

Millennium³² Step by Step Guide to GPC Acquisition & Processing

Purpose: This procedure will guide you through the steps necessary to create methods that will process and report chromatographic data using Millennium32 GPC data. This document also includes steps on how to develop a method that integrates peaks, calibrates narrow standards, quantitates broad unknowns, and generates reports.

Developing a Processing Method is Review- this section will step you through the development of a basic processing method

- 1 In the Millennium 32 login screen, click to select the **Review Data** icon (if the project is already open, then select the **Channels** tab).



- 2 Highlight the **narrow standards** and **broad unknowns**, click the **Review** icon, or right mouse click and highlight **Review**. The review window is displayed (if the project has already been opened, from the menu bar select **Tools – Review**).



- 3 Make sure that a **narrow standard** is selected, then click the **first icon** on the left side of the Review window (**Processing Method Wizard** icon), or from the menu bar select **File – New Method (or open method – an existing one)**.
- 4 In the Processing Wizard dialog box, select **Create a New Processing Method (or Edit Existing One)** and Select **OK** .
- 5 Click in the **Use Processing Method Wizard** box to use the wizard template, then click to select **OK**. Change the **Processing Type** to **GPC**. The wizard will build a processing method that will integrate peaks, calibrate narrow standards and calculate Molecular Weight measurements for Broad unknowns.
- 6 Follow the wizard: Use the mouse to box and zoom in on the area of the chromatogram that you want integration and peak detection to occur. This will automatically inhibit integration for all peaks not shown in the boxed area. Click the **Next** button to continue.

Note: To unzoom **Right Click** - then select **Fullview** from the selection list. Click **Next** button to continue.





- 7 Optionally: select **Minimum area** or **Minimum height** by clicking inside the smallest peak of interest and clicking minimum height. Repeat this step for minimum area if needed. Click the **Next** button.
- 8 The suggested Peak Width and Threshold settings will be displayed for this chromatograms. Click **the Clear Peak and Threshold** if there are varied sized chromatograms. Click the **Next** button.

- 9 Note: Narrow standard identification will be determined by retention time order, so it is very important to determine the proper integration of peaks.
- 10 For Relative GPC Calibration (different polymer for standards than for unknown), leave the calibration type to Relative, Set the Calibration Fit Type, and using the calibration to time or volume. Click **Next**.



Note: Fit types are usually set to **3rd order** or **Bounded fit**. It is important to enter the **molecular weights** in **Quickset** or **Alter Sample** for each narrow standard.
- 11 **Optional:** Determine **Vo** (Void Volume of the bank of columns you are using) and **Vt** (Total retention time of the bank of columns) and enter them in the Column Set dialog box. Click Next.
- 12 Enter in a name for the processing method. Optionally enter in comments. To undo a name, use the right mouse click and click **Undo**. Click **Finish**.
- 13 The narrow standard will be integrated and peaks identified according to the processing method just created.
- 14 In the Review Main window, **select the broad samples**, then select the **wizard icon** (first icon on the left side in the Review window).
- 15 Leave the radio button checked to Edit Existing GPC Processing Method and Keep the Calibration. Click OK.
- 16 Use the mouse to box and zoom in on the area of the chromatogram that you want integration and peak detection to occur. This will automatically inhibit integration for all peaks not shown in the boxed area. Click the **Next** button to continue
- 17 The suggested Peak Width and Threshold settings will be displayed for this chromatograms. Click **the Clear Peak and Threshold** if there are varied sized chromatograms. Click the **Next** button.
- 18 Select **Minimum area** or **Minimum height** by clicking inside the smallest peak of interest and clicking minimum height. Repeat this step for minimum area if needed. Click the **Next** button.
- 19 Let the calibration setting be as is and click **Next** . Let Vo and Vt as is and click **Next**.
- 20 **Enter in the peak name and retention time for a distribution to be calculated.** If multiple distributions are desired, make sure to enter a second peak and retention time. The Retention time window for one component will be at 50%. Click **Next**.

Note: It is important at least one peak is listed in the Distribution table, or molecular weight distributions will not be calculated.
- 21 The Processing Method name will be displayed, then click **Finish**. The broad

unknown integration has been added to the gpc processing method.

- 22 To edit the processing method, or add advanced integration to the processing method, select from the menu bar – **View - Processing Method Layout**.
- 23 To save the processing method to the Method set select from the menu bar, **File - Save As**. Enter in the name for the Method Set and the processing method will be added to the method set.
- 24 **Manually integrate** clicking the **Integrate Icon**. 
- 25 **Manually calibrate** by clicking the **Calibrate Icon**. 
- 26 **Manually quantitate** by clicking the **Quantitate icon**. 
- 27 To view results from manual processing,  click the **Peaks tab** at the **bottom** of the Review window.
- 28 To save **manual processing**, select from the menu bar of the Review window **File - Save Results**.
- 29 To **exit** review, select from the menu bar of the Review window **File - Exit**.

Batch Processing Data using the Process and Report Tool

1. In the project window, click the **Sample set tab**. Click the desired sample set and use the **Right Mouse click** to view as **Channels - or Click on the Channels tab**.
1. Click in **Sample Type** to select **Narrow Standards before Broad Unknowns**. If standards are not first, click again and Standards will be sorted properly.
2. Highlight all standards and unknowns to be processed, using either the World icon, or use ctrl-mouse click. 
1. Click on the **Process** icon or use the Right mouse button and highlight **Process**. 
2. In the Processing area, click on the Use Method Set radio button. Click in the Specified Method Set drop down box to select the method set you created. Click the Clear Calibration button if recalibration is needed.
3. Note: For 2D data selection, you may select Use Specified Processing Method.
4. In the Reporting area check the Reporting box, and click on the Use Specified

Report button. Click in the Specified Reporting Method drop down box to select the reporting method you selected (use Default as a basic report)

5. Click OK and the data will be processed and reported.

Reviewing Results



1. All results will be displayed in the **Results** view.
2. To review results, **highlight the desired results**, and click on the **Review icon**, or **right mouse click** and select **Review**.
3. In the Review window, the data will be displayed with component names. Click the **Peaks tab** below the chromatogram to view peak information for each component.
4. To view the calibration curve, select from the menu bar **Window - Calibration**.
5. To view all details of the results, select Window- Results.
6. To view Peak, Chromatogram, and Calibration in one screen, select Window - Tile Vertically (or Tile Horizontally).
7. Select File - Exit to leave the Review window.
8. To Print Results, click the Results tab, and click to select the desired results.
9. Click on the Print icon, or use the Right mouse click and click Print. Select the desired Report to use and click OK. Your reports will be printed out on your printer.

