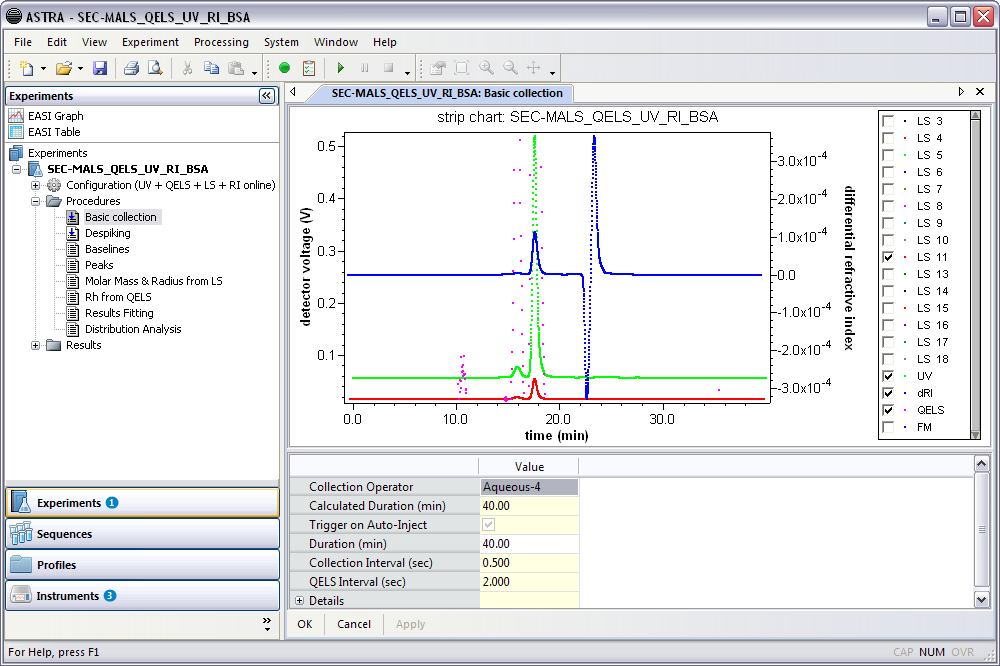
**ASTRA 6 Software Workshops:**

**SEC-MALS Processing and Calculations**

### Quick Processing Guide for a validation standard (e.g. BSA or 30 kDa PS) used to determine light scattering parameters for a new HPLC setup (first injection):

**Basic Collection Window after data collection:**



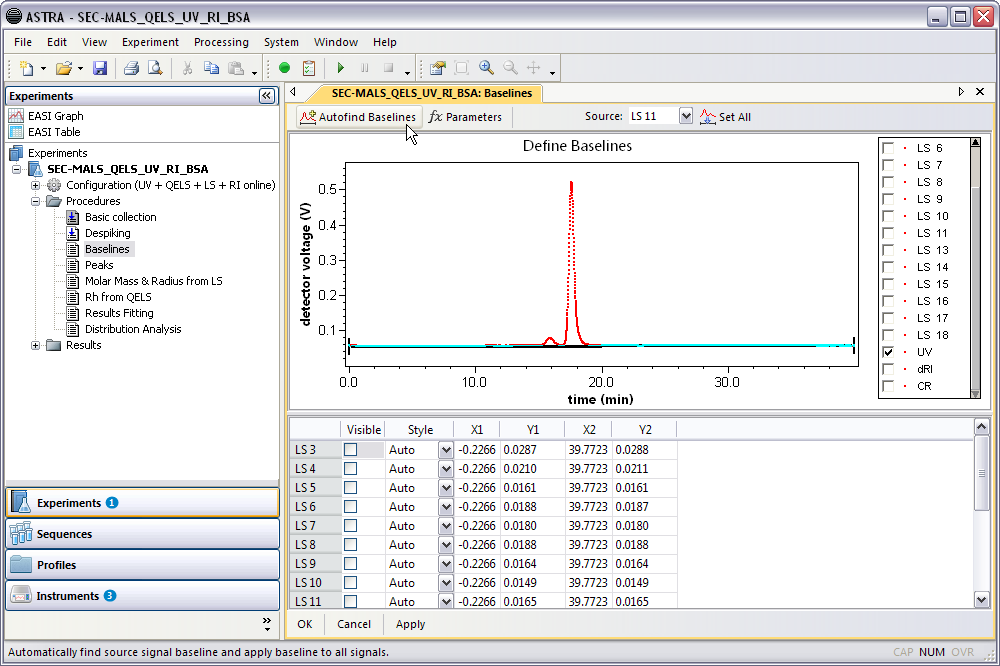
### The processing of a validation standard consists of the following steps:

1. *Setting baselines*
2. *Selecting a peak for Normalization*
3. *Normalization: sample must be an isotropic scatterer (rms radius < 10 nm)*
4. *Alignment (Determine Interdetector Delay): sample must be monodisperse (Mw/Mn < 1.05)*
5. *Band Broadening: sample must be monodisperse*
6. *Adjust peak selection and determine molar mass*

* After performing these processing steps, the experiment can be saved as a **Method** for further use with this particular HPLC setup and mobile phase. Once all these processing steps have been done, you can use this method for all your future data collections.
* This method contains both ***Configuration*** information (Instruments, Normalization, Alignment and Band Broadening) as well as ***Processing*** parameters (if set).
* Refer to the ASTRA manual, tutorials, and ASTRA Processing Workshop for more details.

### 1. Setting Baselines:

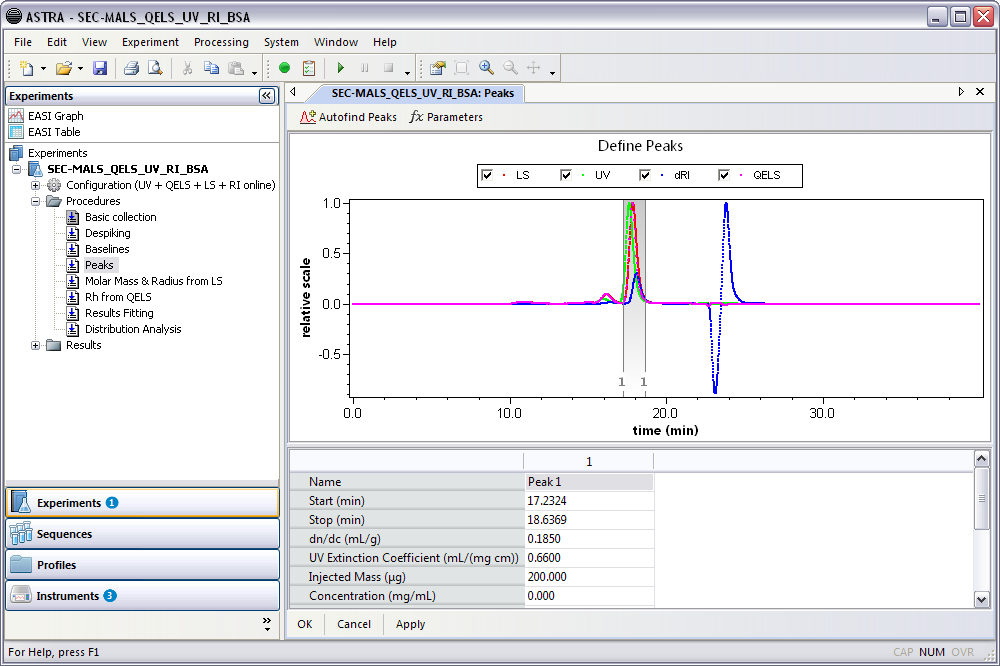
* Click on the **Run** button again to process your data. You will be prompted to set your baselines in the **Define Baselines** window.
* ASTRA 6:
  + Click on the **Autofind Baselines** button above the graph to automatically set baselines for all detectors;
  + Check baselines for all detectors, in particular for the concentration detectors. Zoom into the graph for better viewing. Note that baseline ends do not automatically snap to the data points in the chromatogram.
  + Adjust baselines by clicking on the end and dragging to the desired location, if necessary.
* The ASTRA 5 way:
  + Set baseline for the 90° detector (HELEOS: detector 11, TREOS: detector 2) by clicking with the left mouse and dragging the baselines across the chromatogram.
  + Click on **Set All** above the graph to apply settings to all detectors, check baselines for all detectors, in particular for the concentration detectors. Zoom into the graph for better viewing.
* To undock baselines from the data points in the chromatogram, hold down the SHIFT key while adjusting the baseline.
* Note: The **Despiking Procedure** will be performed automatically. The default despiking setting is **Normal**. Change to **None** or **Heavy** if desired.



* Click **OK** to save your settings. This will automatically take you to the **Peaks** Procedure.

### 2. Selecting a peak for Normalization:

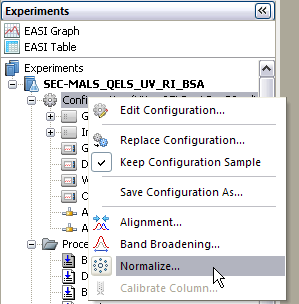
* In the **Peaks** Procedure, select the upper third of the light scattering peak by clicking and dragging across the main peak as shown below (HELEOS: detector 11, TREOS: detector 2)
* Note: if there are multiple peaks in the chromatogram, it is better to set a single peak manually as described above rather than using the **Autofind Peaks** feature in ASTRA 6.



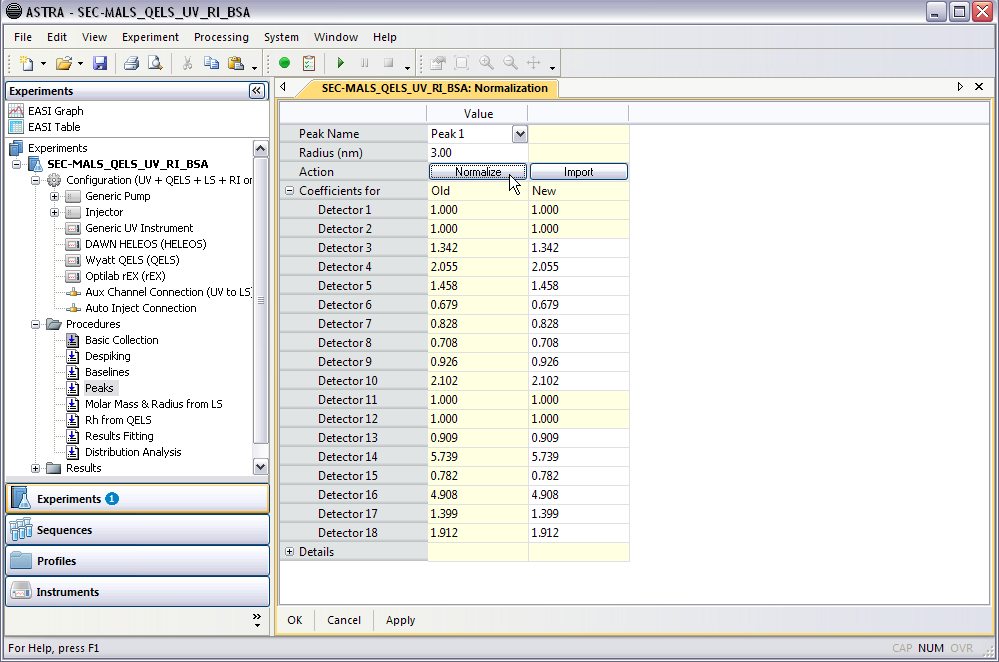
Click **OK**. All procedures have now been run.

### 3. Normalization:

* Right-Click on **Configuration** to select the **Normalization** Procedure:



This will activate the **Normalization** window.

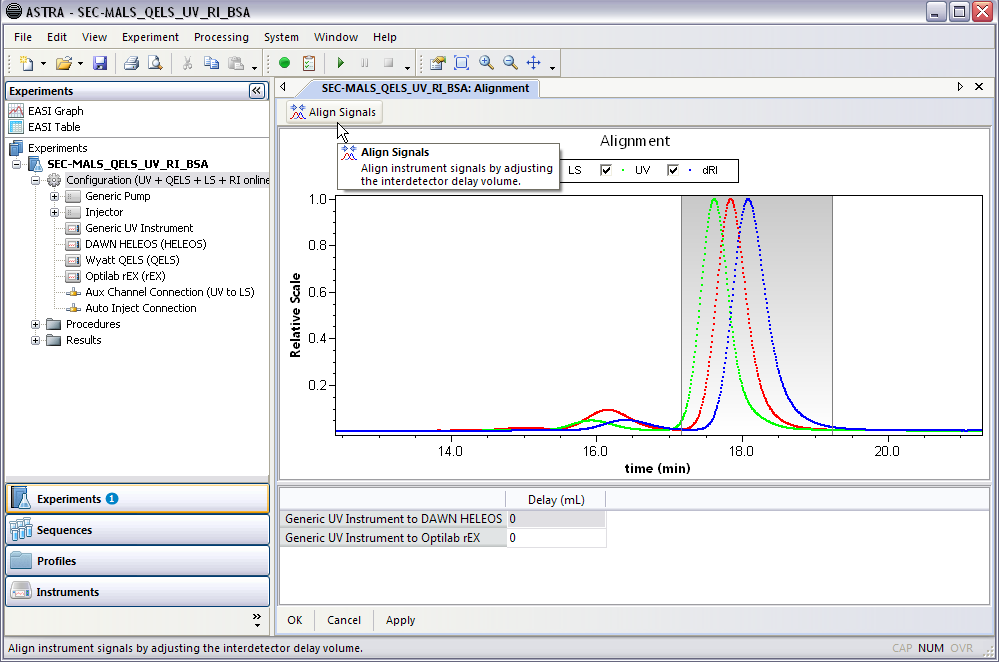


* Select the appropriate **Peak Name** (here: Peak 1) and **Radius** (BSA: 3.0 nm, the type is rms radius) for your sample. ***Note***: you can estimate the radius for a sample of unknown radius as long as the sample is an isotropic scatterer (radius < 10 nm).
* Click on **Normalize**. All detectors except the 90° detector and the QELS detector should be different from 1.000.
* You can also **Import** Normalization Coefficients from another ASTRA data file that is open.  
    
  ***HELEOS users only:***   
  Due to the refraction at the glass/solvent interface, Detector 1 is only available in batch work with a scintillation vial. Detector 2 is only visible in aqueous solvents when using the Fused Silica flow cell. This instrument still has the older K5 flow cell type, so both detectors 1 and 2 have normalization coefficients of 1.000 in this example.  
  Additionally, Detector 12 has been replaced with the QELS optical fiber in this instrument, so the Normalization coefficient is 1.000 for this detector as well.

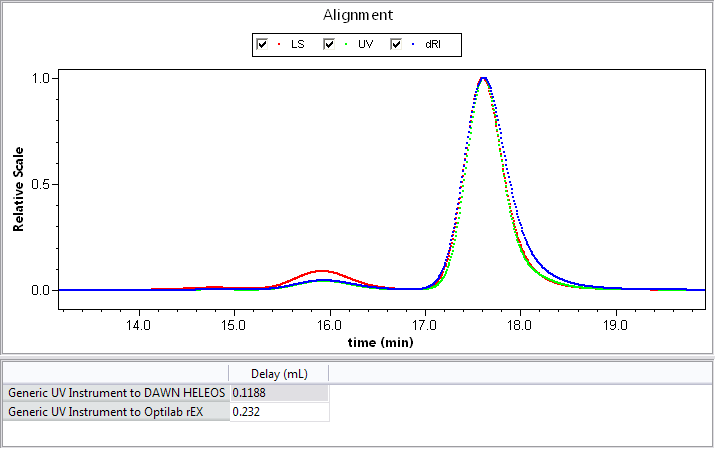
Click **OK** to save your settings and to close the **Normalization** window.

### 4. Alignment:

* Right-click on **Configuration** and select **Alignment**. This will open the **Alignment** window.
* Zoom into the BSA peaks and select a peak region that covers the signal from all three detectors for the monomer peak as shown below. Exact peak ranges are not important for Alignment.



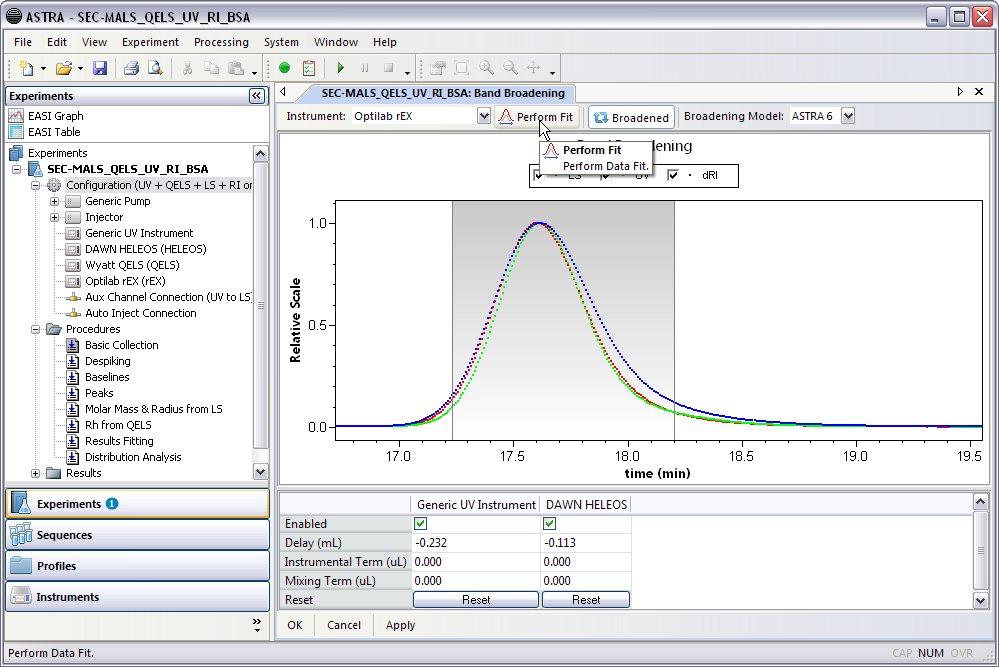
* Click on **Align Signals** above the graph. The **Delays** will be different from zero and the properly aligned detectors will look like this:



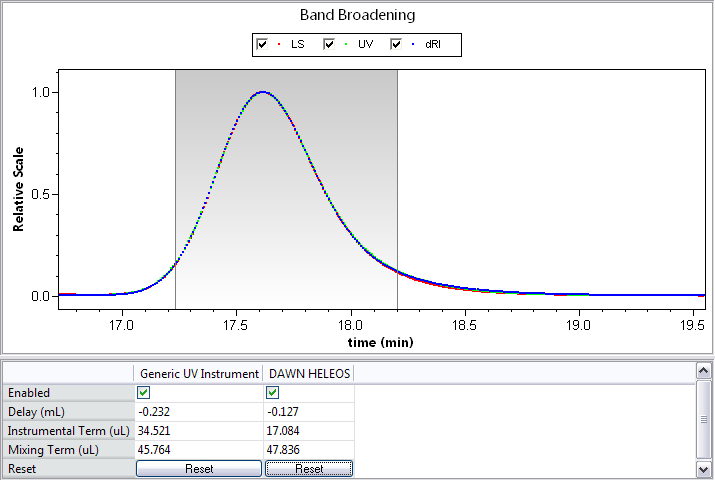
* Peak broadening is particularly visible for the dRI detector. This will be corrected in the **Band Broadening procedure** below. Click **OK** to close the **Alignment** window.

### 5. Band Broadening:

* Right-Click on **Configuration** and select **Band Broadening**. This will open the **Band Broadening** Procedure.
* Select the limits of the BSA monomer 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, which is not the case for this example).

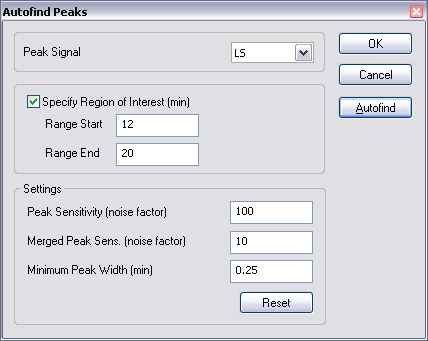


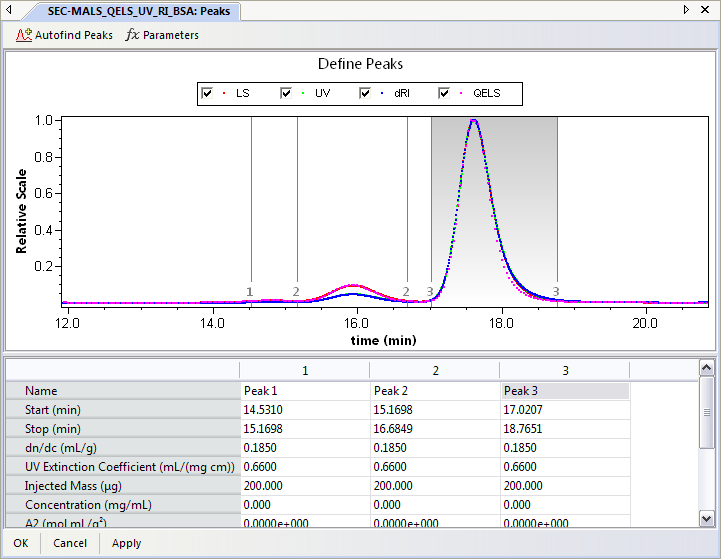
* The Reference Instrument will be automatically selected. Click on **Perform Fit.**
* The properly broadened peak should now look like this:



* You can check that the **Instrumental Terms** should be between 1-40 and the **Mixing Terms** for a UV or LS detector will be approximately 30-80.
* ASTRA 5 users: Note that the peak range selected and the values obtained for band broadening are different in ASTRA 6!
* Click **OK** to save your settings and close the **Determine Band Broadening** window.

### 6. Adjust Peak Selection

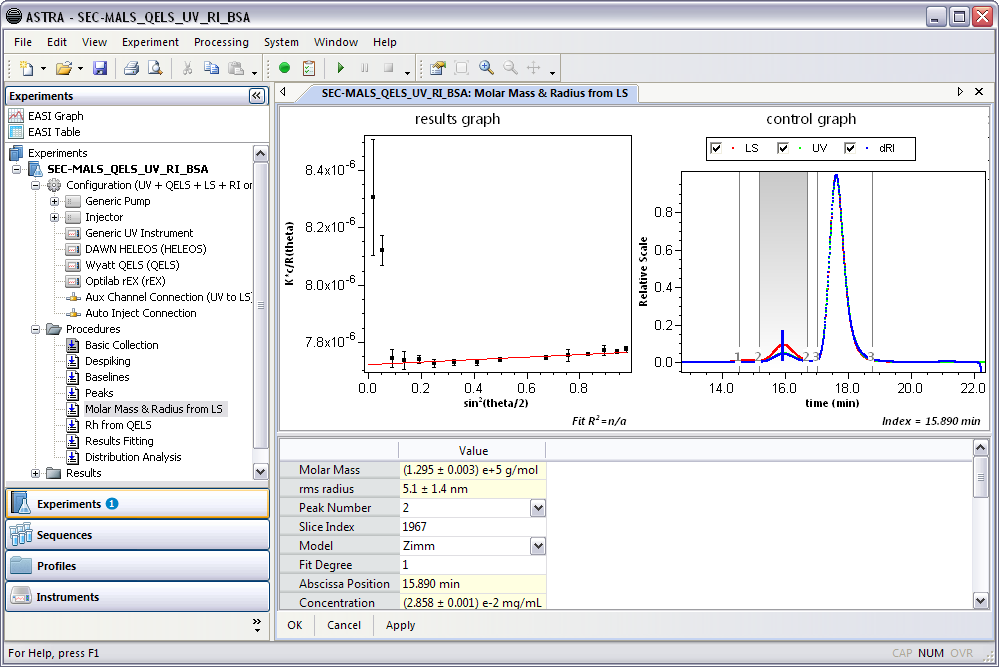
* Double-click on the **Peaks** procedure in the workspace and set peaks for monomer, dimer and trimer:
* ASTRA 6:
  + You can use the **Autofind Baselines** feature above the graph to automatically set baselines for all detectors;
  + Adjust the **Fx parameters**, if necessary. Parameter settings as shown below are good starting points for proteins:  
      
    
  + Click on Autofind (Peaks) to set the peaks.



* The ASTRA 5 way:
  + Click into the graph and select peak regions for monomer, dimer and trimer.
* Click **OK** to close the **Define Peaks** window.

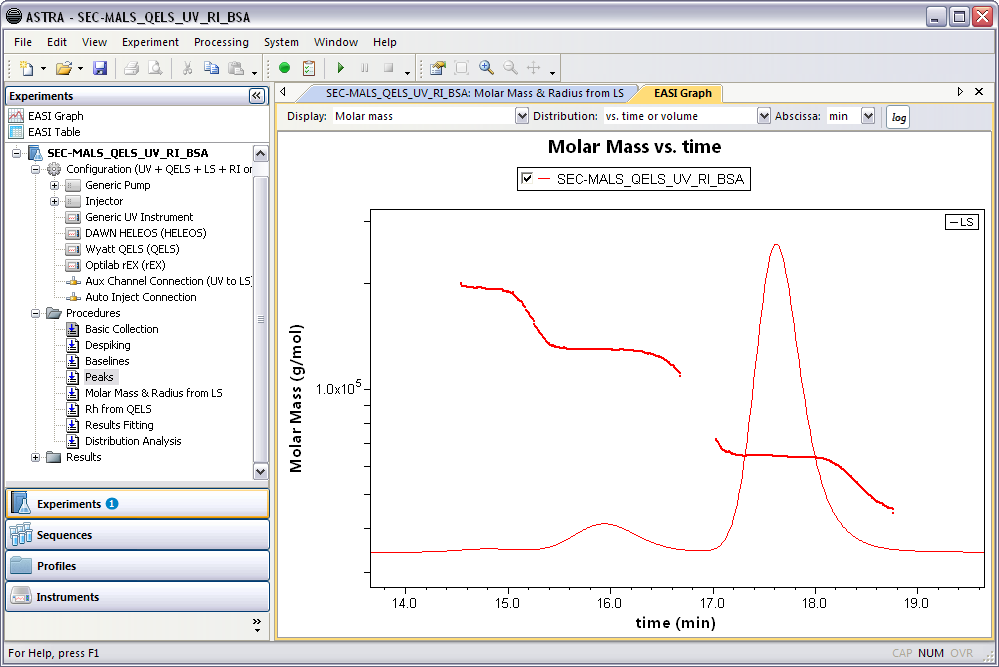
### Determine Molar Mass and Radius from LS:

* Open the **Molar** **Mass and Radius from LS** procedure. All data points in the **Results Graph** (Debye Plot) should form a straight line (within the error bars). Some of the low and high angle detectors might need to get disabled to achieve this (see e.g. detectors 3 and 4 below).
* The molar mass and the radius are displayed for each slice of the selected peak in the chromatogram.

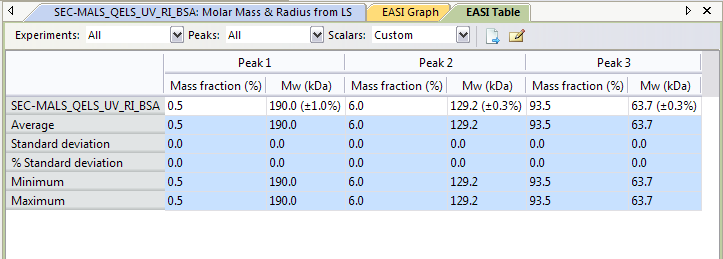


The molar mass can be displayed either graphically or numerically as shown on the next pages.

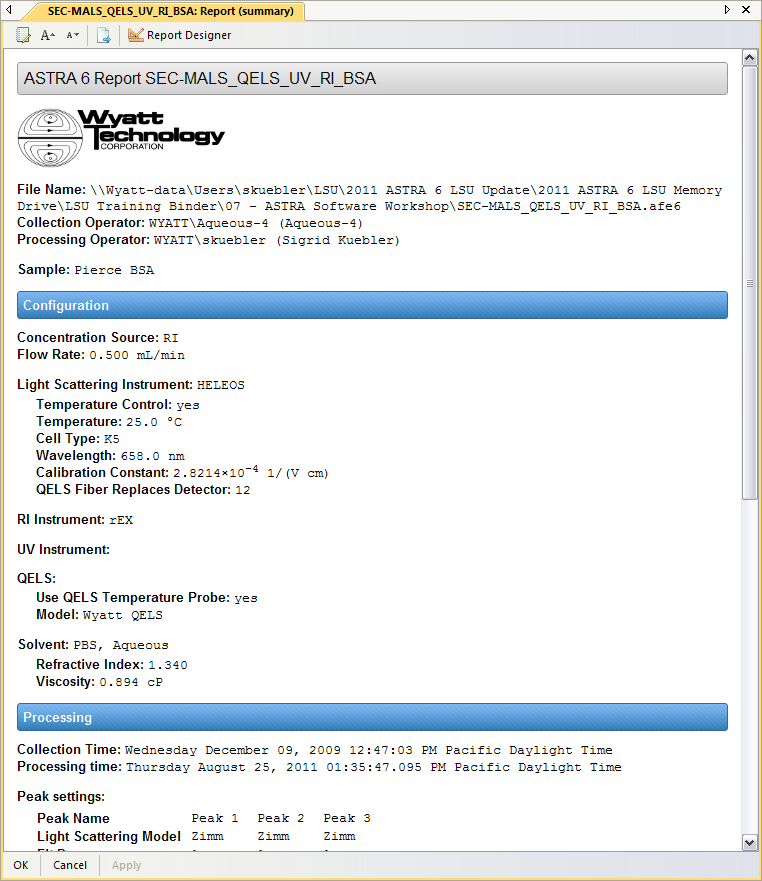
**Graphical display in EASI Graph (also overlay different experiments):**

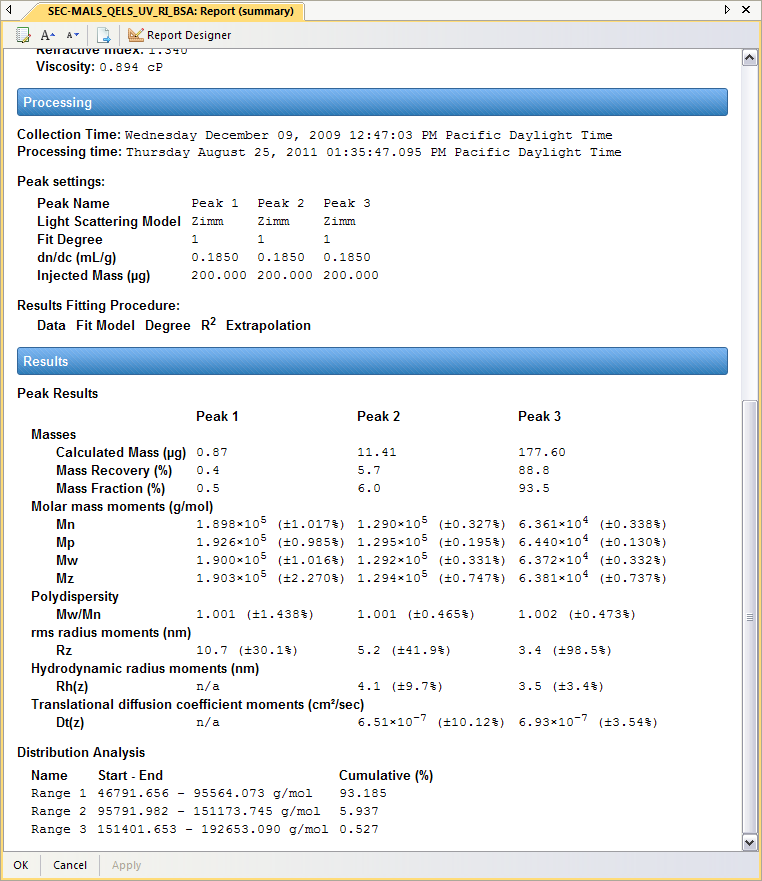


**Numerical display in EASI Table (overlay and perform statistics on results from different experiments):**

****

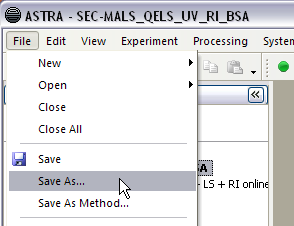
**Summary Report in ASTRA 6 (can be customized with the Report Designer):**





### Running and processing data once a first injection sample has been run and processed (see above)

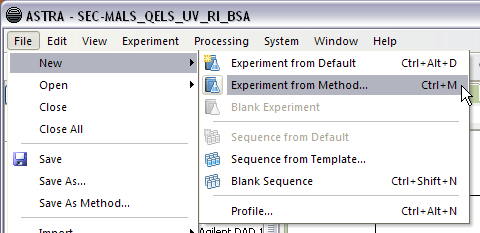
* 1. Save the data file that you have processed above (e.g. SEC-MALS\_QELS\_UV\_RI\_BSA)



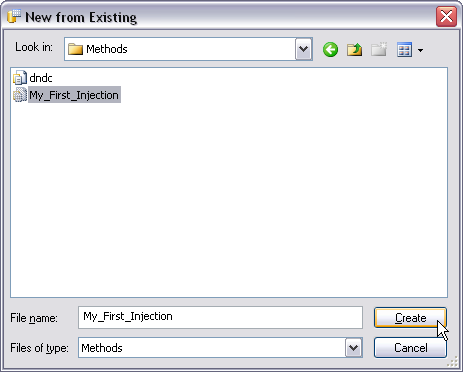
* 1. Also save this file as a Method in the User → Methods Folder (e.g. My\_First\_Injection). You can choose to make this method the default method for your HPLC setup.

|  |  |
| --- | --- |
|  |  |

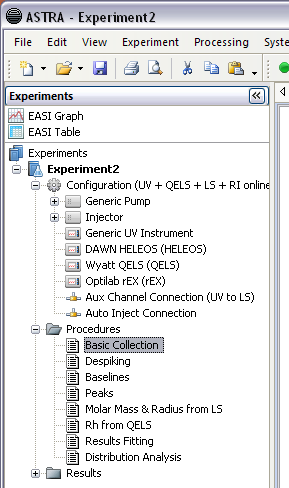
* 1. For the next experiment, select File → New → Experiment from Method or File → New → Experiment from Default (if you checked the Make Default box in the dialog above).



* 1. If you haven’t made the method above your Default Method, navigate to the User → Methods folder, select *My\_First\_Injection* and click on Create:

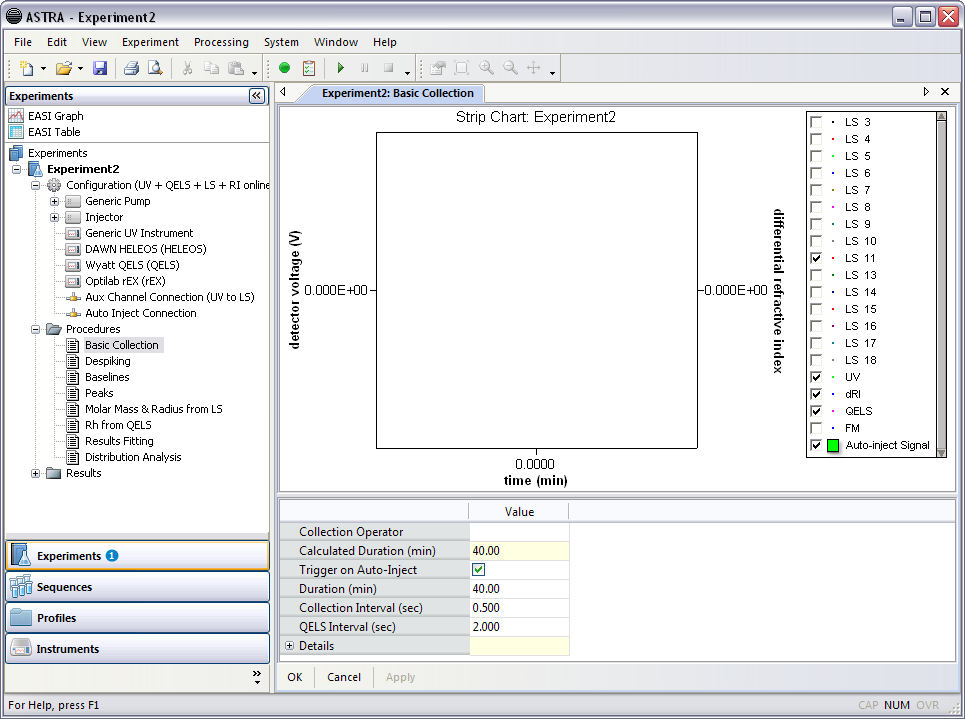


* 1. This will open up a new Experiment in the ASTRA workspace:



**Note that all Configuration parameters, such as your pump, Injector and physical instruments, as well as Normalization Coefficients, Interdetector Delay Volumes and Band Broadening are already set correctly in this method for your current HPLC system setup.**

* 1. Set Duration and Collection Interval for your new experiment and click on the “Run” icon to start data collection. You may want to adjust parameters, such as dn/dc or UV extinction coefficients as well (can be also done post-acquisition).



* 1. After data collection is completed, your data should be processed automatically. If not, you will need to click the “Run” icon again to process your data.

***You will now only need to:***

* + - set or adjust baselines,
    - select peak limits and adjust parameters, e.g. dn/dc, UV extinction coefficient, …
    - and view your results!

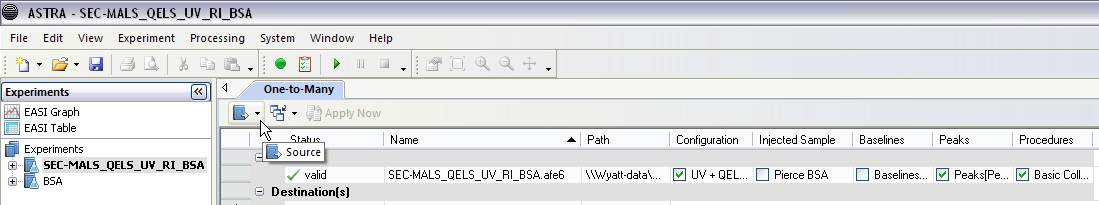
***Notes:***

* If your method contains processing information such as baselines and peak settings, the data file will be automatically processed. If you do not want processing parameters in your methods, delete those and then save this data file as a *method*.
* If you change your HPLC setup (e.g. tubing between detectors, add detectors) or change your mobile phase, you may need to renormalize or determine Interdetector delay volumes and Band Broadening Parameters. Please refer to Section 6 of your LSU course binder for more information.

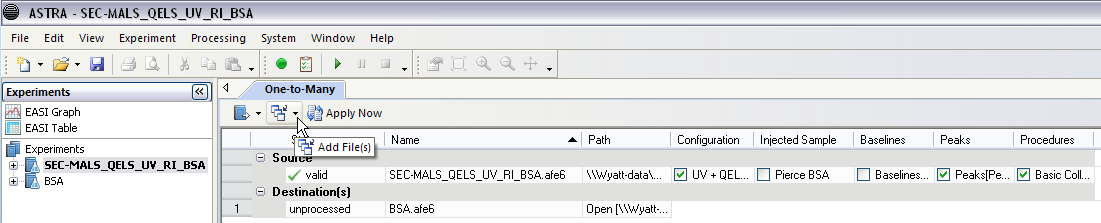
### Applying Normalization Coefficients, Interdetector Delay and Band Broadening parameters after a data file has already been run:

Using One-to-Many (preferred method):

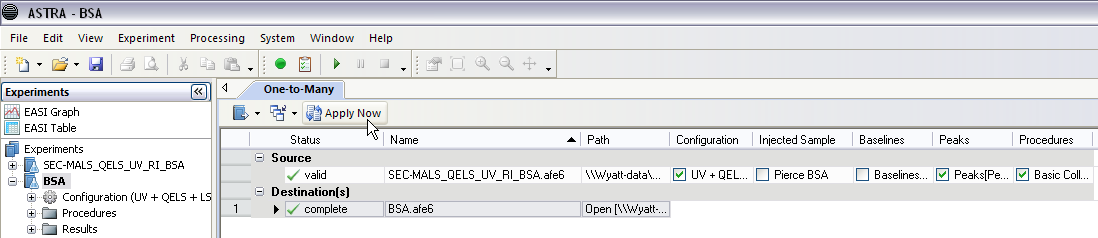
1. Select **File** → **One-to-Many** (CTRL+B)
2. Select a **Source**. The Source file does not need to be open in ASTRA.



1. Specify which parameters you like to transfer, e.g. **Configuration**, **Peaks** and **Procedures** in our example.
2. Select one or more **Destination** files.



1. Push the **Apply Now** button. Once the **Destination** data files have all been processed, the status will be shown as “complete”.



***Notes:***

* **One-to-Many** is the preferred method to transfer settings from one file to another in *ASTRA 6*. ASTRA 5 users will be more familiar with *Replacing Configurations* as described below.
* On existing data, applying *Methods* (ASTRA 5: *Templates)* only apply to *Procedures*, not settings such as Normalization Coefficients, Interdetector Delay, Band Broadening parameters, Calibration constants, solvents, etc. which are part of the *Configuration* of an Experiment.
* To change these *Configuration* parameters, the *Configuration* of the existing data file will need to be *replaced (see below)*.

Replacing Configurations

1. Save the configuration of a data file that has the desired settings in the **User** → **Configurations** folder (e.g. “*BSA\_configuration*”):

|  |  |
| --- | --- |
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2. Open the data file that you want to apply this **Configuration Profile** to and choose **Replace Configuration** with the desired **Experiment Configuration** (“*SEC\_MALS LSU*” in the **User** → **Configurations** folder):

|  |  |
| --- | --- |
|  |  |

1. View the Replaced Configuration of your data file:

|  |  |
| --- | --- |
|  | ***The data file  “BSA”  will now contain the desired Configuration parameters, such as Normalization Coefficients, Interdetector delay volumes, Band Broadening parameters, etc.*** |

**ASTRA FAQs**

**What are the “illegal” characters Astra prohibits from file names?**

The following characters are not allowed as part of file names:

The colon:                     :  
Question Mark:              ?  
Quote:                          "  
Asterisk:                       \*  
Forward Slash:              /  
Back slash:                   \  
Less than:                     <  
Greater than:                 >  
Pipe:                             |

These are reserved by the operating system to indicate path information, disk/device, or are ‘wild card’ characters used for pattern matching of file names.  The standard file browser dialogs will reject these characters, as will the fields containing components of file names (such as the ‘name’ portion of the sample set grid).

**Where does Astra get the names for the “Collection” and “Processing” operator entries in the reports?**

**In ‘basic’ mode:**

* The “Processing Operator” is determined at the time the report is generated, and is retrieved from Windows as the “currently logged in user”.  So on the “Aqueous-1” machine, this will show up as “Aqueous-1”.
* The “Collection Operator” is determined at the time the “StartCollection” message is generated, and is again retrieved from Windows as the “currently logged in user”.   This is then embedded in the collected data, and retained forever after.

**In 21CFR mode:**

* The “Processing Operator” is determined at the time the report is generated, and is the login information for the user currently logged into Astra.  It ignores the Windows “currently logged in user”.
* The “Collection Operator” is determined at the time the “StartCollection” message is generated, and is again based on the login information for the user currently logged into Astra.  It ignores the Windows “currently logged in user”.   This is then embedded in the collected data, and retained forever after.