

Millennium³² 4.00 Step by Step

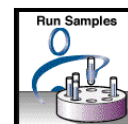
To PDA (Non-Regulated)

Purpose: This procedure will guide you through the steps necessary to create methods that will acquire, process and report PDA data using Millennium³² version 4.00.

Starting Millennium³²

1. From the **Start** menu bar, click **Start - Programs - Millennium³² - Millennium³² Login**.
2. In the User Name area enter in **XXXXXX**, and the Password **XXXXX** and click **OK**. You will be logged into Millennium³² and all screens will be active.

Running Samples - Creating the Instrument Method and Method Set



- 1 From the Millennium³² main window, double click on the **Run Samples** icon (or **right mouse click** and select the system you prefer).
- 2 In the Run Samples dialog box, click to select the system desired to collect data, then click **OK**. The system will be connected to the **Bus Lace** and the Run Samples window will be displayed.
- 3 In the **menu bar of the Run Samples window** select **Edit – New Method Set**. Select **Yes to Use the Wizard to Create this Method Set**.
- 4 In the Select Instrument Method dialog box click on "**Create New**" to develop a new instrument method.
- 5 Click on each instrument and edit each instrument's parameters. **File - Save**. Enter in the **Name** and description of the instrument method, then click **Save**. Select **File - Exit**.
- 6 In the **Select Instrument Method** dialog box, highlight the desired method. Click **Next**.
- 7 Select a **Processing Method** from the Processing Method from the drop down list. If desired, select a **Report Method**. Click **Next**.
- 8 Click **Define PDA Derived Channel**. Make sure Single Wavelength is selected and enter the desired wavelength in the Wavelength box. Click **Next**.
- 9 The derived channel is assigned a wavelength name. Click **Next**. The **Derived Channel Summary** appears (optionally repeat steps 8 & 9 for multiple wavelengths selections).
- 10 Click **Next**. In the **Name Method Set** dialog box, enter in a name and comment for the **Method Set**. Click **Finish**. Select **File – exit**
- 11 In the Samples Table fill out the information for each row, including the sample name, the Method Set to use, the Sample Type (Samples Standards etc), injection volume, run time, etc.

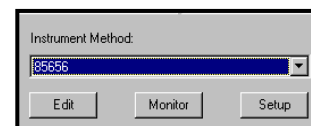
Millennium³² Step by Step Guide to PDA for Version 4.00

12. To enter amounts, click the **Amounts** icon. Enter in a component name, concentration, and unit for each standard vial. Select **File – Exit** after entering concentrations.



11. Make sure the **Run Mode** is set to **Run and Process** or **Run and Report**. This will ensure that the wavelength is extracted.
12. From the Menu Bar select **File - Save Sample Set Method**. This will be saved as a template for future acquisition of samples.

13. To setup your instruments before acquisition: Click in the Instrument drop-down box to select your instrument Method and click **Setup**.



14. To acquire Samples click the **4th icon (Green light)**.



15. Select a Printer to use. Click **OK**.
16. Enter in a name for the Sample Set. Click the Run Samples button.
17. After all acquisition has occurred, select **File - Exit** to exit out of Run Samples.

Developing a Processing Method in Review

1. In the Millennium 32 browse project window, select the desired project, and then select the **Channels** tab.

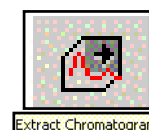
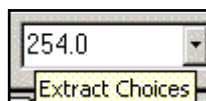


2. Highlight a standard channel and click the **Review** icon, or right mouse click and highlight **Review** (if the project has already been opened, from the menu bar select **Tools – Review**).



3. Select **View - 3D Layout** from the menu bar.
4. If a derived wavelength and a generic processing method were included in the acquisition method set, select **File, Open, Method Set** and select the desired method set. The method set will be automatically applied and the extracted wavelength chromatogram will be displayed.
5. The chromatogram at the extracted wavelength appears with “**default**” integration. Proceed to step 7.

To manually extract Chromatograms:



6. Enter a specified wavelength in the Extract Choices drop down list, and then click the Extract Chromatogram icon.

7. Click the **Processing Method Wizard** icon on the left hand side of the Review window icon display, or from the menu bar select **File – New Method**.



8. Select **Create a New Processing Method** and Select **OK**. Make sure that **PDA** is the Processing Type and click **Use Processing Method Wizard**, click **OK**.

Millennium³² Step by Step Guide to PDA for Version 4.00

9 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. Click the **Next**

Note: To unzoom **Right Click** - then select **Full view** from the selection list

10 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.

11 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.

12 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 **Next**.

13 Click in the "**quantitate by**" drop down box to select **area** or **height**. Click in the amount – concentration drop down box to select **concentration** or **amount**. Click in the calibration fit type to select the type of calibration curve used (default to **linear**). Click **Next**.

14 If using a standard, **click the drop down box to select the peak name**. If using an unknown, enter in the compound names and make sure they match the component name entered in the **Amounts** from **Run Samples**.

15 Select the **Type of Calibration** (skip and click **Next** if not calibrating). Click **Next**.

16 The **PDA Purity/Matching** window appears. Select **Yes** for peak purity testing and **No** to library matching. Click **Next**.

17 The **PDA Spectral Contrast** window appears. Select a noise interval. **Note:** examine the Maxplot chromatogram displayed and make sure that the region you select is free of all peaks. Click **Next**.

18 Enter in a name for the processing method. Enter in comments. Click **Finish**.

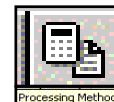
19 The chromatogram will be integrated, peaks identified, and UV spectra displayed according to the **processing method** just created.

20 Create a spectral library and add all displayed spectra into the Library:

- a. From the menu bar, select **Library – New Library**.
- b. Enter a **name** for the library and click **OK**.
- c. From the **Library** pull down menu select **Add to Library XXXXXX**.
- d. The name for the first spectrum is listed in retention time order and the name is taken from the peak name you entered using the wizard. Click **OK**. Repeat for all remaining spectra.

Millennium³² Step by Step Guide to PDA for Version 4.00

21 Click the **Processing Method** icon and select the **PDA Library Search** tab. Select the name of the library to search against.



22 To make changes to the Peak Purity section, select the **Peak Purity tab** of the Processing Method. To make changes to the Components or calibration type, select the **Component tab** of the Processing Method.

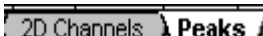
23 Click the review main window icon to return to the chromatogram and spectra screen.



24 From the **File** pull down menu, select **Save Processing Method**. 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.



25 Manually Process by clicking on the **Integrate, Calibrate, and Quantitate Icons**.

26 To view results from manual processing, click  the **Peaks tab** at the **bottom** of the Review window. To view the spectrum index (chromatogram and spectra) select the Spectrum Index tab displayed next to the contour plot tab on the left hand side.

27 To view the Purity Results, select the Results icon, and then select Purity & Purity Plot, Library & Library Plot. View the Purity/Library results.



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 Sets tab**, or click the desired channels sample set and use the **Right Mouse click** to view as **Channels** - or click on the **Channels** tab.

2. Highlight all standards and unknowns to be processed – making sure that standards are selected before unknowns, and click on the **Process** icon or use the Right mouse button and highlight **Process**.



3. 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, and make sure that that **How: is selected to Calibrate and Quantitate**.

Millennium³² Step by Step Guide to PDA for Version 4.00

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. Peak Purity and Library information will be displayed if processing was activated.
4. To view the calibration curve, select from the menu bar Window - Calibration. To view all details of the results, select Window- Results.



5. To Print Results, click the Results tab of the project window, and click to select the desired results. 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.
6. To Preview or change a report before printing out a Result, click the Results tab of the project window, and click to select the desired results. Click on the Preview/Publisher icon, or use the Right mouse click and click Print/Publisher. Select "Use a Report Method that was appropriate for the selected data". The result will be displayed in the Preview window.



7. Print the Report by using the Print icon, save the report as a PDF file by using Save Report Icon, or email the Report using the Send Mail icon.



Millennium³² Step by Step Guide to PDA for Version 4.00