

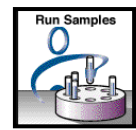
Millennium³² 3.20 PDA 2D Simplified Step by Step (Regulated)

Purpose: This procedure will guide you through the steps necessary to create methods that will acquire, process and report LC data using Millennium³² version 3.20, including acquiring an extracted wavelength and deleting the 3D data.

Starting Millennium 32


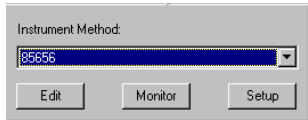

1. From the **Start** menu bar, click **Start - Programs - Millennium 32 - Millennium 32 Login**.
2. In the User Name area enter in **XXXXXX**, and the Password **XXXXX** and click **OK**. You will be logged into Millennium32 and all screens will be active.

Running Samples - Creating the Instrument Method and Method Set






- 1 From the Millennium 32 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 If You have already created a method set, then skip steps 4 – 12 and proceed to step 13 (Load Existing Sample set Method).
- 4 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**.
- 5 In the Select Instrument Method dialog box click on "**Create New**" to develop a new instrument method.
- 6 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**.
- 7 In the **Select Instrument Method** dialog box, highlight the desired method. Click **Next**.
- 8 Select a **Processing Method** from the Processing Method from the drop down list. Makes sure to select some processing method or the derived channel box will not be displayed.
- 9 If desired, select a **Generic Report Method**. Click **Next**.
- 10 Click **Define PDA Derived Channel**. Make sure **Single Wavelength** is selected and enter the desired wavelength in the **Wavelength** box. Click **Next**.
- 11 The derived channel is assigned a wavelength name. Click **Next**. The **Derived Channel Summary** appears. Click **Next**.
- 12 Click **Next**. In the **Name Method Set** dialog box, enter in a name for the Method Set. Click **Finish**.

Millennium³² Step by Step Simple Guide to PDA 2D Version 3.20

- 13 Click in the **Save Extracted Channels Box**, and Click in the **Delete Channels after 3D extraction**. This will save the desired wavelength and delete the #D Data if using run and report. Select **File – Exit** to leave the **Method Set** editor.
- 14 In the Samples Table fill out the information for each row, including the sample name, the **Method Set** to use, the **Sample Function** (Inject Samples, Inject Standards, etc), injection volume, run time, etc.
- 15 To enter amounts, click the **Amounts** icon. Enter in a component name and concentration for each standard vial. Select **File – Exit** after entering concentrations. 
- 16 Make sure the **Run Mode** is set to **Run and Process** or **Run and Report (You must be in Run and Report Mode for extracted channels to work)**. From the Menu Bar select **File - Save Sample Set Method**. This will be saved as a template for future acquisition of samples.
- 17 To setup your instruments before acquisition: Click in the Instrument drop-down box to select your instrument Method and click **Setup**. 
- 18 To acquire Samples click the **4th icon (Green light)**. 
- 19 Select a Printer to use. Click **OK**.
- 20 Enter in a name for the Sample Set. Click the **Run Samples** button.
- 21 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 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 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**. 
 - 4 Select **Create a New Processing Method** and Select **OK**. Select **LC** from the Processing Type drop down list and click in the **Use Processing Method Wizard** box, then click **OK**.
 - 5 Follow the wizard template by: Use the mouse to box and zoom in on the narrowest peak of interest, and the **Peak Width** will be automatically set based upon the boxed area.
- Note: To unzoom **Right Click**-then select **Fullview** from the selection list. Click **Next** button to continue.
- 6 Use the mouse to box and zoom in on the highest baseline noise area without peaks and the **Threshold** will be set based upon the boxed area.

Millennium³² Step by Step Simple Guide to PDA 2D Version 3.20

- 7 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.
- 8 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**.
- 9 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**.
- 10 Enter in each **peak's name**. Compound names should match the name that was entered in the **Amounts** from **Run Samples**.
- 11 Enter in an amount for single point calibration for each peak (if multiple points are to be entered, just click next). To copy down duplicate information for units and amounts use **Ctrl – D**. Click **Next**. Multiple component amounts are entered in **Run Samples, Processing Method or Alter Sample**.
- 12 Select the **Type of Calibration** (if desired – click **Next** if not calibrating). Click **Next**.
- 13 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**.
- 14 The chromatogram will be integrated and peaks identified according to the processing method just created.
- 15 To enter in multiple concentrations of a component in the processing method, or add advanced integration to the processing method, select from the menu bar – **View - Processing Method Layout**.
- 16 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. You must also enter in a comment line.




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

- 18 To view results from manual processing, at the **bottom** of the Review window,  click the **Peaks** tab

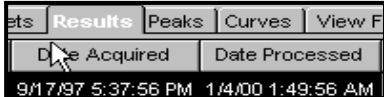
- 19 To save **manual processing**, select from the menu bar of the Review window **File - Save Results**. Enter in a comment line, then select **Ok**.

- 20 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. Click the desired 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, 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.
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. 