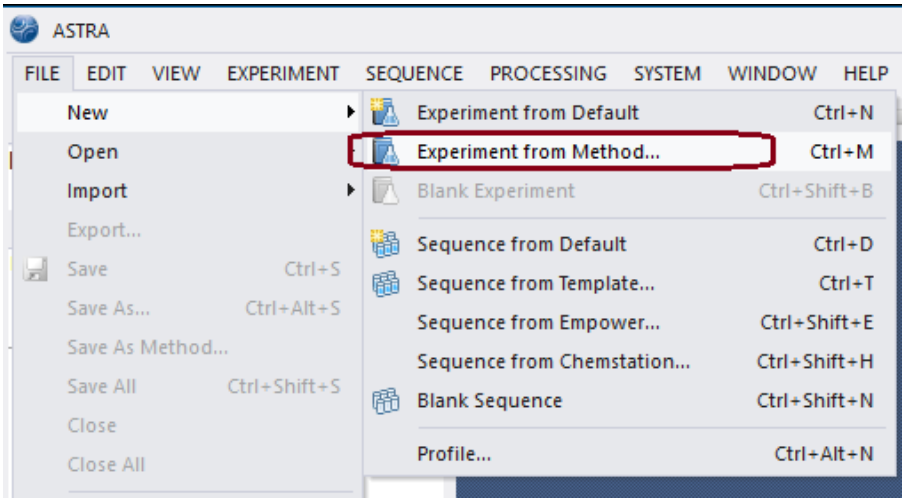
Running a Single Injection

Use this mode when collecting data from only one injection or recording solvent data. You need to have previously created and saved an ASTRA method.

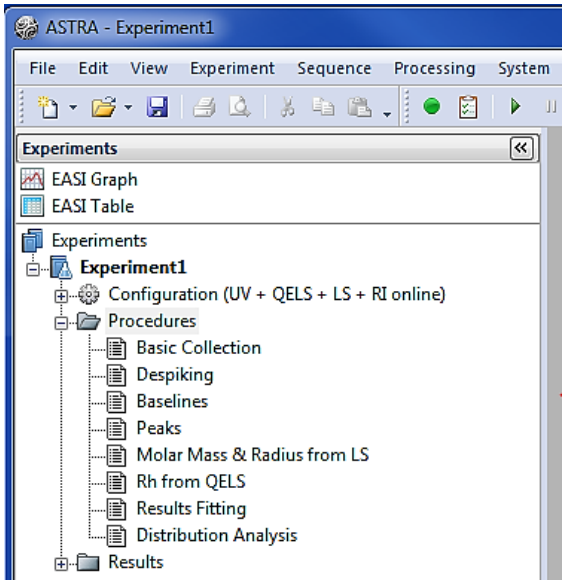
1. If you have created a default method that you would like to use for data collection, open ASTRA and click the **Run Default** button 

in the upper left of the window. This will create an experiment from the default method and run it. If you are not using a default method, proceed to steps 2 through 5 below.

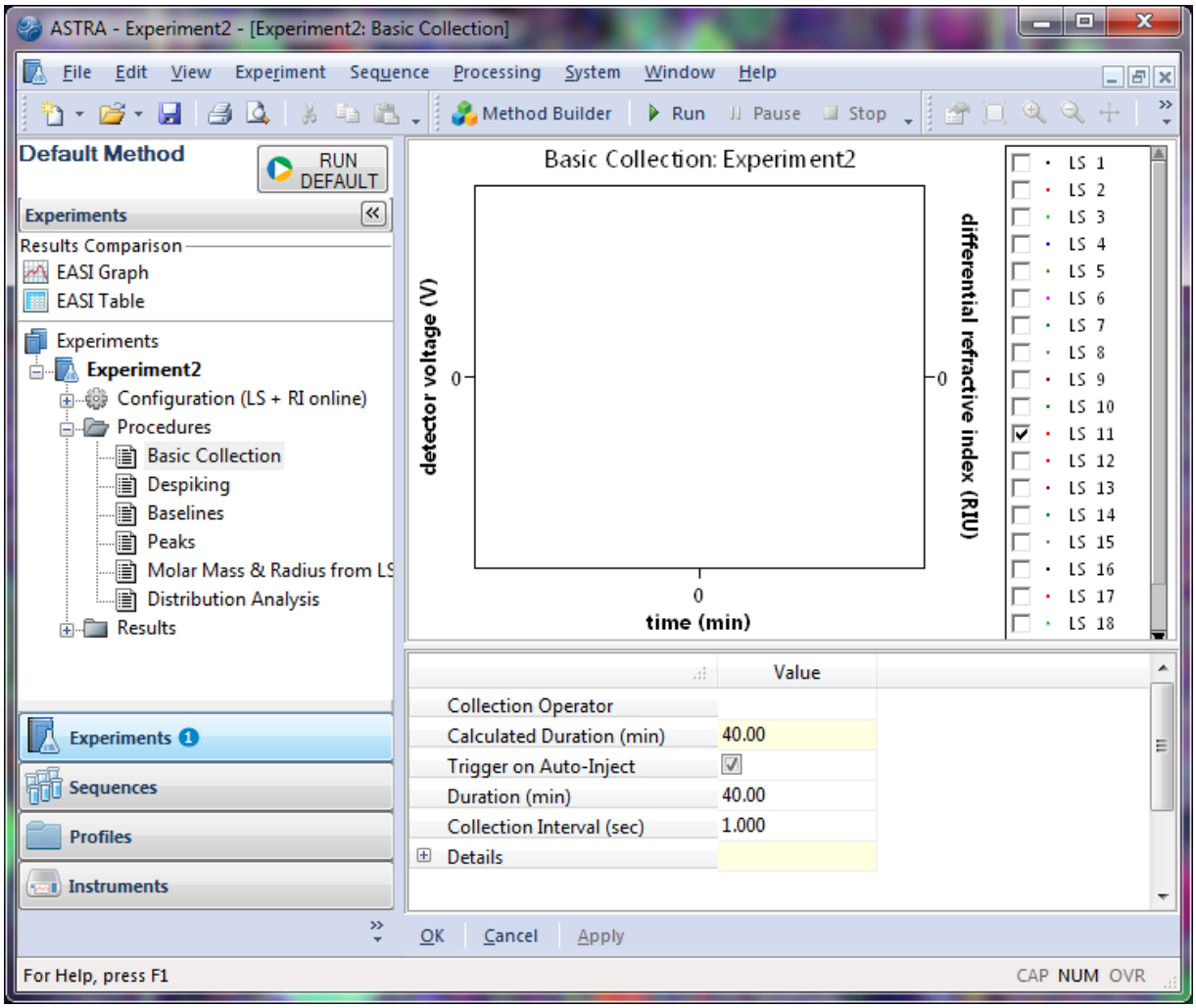
2. Go to **File → New → Experiment from Method…** and navigate to your previously created method. This will open the method in the ASTRA left-hand side workspace.



3. Expand the **[+] Procedures** section of the method by clicking on the **[+] sign** or double-clicking on **Procedures**.



4. In the **Procedures** section, double-click on **Basic Collection** to open the data collection view.

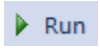


   a. If you will be using an autosampler or a manual injection valve with a contact closure connection to trigger the injection start, place a check mark in the **Trigger on Auto-Inject** box in the table below the graph. In this mode, data collection will begin automatically when the injection is made.

   b. If you would like to begin the data collection manually, make sure there is *no* check mark in the **Trigger on Auto-Inject** box.

   c. Change the **Duration (min)** to the desired experimental run time in minutes.

   d. Click the **Apply** button near the bottom of the page to accept the above changes.

5. To begin data collection, click the **Run** button 

near the top of the ASTRA page.

   - If you enabled the **Trigger on Auto-Inject** option, a message *"Waiting for auto-inject signal"* will appear. Data collection will automatically start once your sample has been injected and the contact closure signal has been sent from the autosampler or injector.

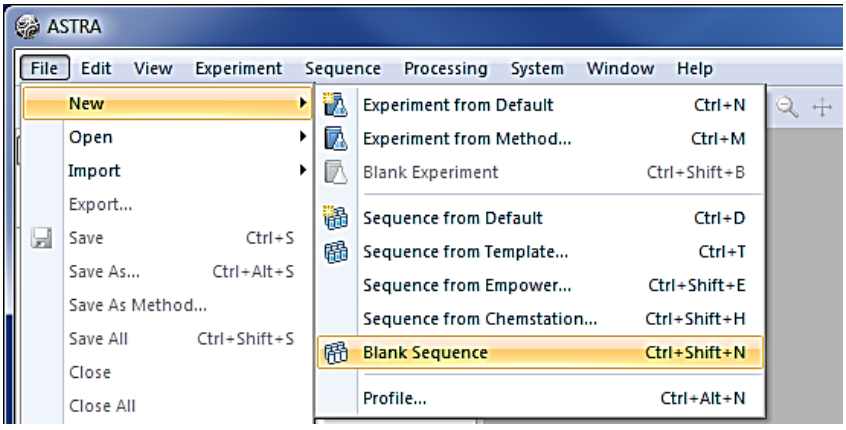
Note: On occasion, ASTRA is not done preparing the experiment at the time the autosampler makes the injection. If this happens, ASTRA will miss the injection signal and you will lose data. This can happen if the computer running ASTRA is slow or if the network connection is slow. To ensure that data is collected, click the Run button well before the injection is performed; ASTRA will not collect until the injection signal arrives.

- If **Trigger on Auto-Inject** was not selected, a message *"To begin collection, press OK and then inject sample"* will appear. Data collection starts when you click **OK** in the dialog box or hit **Enter**, after which you can inject your sample.

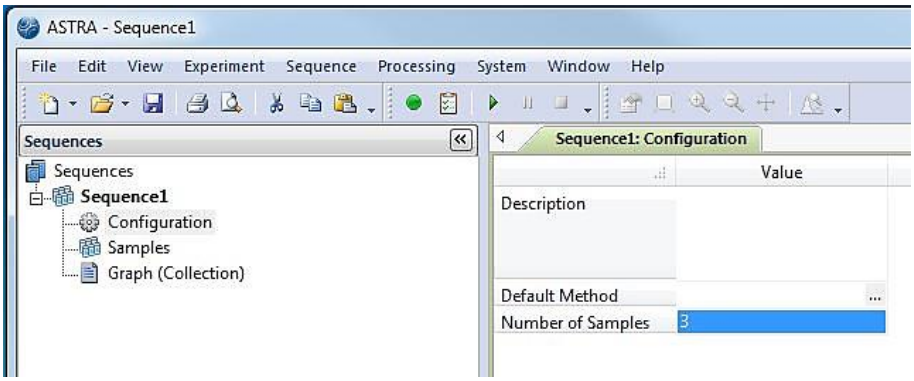
Running a Sequence

Use this mode to collect data from multiple samples automatically. You need to have previously created and saved an ASTRA method.

1. Open ASTRA and navigate to **File** → **New** → **Blank Sequence**. This opens a blank sequence in the ASTRA workspace on the left-hand side of the ASTRA display.



2. Double-click on the **Configuration** in the ASTRA workspace.

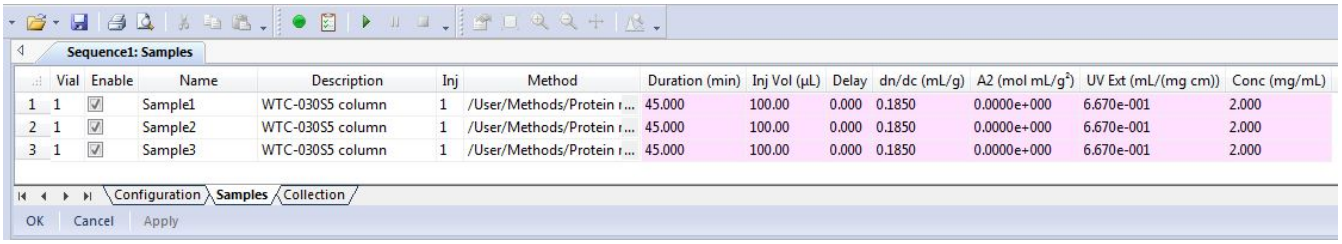


3. In the **Default Method** field, click the browse button **[...]** and then navigate to and select the ASTRA method to be used for the sequence.

4. In the **Number of Samples** field, manually enter the number of samples to be run in the sequence.

5. Click the **Apply** button near the bottom of the window to save your changes.

6. Next, click the **Samples** tab near the bottom of the window to open the information page for the samples in the sequence. There should be a row for each of the samples. (The number of rows corresponds to the number of samples entered in step 4).



7. You can set the following properties for a sample. (Any values you enter in these fields will override the method settings.)

   Vial: The behavior of this field depends on whether HPLC control is enabled.

   With HPLC Control: This field is the vial/well position of your sample in the autosampler tray. This must be set if using HPLC control. It will be populated by what is present in the Basic Collection Procedure in the Method, but if edited here, that will supersede the Method's vial.

   No HPLC Control: This field is informational only. You may leave it at 0, or it can be used to indicate a specific vial/well position in the autosampler. You need not use this field.

   Enable: Checking this box includes the sample in the sequence run. If no check mark is present, then the sequence will skip this line and proceed to the next one. (The default is to have this checked.)

   Name: This is the file name that will be used when the file is saved. If no name is specified, ASTRA will automatically generate a name. Do not use any special characters (/ % * \ etc.).

   Description: This field is used to enter any relevant sample information.

   Inj: This field indicates the number of injections to be made for this sample.

   Method: This field defaults to the method you selected in step 3 above. If you want to select a different method for a sample, use the **[…]** browse button and locate and select the intended method.

   Flow rate: This is only present if HPLC control is enabled. It is populated from the method and is read-only in the Samples view of the Sequence.

   Duration: This is the ASTRA run time. If there is no HPLC control, it should match the run time specified for the sample in the chromatography software sequence that will manage the actual injections. Caution: If HPLC control is not enabled and the duration entered here is longer than that in the chromatography software sequence, a sample could be injected before ASTRA is ready to collect data, and data could be lost.

   Inj Vol: ASTRA uses this field, in µL, in combination with the **Conc** value (below) to calculate mass recovery. The behavior of this field depends on whether HPLC control is enabled.

   With HPLC Control: This is also the amount in µL that will be injected by the autosampler.

   No HPLC Control: This field is optional.

Delay: This optional field allows delays between injection and the start of data collection. It is typically recommended to leave this at 0.0.

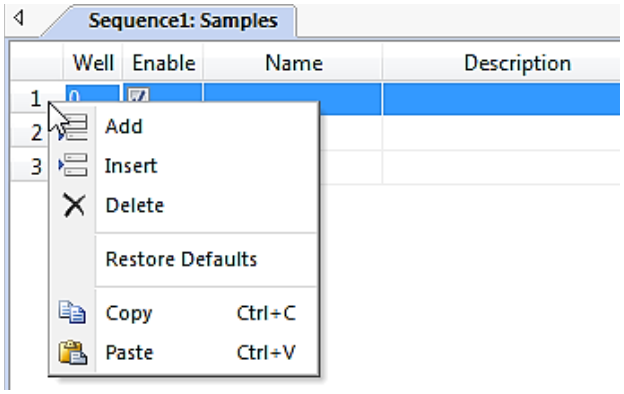
dn/dc: This field is used to enter the dn/dc of your sample in units of mL/g (if different from the value in the method applied in step 3).

A2: This optional field is used to enter the second viral coefficient, if known for the sample, and is used to correct for non-linear effects due to concentration. The value is typically left as 0.0 because the concentrations observed in SEC are typically too low to see any effect.

UV Ext: When using a UV detector as a concentration source, this field is used to enter the UV extinction coefficient for the sample in units of mL/(mg·cm).

Conc: This optional field is used to enter the sample concentration in mg/mL. ASTRA uses this field in combination with the **Vol** value (above) to calculate mass recovery.

8. You can add additional samples to the end of the sequence by right-clicking in the sequence table and selecting **Add**. Similarly, by clicking on a sample number to highlight it and then right-clicking, you can **Delete** that sample or **Insert** a sample above it. In addition, the **Copy** and **Paste** features allow you to copy information from one sample to another.



9. Click **OK** near the bottom of the page to save the changes you made to the sequence.

10. Click the **Run** button  near the top of the ASTRA window (**Ctrl+R**) to begin the sequence collection. After a sample run has completed, its row in the Samples tab is shown with a blue background.

Note: Be careful ***not*** to click the **Run Default**  button in the upper left – this will only collect data from a single injection using your default method.

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11. In the **Save** window that will appear, navigate to the location where you want to save the ASTRA data. Enter a name for the sequence in the **File name** field and click **Save**. The data files generated by the sequence will have the format "**Name[SequenceName]**.afe7".

12. The message *"Waiting for auto-inject signal"* will appear.

    With HPLC Control: ASTRA will begin collecting when the autosampler makes the injection. No further action is required to collect.

No HPLC Control: Use your HPLC or UHPLC software to begin the sample injections.

Note: Data from each ASTRA experiment in the sequence are saved in individual files in the location specified above in step 11.

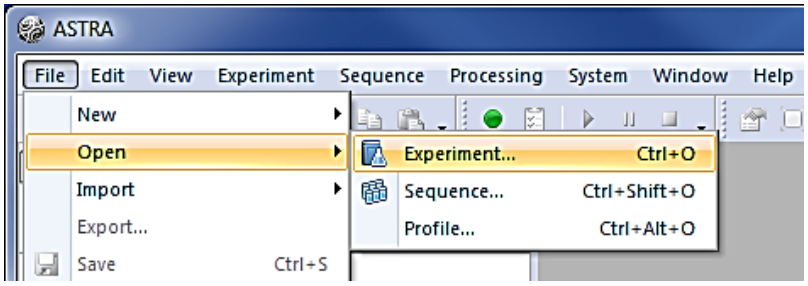
13. You can use this sequence to create new sequences by right-clicking on the sequence name and selecting **Save as Template**.

14. To create a sequence from a previously saved sequence template, navigate to **File** → **New** → **Sequence from Template…** and find the desired template.

Processing SEC-MALS Data

This section explains how to process your ASTRA SEC-MALS data after you have collected an ASTRA experiment and saved it. It assumes that all procedures for setting system parameters such as normalization, alignment (interdetector delay), and band broadening have been performed and incorporated into your method.

1. If the experiment isn't already open in ASTRA, navigate to **File** → **Open** → **Experiment…** to find the desired experiment. This opens the experiment in the ASTRA workspace on the left-hand side of the display.



2. Click on the name of the experiment in the workspace to select it.

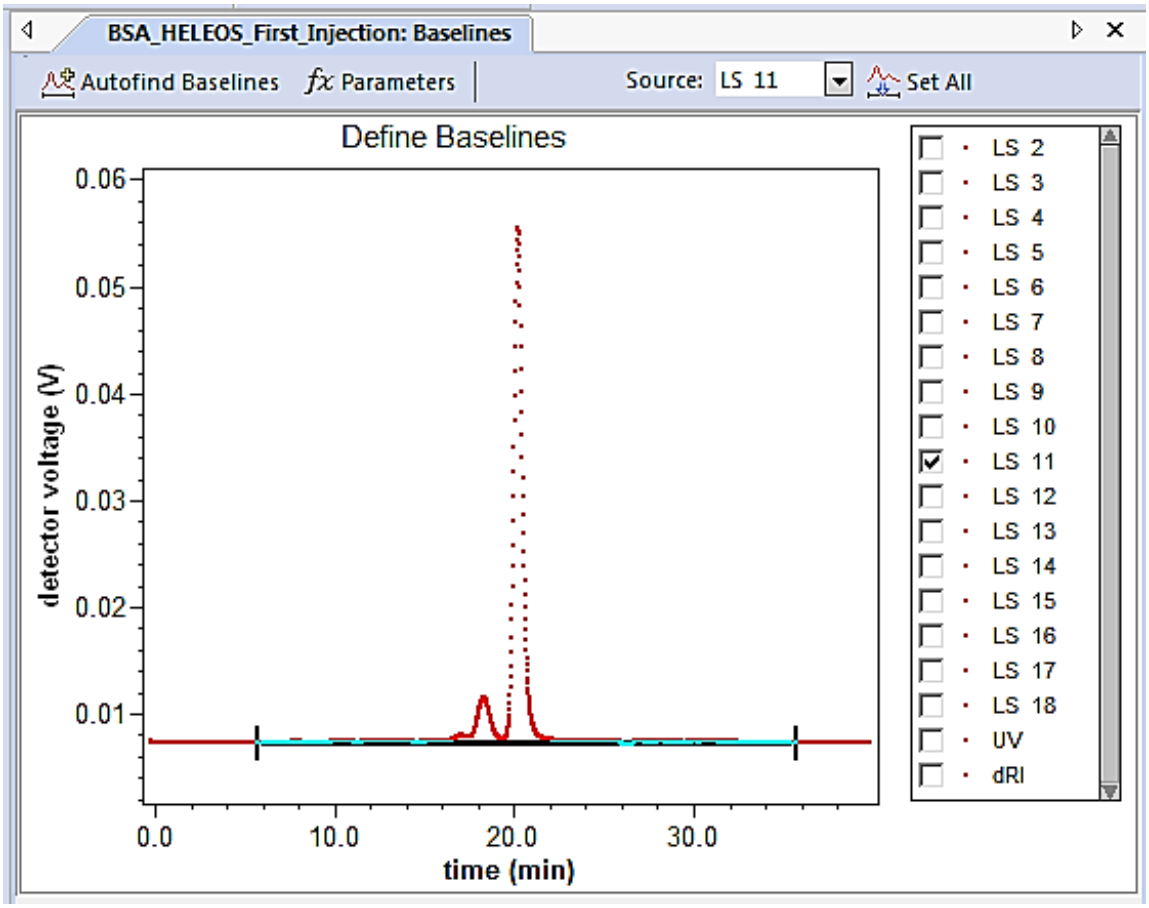
3. If the experiment has not been automatically processed already, click the Run **Run** button near the top of the ASTRA display. The message *"'YourExperimentName' does not have all of necessary baselines defined. Please specify appropriate baselines before attempting to analyze data"* should appear indicating that the baselines need to be set. Click **OK** to close this window and the **Define Baselines** view will appear. Note: if Experiment Builder mode is enabled, you may need to double click on the Baselines Procedure in the tree on the left.

Note: The **Run** button Run has two functions. The first time it is used on an experiment, it begins the actual data collection. If you highlight an experiment and click **Run** after data has been collected, it prompts you to process the data if it has not been processed already.

Hint: Zoom in on baselines and peaks by holding the **Ctrl** key and using the mouse to draw a box around the region. Step backward from the zoomed in view by holding **Ctrl** and right-clicking on the graph. To ensure you don't miss the ends of the baseline or top of a peak, you can draw your box outside of the edge of the graph.

Defining Baselines

1. Click the Autofind Baselines button near the top of the window to automatically set the baselines for all detectors at once. Alternatively, draw baselines for the **source** detector. (The **source** is set to the 90° detector by default: LS 2 for a 3-angle instrument and LS 11 for an 18-angle instrument). Left-click and hold onto the ends of the baselines. Drag them from well before any peaks to well after any peaks. The baselines should be set in a flat region where the detector signal comes from pure solvent (mobile phase). Click Set All to apply the **source** baseline to all other detectors.



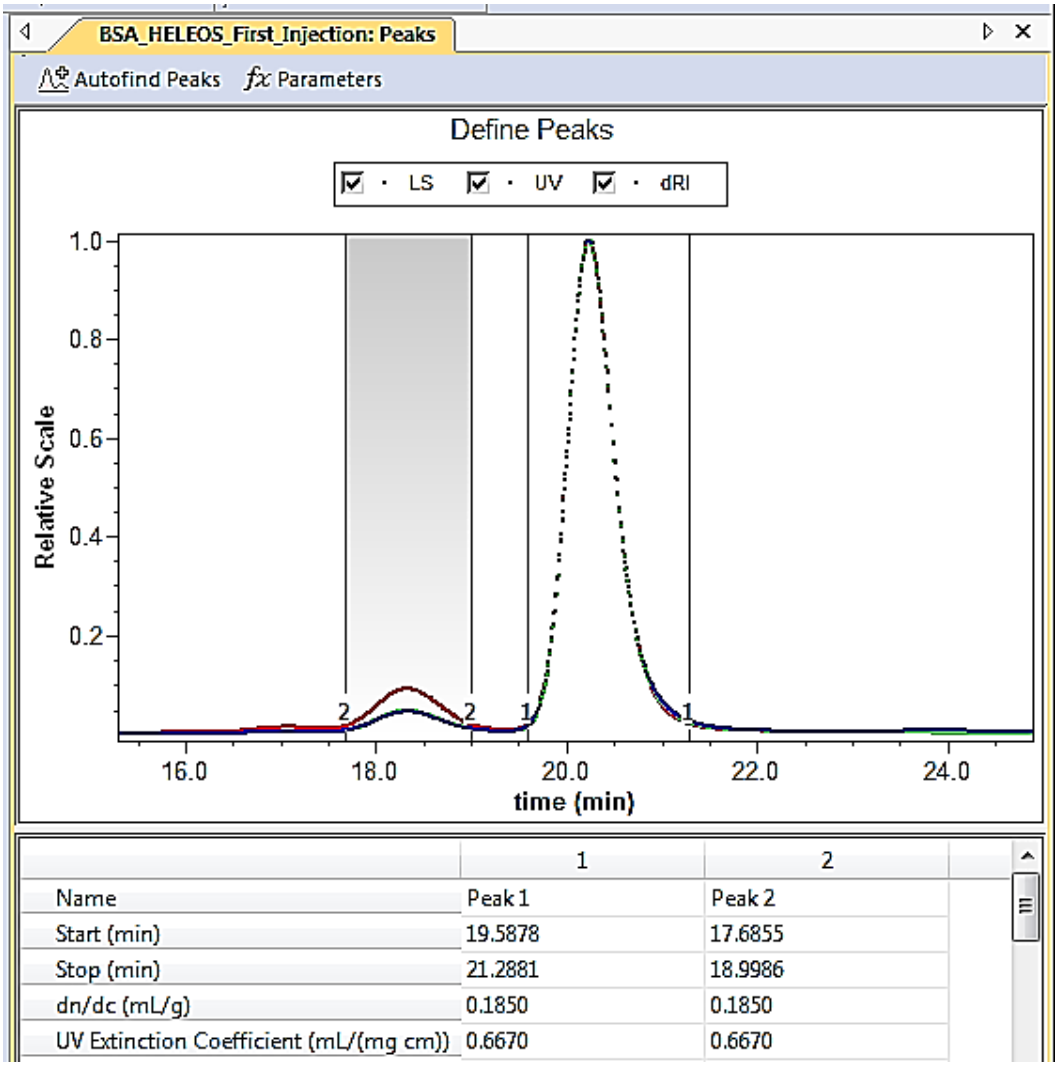
2. Zoom in (press the **Ctrl** key and draw a box with the mouse) to examine the baseline for all LS detectors, the RI detector, and the UV detector (if present). If necessary, adjust the baseline limits for any single detector.

3. Once you have set and evaluated the baselines for all detectors, click **OK** near the bottom of the page to accept these baselines. A message should appear: "*'YourExperimentName' does not have any peaks defined. Please specify peaks covering the regions of your data you wish to have analyzed*". Click **OK**. The **Define Peaks** view will appear. Note: if Experiment Builder mode is enabled, you may need to double click on the Peaks Procedure in the tree on the left.

Defining Peaks

1. Manually define the peaks by left-clicking and dragging to place the limits around each peak of interest.

2. Alternatively, click the Autofind Peaks button near the top of the **Define Peaks** view. Adjust the peak limits if necessary by clicking and dragging on the edge of each peak. In the grid below the plot, enter a **dn/dc** value if the correct value is not already displayed. Enter a **UV Extinction Coefficient** if a UV detector is being used (optional). Click the **OK** button at the bottom of the page.



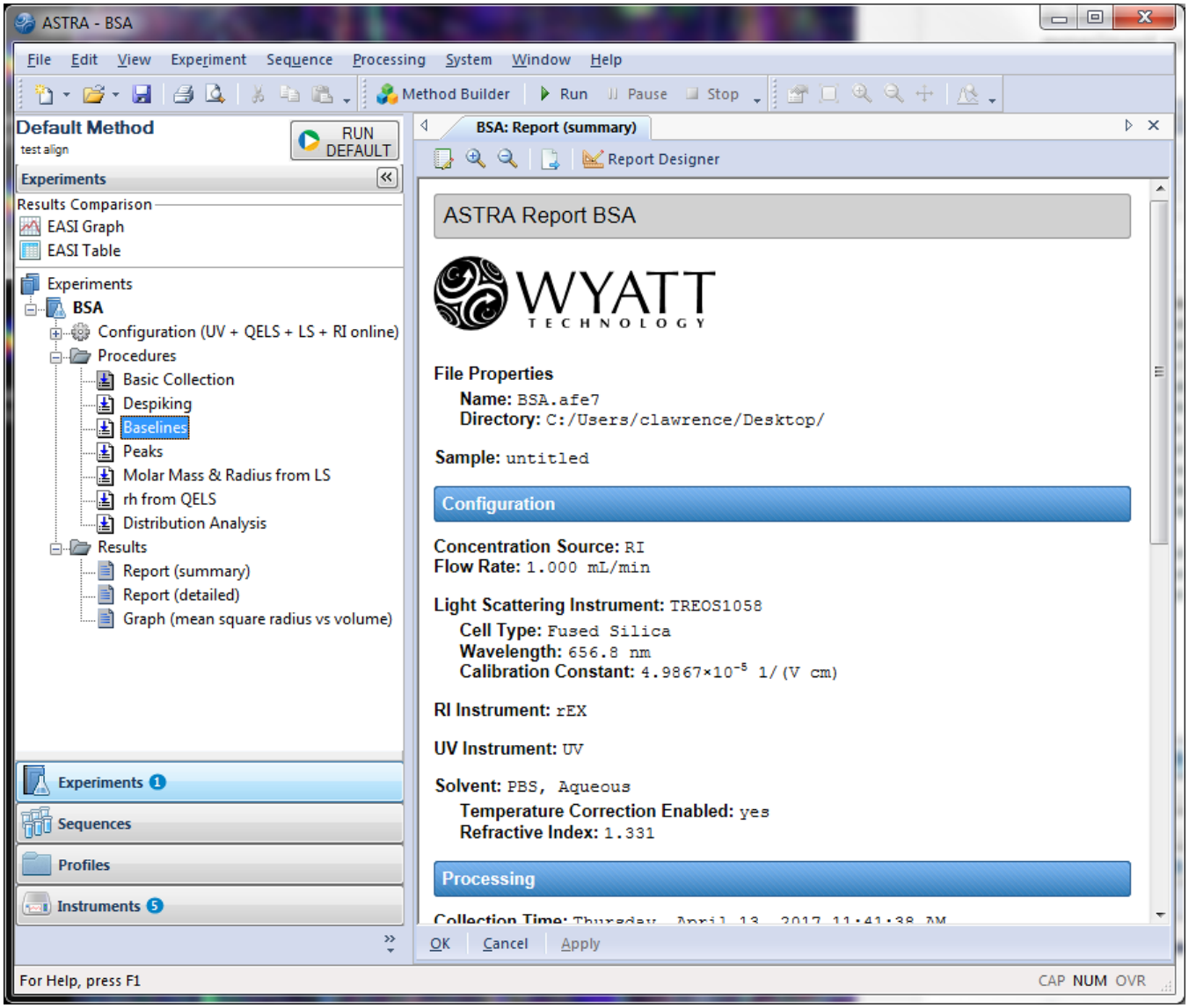
	1	2
Name	Peak 1	Peak 2
Start (min)	19.5878	17.6855
Stop (min)	21.2881	18.9986
dn/dc (mL/g)	0.1850	0.1850
UV Extinction Coefficient (mL/(mg cm))	0.6670	0.6670

Hint: To delete a peak you mistakenly selected, simply click on the peak to highlight and press the **Delete** key. You may also click on the column for the peak under the graph and press the **Delete** key.

You are now ready to look at your results.

Results

In the workspace panel, click the **[+]** in front of **Results** to expand it. Double-click on **Report (summary)** to view the experiment results.

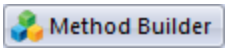


Save

To save the experiment with the above changes, click on the name of the experiment to select it. Go to **File** → **Save** or **File** → **Save As**... to save it with a new experiment name.

Part II: System Setup

Creating a New Method



A method is required to run an experiment and collect data and to process the data. If this is the first time you have opened ASTRA, you will be prompted to make a **Default Method**. Otherwise, open ASTRA and select the Method Builder from the top toolbar.

Method Builder – Step 1:

1) Enter a **Name** for your user profile. This will create a folder in the system database in which your methods will be saved.

2) Enter the **Sample** name

3) Select the **Application**. This will determine what analyses are included in the method.

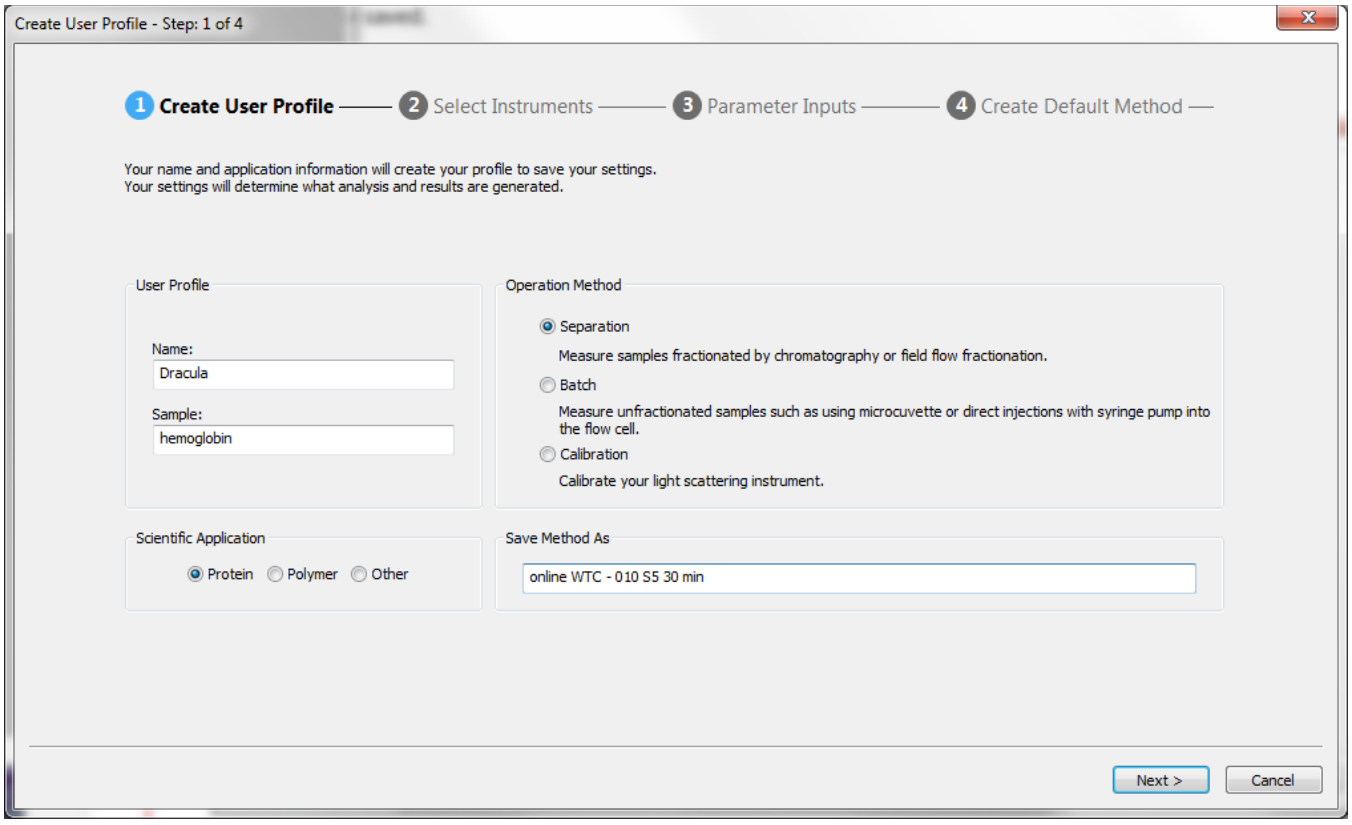
4) Set the **Operation Method**.

   Separation: Select this mode if your sample will flow through multiple instruments (often the case for SEC-MALS use).

   Batch: Use this mode for unfractionated samples delivered to a single instrument.

   Calibration: This mode is for calibrating your light scattering detector with toluene. If calibrating an RI instrument, the Method Builder, as of ASTRA 7.1.0, does not support this. But RI calibration methods can be accessed in the system database; select File → New → Experiment from Method and navigate to /System/ Methods/RI Measurement/Optilab rEX Specific.

5) Choose a name for the method in **Save Method As**.



6) When all fields have been entered, click **Next**.

Method Builder – Step 2:

ASTRA will automatically detect available instruments on the network. Check all instruments present in your system that you want to use. Instrument serial numbers will appear for each Wyatt instrument selected.

HPLC: This is only applicable if the ASTRA HPLC Service has been installed. It is only available in Separation mode.

UV: You must specify which **Aux Channel** the **UV Detector** signal will be sent to. Refer to the HPLC connection guides (listed under **Related Technical Notes** at the beginning of this guide), or to the manual of the corresponding Wyatt instrument, for more information.

Light Scattering:

Select the **Flow cell**. For TREOS and HELEOS instruments, this is usually Fused Silica. In a µDAWN, this is usually Vertical. F2 is a typical flow cell for high temperature applications.

ASTRA will automatically detect the presence of a **DLS** detector. The default detector position, 12 for a HELEOS, will be prepopulated. If the detector has been moved, this needs to be selected in the pulldown to the right of the DLS box. (For three-angle detectors like TREOS or µDAWN, the DLS angle is fixed).

The **Calibration Constant** must be entered. This will be the calibration constant from the Certificate of Performance or as determined from a more recent calibration measurement. At the time of this writing, TREOS II instruments will have the original calibration constant stored on the instrument. To use this, click Use factory default.

Viscometry: The viscometer can be either a Wyatt ViscoStar or generic. If it is generic, provide the host Wyatt instrument and Aux Channel receiving the signal from the viscometer. If the dilution factor is known, enter it here.

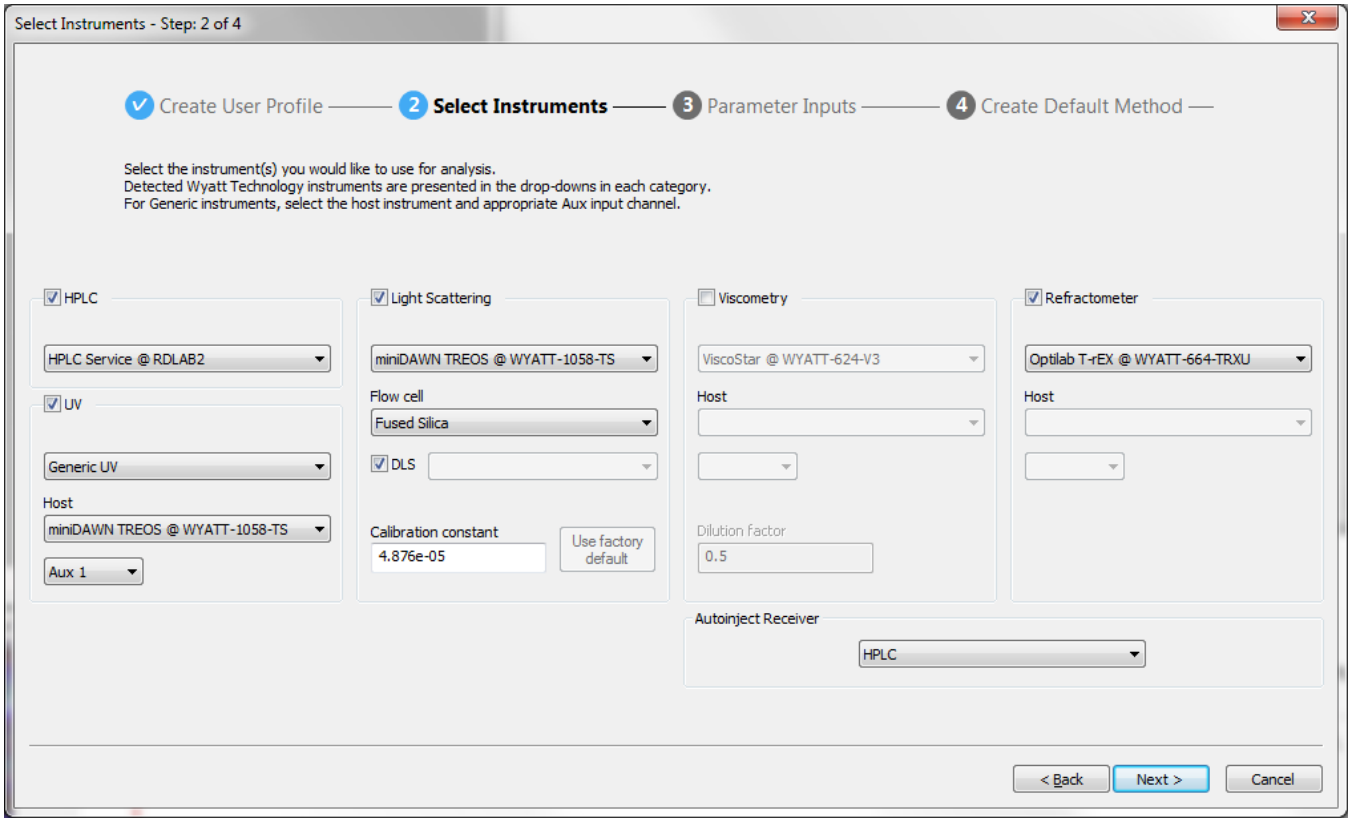
Refer to the HPLC connection guides (listed under **Related Technical Notes** at the beginning of this guide), or to the manual of the corresponding Wyatt instrument, for more information.

Refractometer: The refractometer can be either a Wyatt Optilab or generic. If it is generic, provide the host Wyatt instrument and Aux channel receiving the signal from the refractometer. Refer to the HPLC connection guides (listed under **Related Technical Notes** at the beginning of this guide), or to the manual of the corresponding Wyatt instrument, for more information.

Note: If you are using multiple generic instruments (for example generic UV and RI detectors), different Aux channels must be used for the different instruments.

Autoinject Receiver: If using an autosampler or manual injection valve with a contact closure connection to trigger the injection start, select which device is receiving the injection signal.

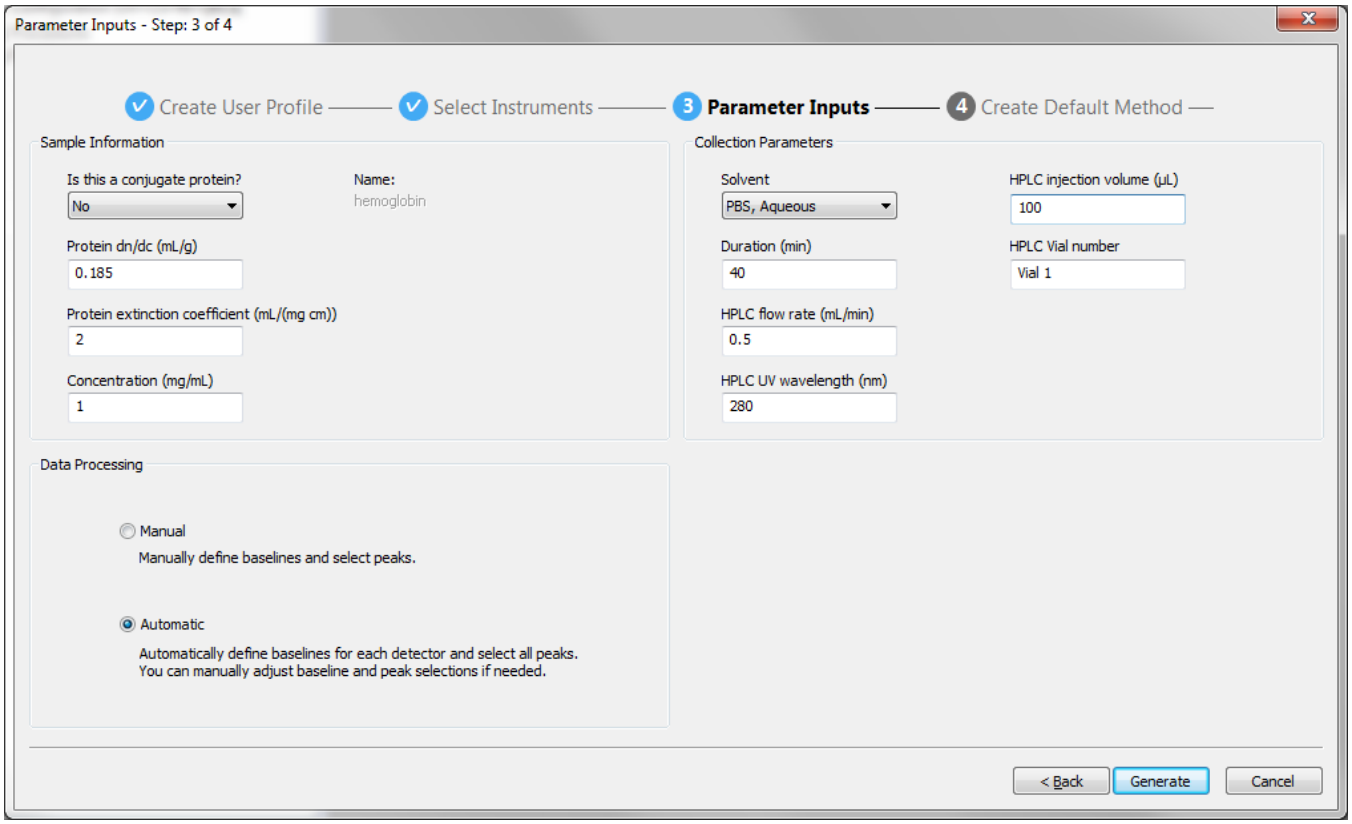
When HPLC Service is enabled, the Autoinject Receiver should be set to HPLC.



Click **Next** when all instruments have been selected.

Method Builder – Step 3:

The options in Step 3 depend on your selections in Steps 1 and 2. For example, selecting options for a conjugate protein results in fields for specifying dn/dc values and extinction coefficients of two components.

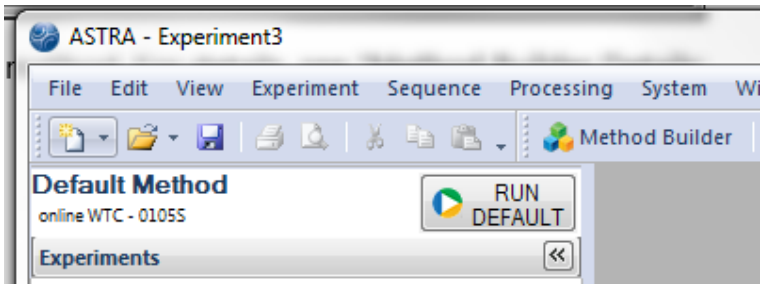


You can click Back to change prior settings or Next to create the method. For details, see "Method Builder Details: Step 3 of 4" on in the ASTRA User's Guide.

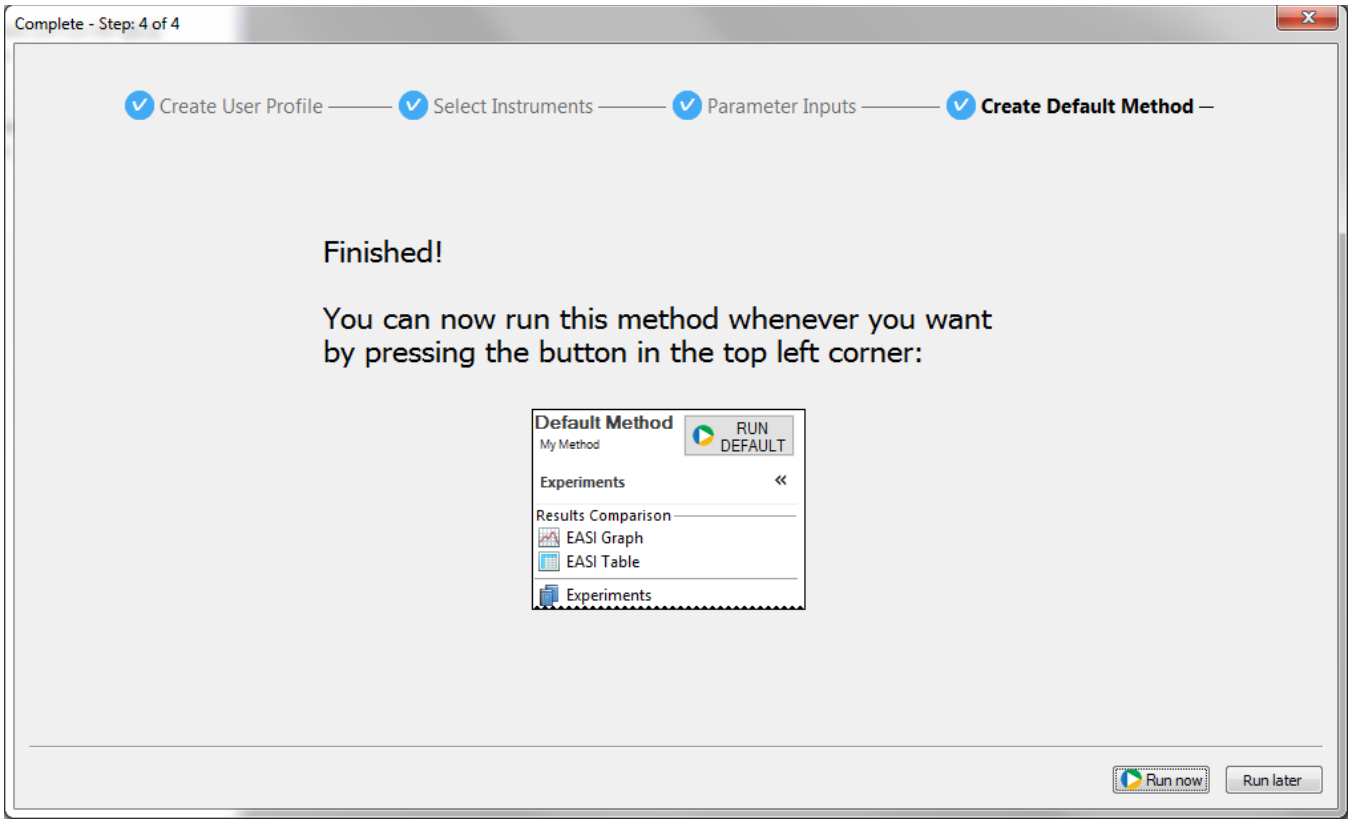
When all information is complete, click Generate.

Method Builder – Step 4:

In this step, the wizard sets the method you created as the default method. The method can be manually run from the default method interface at the top of the ASTRA workspace. For details, see "Method Builder Details: Step 4 of 4" In the ASTRA User's Guide.



Note: Calibration methods will not be set as the default method.

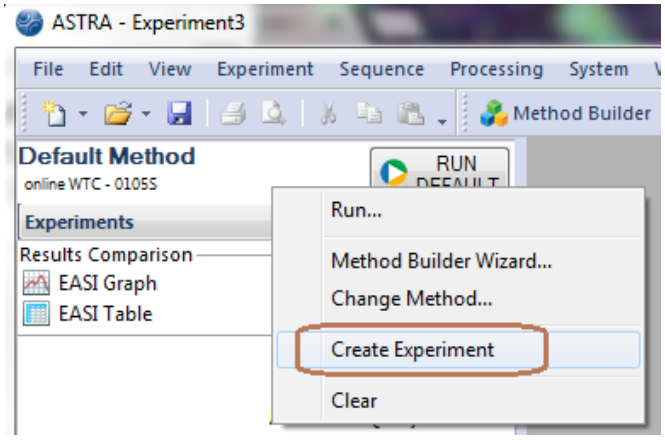


Click **Run now** to create a new experiment using the newly created method and to immediately start a collection.

Click **Run later** to store the method for future use. The default method can be quickly run from the main ASTRA window.

Editing a Method

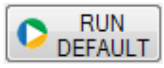
You may want to make changes to a method. For example, you might need to change the duration or the sample information. You can edit your newly generated experiment, which is already open in ASTRA if you selected Run now in Step 4 of the previous section. If Run later was selected, an experiment from that method can be opened by right clicking on the method name and selecting **Create Experiment**.



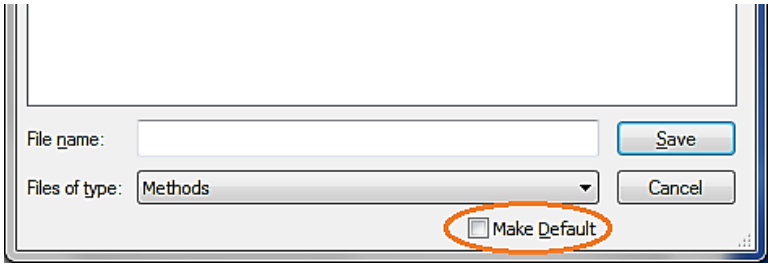
Once you have the desired experiment open in the left-hand workspace, make changes as needed. Correct settings are required for proper data processing. When you are finished modifying the settings, save this experiment as a method. You can then use this method to run experiments with the new settings.

Saving the Changes

1. To save the edits you've made to the method above, click on **File** and select **Save as Method...**. You can choose a new name or overwrite the previous method. If the Method Builder was used to the create the method, it will be located in /Method Builder/<user name>/<method>.

2. When saving, you can also check the **Make Default** box to save the method as your **Default Method**. This will associate this method with the **Run Default** 

button.



Setting System Parameters

Normalization, Alignment (Interdetector Delay), and Band Broadening

This section helps you setup system parameters in ASTRA, including normalization, alignment (interdetector delay), and the band broadening correction. It assumes that SEC-MALS data has been collected and processed (baselines defined and a peak region set) for a sample suitable for setting normalization and the interdetector delay volume. Suitable samples include Bovine Serum Albumin (BSA) for an aqueous mobile phase or a 30 kDa monodisperse polystyrene (PS) standard for an organic mobile phase.

Note: *Alignment* and *band broadening* need to be performed when the system is installed. They only need to be redetermined if the tubing connections (length or inner diameter) between the instruments are altered.

Normalization needs to be performed once in a given solvent used for MALS analysis. It only needs to be redetermined if a different solvent (mobile phase) is used. *Normalization* can be performed before or after alignment and band broadening.

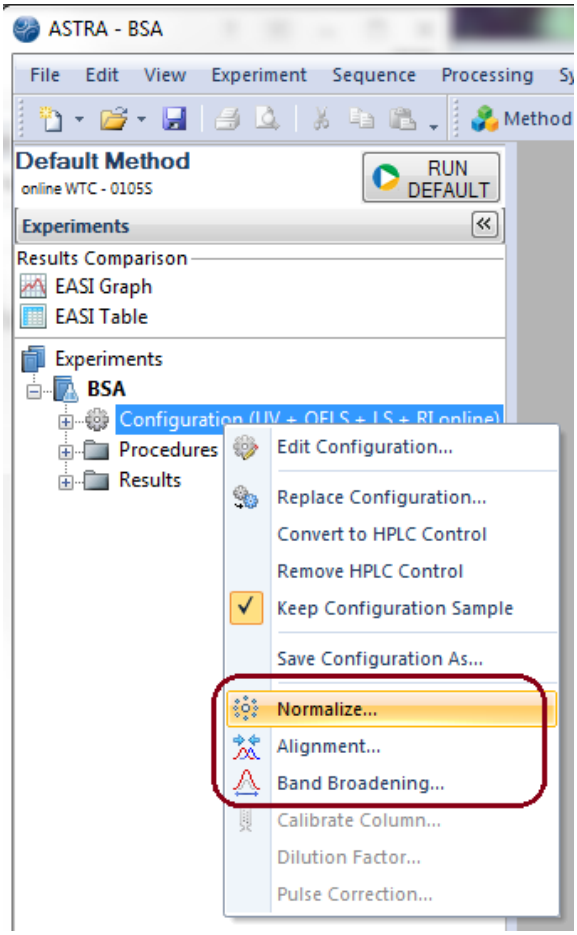
Regular checks of alignment, band broadening, and normalization are recommended, e.g., on a weekly or monthly basis.

1. Open the ASTRA experiment to be used for the determination of the instrument delay volumes, band broadening, and the MALS normalization coefficients.

2. Ensure that the baselines and peak regions have been properly set if you have not done so previously. (Refer to the section on **Processing SEC-MALS Data**.)

3. Click on the name of the **Experiment** to select it.

4. Right-click on the **Configuration**.



Normalization

Normalization requires you to set baselines and define a peak. If a peak has not already been defined, double-click the left mouse button on **Procedures** and then double-click the left mouse button on the **Peaks** procedure. Click and drag the peak limits to select the section of maximum peak height of the light scattering signal; you can return to the **Peaks** procedure after normalization and choose a wider peak region. Click **OK** to save your peak settings.

1. Right-click on the **Configuration** and navigate to **Normalize...** This should open the **Normalization** view.

2. Enter the following information:

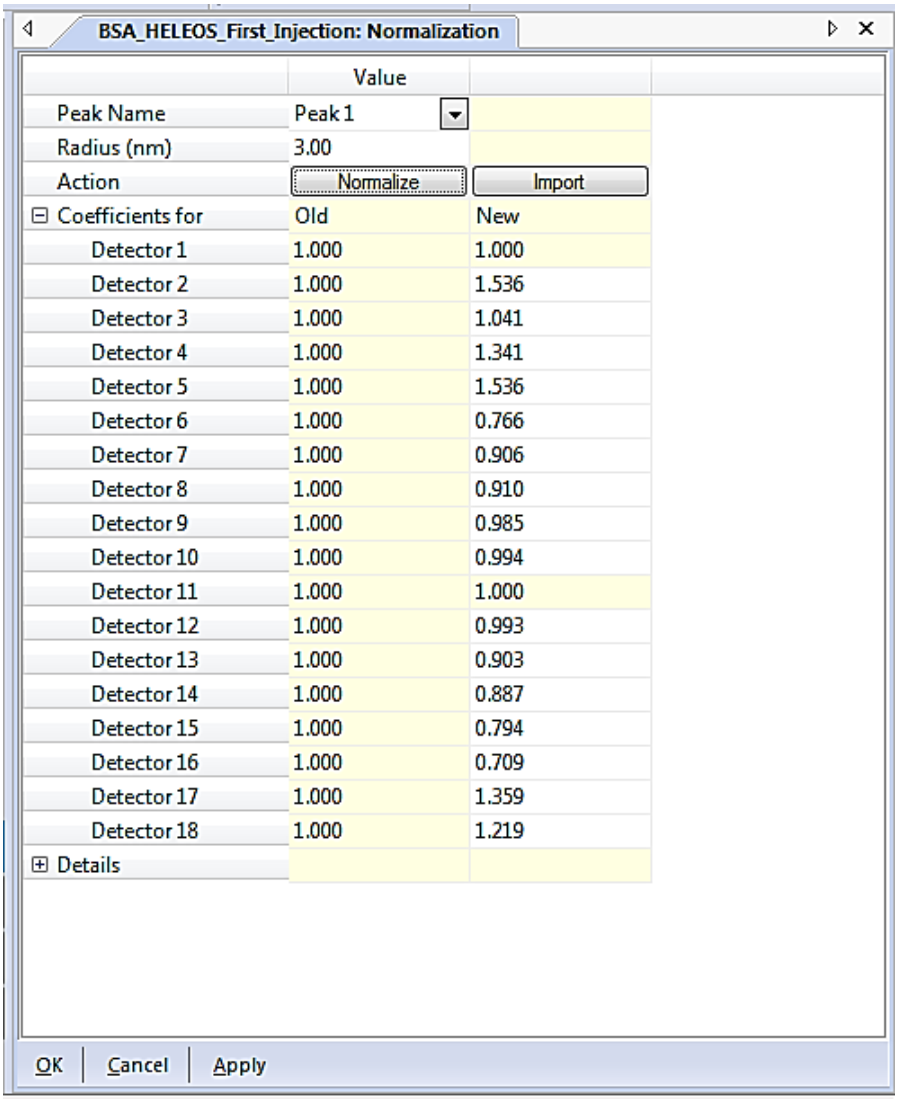
   Peak Name: Peak 1

Radius (nm): 3.00 for BSA or 6.00 for 30 kDa PS.

3. Click the **Normalize** button to compute the normalization coefficients.

4. Click **OK** to accept these constants.

The other detectors are normalized using the 90° detector which will be set to 1.000.



Note: Detector 1 in the DAWN HELEOS is blocked by the flow cell manifold and is only accessible when performing batch experiments in scintillation vials. Thus, detector 1 is not normalized when using the MALS flow cell and set to a value of 1.000.

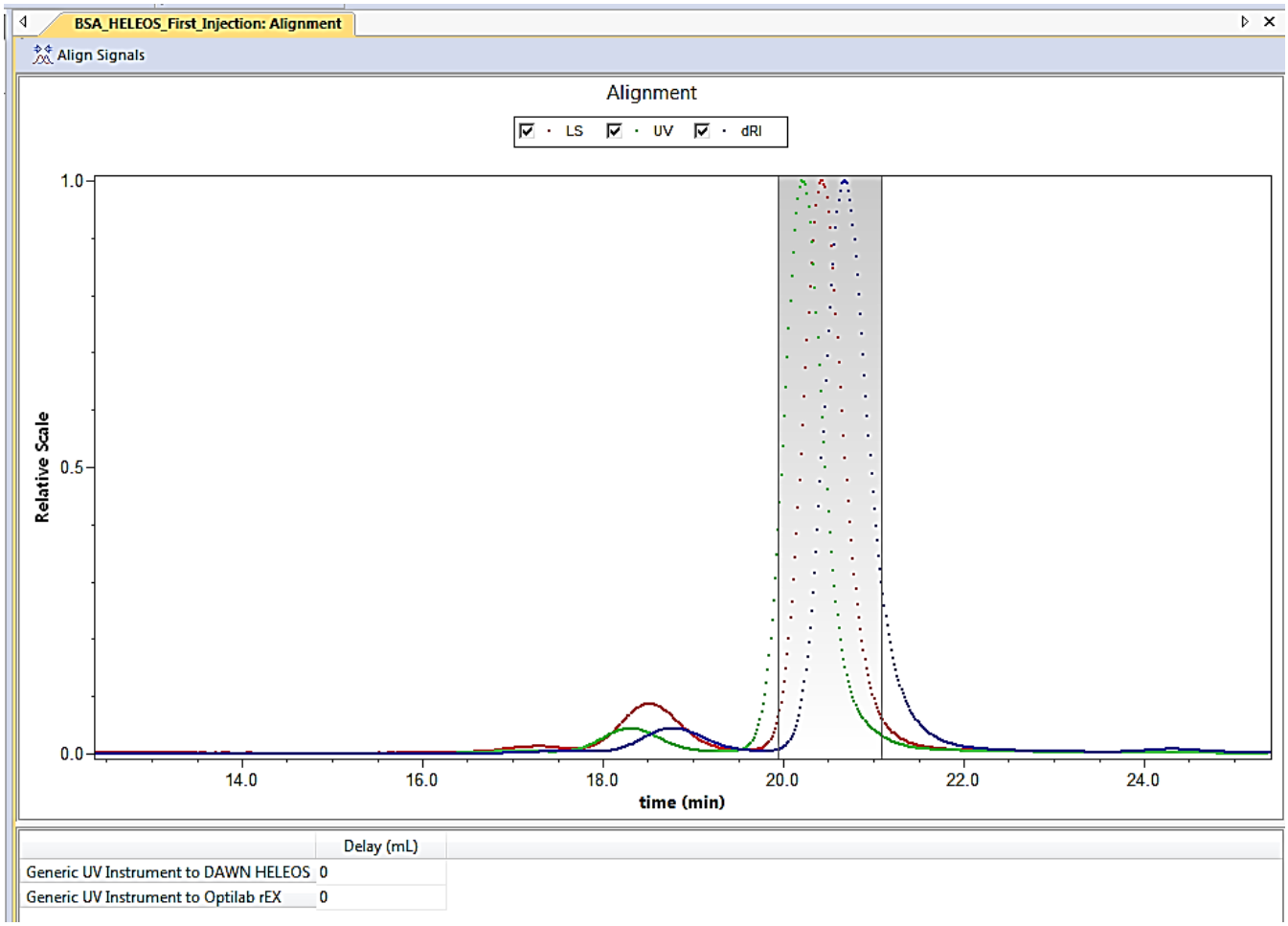
Similarly, if the DLS (QELS) fiber has been installed in the HELEOS (typically Detector 12 or 16), the DLS detector is not normalized and set to a value of 1.000.

Alignment

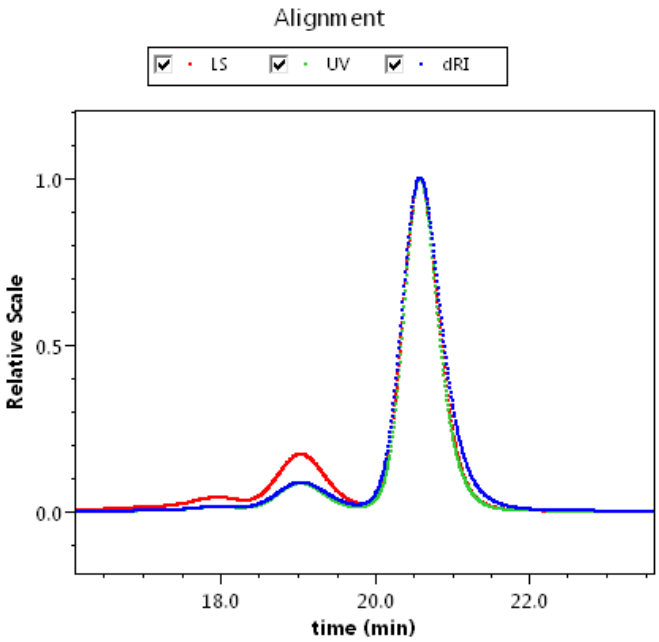
1. Right-click on the **Configuration** and navigate to **Alignment…** This opens the **Alignment** view.

2. In the plot, click and drag the peak guides around a region that includes the apex of the main peak from all detectors.

Hint: It may be helpful to zoom in on the peak first by holding **Ctrl** while pressing the left mouse button to draw a box around the peak region. Press **Ctrl** and right-click the mouse button to step backward from zoom.



3. Click the Align Signals button above the plot. The peaks from all instruments should now be overlaid, and Delay volumes in the table below the graph nonzero.



4. Click the **OK** button near the bottom of the window to save these delay volumes.

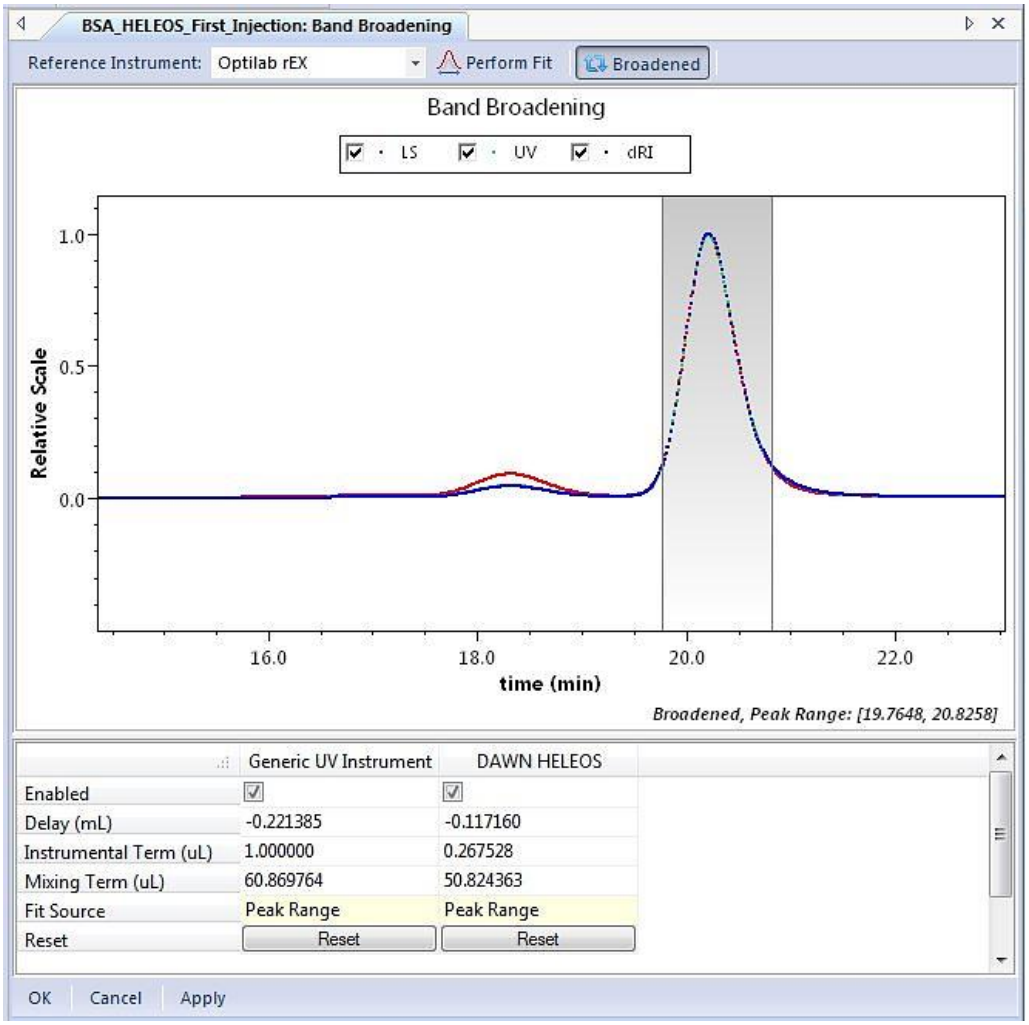
Band Broadening

1. Right-click on the **Configuration** and select **Band Broadening…**.

2. Select the **Instrument** that has the broadest signal. This is the reference instrument against which others will be compared. (ASTRA automatically selects the furthest downstream instrument, as it typically has the greatest broadening. Usually, this is the RI detector; however, if there is a viscometer in the flow path, choose that as the reference.)

3. Make sure **Enabled** is checked for all instruments. (This is the default.)

4. Click and drag to select a range that spans the major peak as shown. (Make sure that you cover the main peak region and that there is no overlap from adjacent peaks, such as the dimer peak co-eluting partially with the monomer peak—not the case for this example).



5. Click Perform Fit near the top of the window. Typical correction terms for standard SEC-MALS are 1.0 µL (mixing term) and 50 µL (instrument term). For UHPLC systems using the µDAWN, typical terms are 1.0 µL (mixing term) and 5.0 µL (instrument term). Use the **Reset** button to use these typical terms as starting points for a fit.

6. Use the Broadened button to toggle the broadening on and off. If the match between the peaks is not good, repeat the fit.

7. Click **OK** to save the band broadening correction.

8. Right-click on the name of the experiment and select **Save** to save this experiment with the above changes.

Saving the System Parameters

1. To save the normalization, alignment, and band broadening parameters as an ASTRA method, click on the name of the experiment, right-click, and select **Save as Method…**.

2. Give this method a new name.

3. Use this method to collect ASTRA data in the future.

Note: The above method will contain the peak regions and the baselines used in this file. You may wish to generate a method that does not contain baselines and peaks. To do this, before saving as a method, manually delete the peak regions and all baselines.

Congratulations!

You have successfully created a method, run an experiment, and analyzed the collected data. Also, you now have a method saved for running future experiments.

If you are interested in more advanced data analysis, such as performing branching or protein conjugate analysis, please see our technical notes on our customer support website. Login at www.wyatt.com/Support.

If you have any questions, please email support@wyatt.com or call Wyatt's support team at 805-681-9009, option 4 (within the US and Canada). You can even send us your data file!

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.

Appendix

HPLC Control

The instructions in this document vary in some places depending on whether or not HPLC control has been enabled in ASTRA. If it is enabled, there are a few indicators:

1) There will be a molecule icon  in the system tray. When not connected to an HPLC stack, it is yellow.

2) Right clicking on Configuration, and selecting Convert to HPLC control will replace the generic instruments under Configuration with instruments that contain "HPLC Device" in the name.