**ASTRA 5 Quick Guides**

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A quick guide to collect data in ASTRA 5.3.4.x using **Sample Sets**. This guide assumes that the ASTRA 5 collection template has already been created and saved.

**Sample Sets: Open ASTRA 5.3.4.x**:

1. **FILE < NEW < Blank Sample Set** This should open a blank sample set in the ASTRA 5 workspace on the left hand side of the ASTRA display.
2. Double click on the word “**Configuration**” in the workspace to open Configuration in the right hand window.
3. In the “**Default Experiment Template**” field click the browse button […] and navigate to and select the ASTRA 5 experimental template to be used for data collection.
4. In the “**Number of Samples**” field manually enter the number of samples (vials) to be run.
5. Click the “**APPLY**” button near the bottom of the window.
6. Click the “**Samples**” tab near the bottom of the window to open the sample info page. There should be a row for each of the samples.
7. Complete each field as:

* **Well**: This field is not used – just leave it at 0
* **Enable**: Make sure there is a check mark
* **Name**: This is the file name that will be used when the file is saved on the hard drive. Do not use any strange keys (/ % \* \ etc.)
* **Description**: This is a free field. Use it if you wish to enter sample info.
* **In**j.: This is the number of injections to be made of this sample.
* **Template**: This will be the template that you selected in step 3 above.
* **dn/dc**: Enter the dn/dc of your sample. (optional)
* **A2**: Leave at 0.0
* **UV Ext**: If using a UV detector enter the UV extinction coefficient in units of (mL/g cm) (optional)
* **Conc**.: Sample Concentration in g/mL (optional)
* **Vol**: Injected volume in mL. (optional)
* **Delay**: Leave this at 0.0
* **Duration**: This is the ASTRA “run” time. Change if needed.

1. Click “**APPLY**” and then “**OK**” near the bottom of the Window.
2. Click the “**Run Sample Set**” icon near the top of the ASTRA window. (looks like 4 vials).
3. A “Save” Window should be displayed. Navigate to the location on the hard drive where you want to save the ASTRA data and enter a name for the Sample Set in the “File Name” field. Click “**SAVE**”.
4. A message “**Waiting for auto injection**” should be displayed. Use your HPLC software begin the sample injections.

As each ASTRA experiment is collected it will be saved to the hard drive at the location specified above in step # 10.

**ASTRA 5 Quick Guide – Processing Data – HPLC/MALS data**

A quick guide for the processing of ASTRA 5.3.4.x MALS/HPLC data. This guide assumes that the ASTRA 5 experiment has already been collected and saved. This also assumes that the detector delay volumes (alignment) and normalization have been completed and incorporated in the template that was used for data collection.

**Open ASTRA 5.3.4.x:**

1. **FILE < OPEN < EXPERIMENT** and navigate to the desired ASTRA 5 experiment. This should open the experiment in the ASTRA workspace on the left hand of the ASTRA display.
2. Click on the name of the experiment in the workspace to bring the focus to this experiment.
3. Click the “**Run Experiment**” icon (looks like an arrow head) near the top of the ASTRA display. The message “Some of the necessary parameters are not set for the Define baselines procedure….” Should appear. Click OK. The Define baselines window should appear.
4. In the legend of the plot select the 90 degree detector (det # 2 for a 3 angle detector and det # 11 for an 18 angle detector). In the plotted data click and drag a baseline from well before any peaks to well after any peaks.
5. Scroll to the last line in the grid below the plot and click the “**Autobaseline**” button. This will set the baselines for all detectors. Examine the baseline for each detector and change if needed. If changes are made to any of the baselines **DO NOT** click the “**Autobaseline** button again. It may be advisable to zoom in on the baselines to evaluate the baseline selection and make changes as desired.
6. When the baselines for all detectors have been set and evaluated, click “**APPLY**” and then “**OK**” near the bottom of the page to accept these baselines.
7. The message “Some of the necessary parameters are not set for the Define peaks procedure….” Should appear. Click the “**OK**” button. The Define peaks window should be displayed.
8. In the Define Peaks window plot click and drag the desired peak region. In the grid below the plot enter a dn/dc value if the correct value is not already displayed. Enter a UV Extinction coef if a UV detector is being used. (optional). Click the “**APPLY**” and then the “**OK**” button.
9. Click the **[+]** in front of Results in the workspace to expand the results. Double click on “**Report (summary)**” to view the results.
10. To save the experiment with the above changes click on the name of the experiment to bring the focus to this experiment and go **to FILE < SAVE** **or FILE < SAVE AS**.. to save with a new experiment name.

**ASTRA 5 Quick Guide – Setting Detector Delay Volumes (Alignment) and Normalization**

A quick guide for setting the detector delay volumes (detector alignment) and normalization in ASTRA 5.3.1.x. This guide assumes that HPLC/MALS data (an ASTRA 5 experiment) suitable for setting the delay volume and normalization has been collected and processed (baselines set and a peak region set). The sample should be Bovine Serum Albumin (BSA) for an aqueous mobile phase or a 30 kD narrow Polystyrene standard for an organic mobile phase.

**Open ASTRA 5.3.4.x**

1. Open the ASTRA 5 experiment to be used for the determination of the detector delay volumes and the MALS normalization coefficients. (A BSA or a narrow 30 kD polystyrene sample)
2. Process the data: Set the baselines and peak region **if not previously set**.
3. Click on the name of the experiment to bring the focus to this experiment.
4. **Alignment:** Right mouse click and navigate to: **MANAGE < CONFIGURATION < ALIGMENT**. This should open the “Determine Interdetector delay” view.
5. In the plot click and drag a region where the peaks from the different detectors should all overlay. (The peak must be a monodisperse sample like BSA monomer or a 30 kD polystyrene in toluene. Click the “Determine Delays” button below the plot. The peaks from all the detectors should now be overlaid. Click the “OK” button near the bottom of the page to save these delay volumes.
6. **Normalization**: Right mouse click on the name of the experiment to bring the focus to this experiment.
7. Right mouse click and navigate to: MANAGE **< CONFIGURATION < NORMALIZE**. This should open the Normalization profile.
8. Enter: a) Peak Number = 1, b) Radius = 3 for BSA and 6 for 30 kD PS, c) Radius Type = rms, Click the **NORMALIZE** button to compute the normalization coefficients. Click “APPLY” and then “OK” to accept these constants.
9. Click on the name of the experiment to bring the focus to this experiment. Right mouse click and select “Save” to save this experiment with the above changes.
10. To save the interdetector delay volumes and the normalization coefficients in the ASTRA 5 template click on the name of the experiment, right mouse click, and select “Save as template”. Give this template a new name. Use this template to collect ASTRA data in the future. **Note**: The above template will contain the peak regions and the baselines used in this file. Normally a template should not contain any peak regions or baselines. Before saving as a template manually delete the peak regions and all baselines before saving as a template.

**ASTRA 5 Quick Guide – Single Injection**

A quick guide to collect data in ASTRA 5.3.4.x for a single injection (when not using sample sets). This mode may be used to collect baseline data when no injection is made or when data from only one injection is to be collected. This guide assumes that the ASTRA 5 collection template has already been created and saved.

**Open ASTRA 5.3.4.x**

1. Go to: “File < New < Experiment from template” and navigate to your previously created collection template. This will open the template in the ASTRA left hand side workspace.
2. Expand the **[+] Procedure** section of the template by double clicking on the **[+] Procedure**.
3. In the [+] Procedure section double click on the first entry – “**Basic collection**” to open the basic collection profile.
   1. If an autosampler will be used or a manual injection valve with an autoinject feature will be used then place a check mark in the “**Trigger on Auto-Inject”** box in the table below the strip chart plot. In this mode data collection will begin automatically when the injection is made.
   2. If you wish the data collection to begin from a keyboard command ensure that there is **no** check mark in the “**Trigger** **on Auto-Inject”** box. In this mode the ASTRA data collection will start from a keyboard command and thus may be started at anytime.
   3. Change the “**Duration”** entry to be the desired run time or the total time data is to be collected.
   4. Click the “**Apply**” and then the “**OK**” button near the bottom of the page to accept the above changes.
4. To begin data collection click the “**Run Experiment**” icon near the top of the ASTRA page.
   1. If data collection does not begin immediately a message window mayl appear suggesting to select **OK** to begin collection.Click OK to begin data collection..
   2. If the “**Triger on Auto-Inject**” option had been selected a message “Waiting For Autoinjection” should appear. Inject the sample to automatically begin data collection.
   3. If the “**Trigger on Auto-Inject**” option had not been selected then data collection will begin when the “OK” button is selected.