

Empower QuickStart LC

Step by Step

Objective:

This procedure will step you through the basics of running 2D analysis with Empower using the QuickStart interface. You will setup an instrument method, method set, and sample set to prepare to run a sample. Make a processing method in review and setup basic analysis. Batch process the sample set, and the preview the result for a peak area and amount.

Starting Empower

1. _____ Login to Empower



- a. Double click on the Empower icon.
- b. For User Name: **System**, For: Password: **Manager** - click **OK**. In the Requested Interface select: QuickStart.




- c. Click Ok and select the desired Project and system then select **OK**. You will be logged into the QuickStart interface of Empower.


2. _____ Customizing QuickStart

- a. QuickStart will be displayed with "Empower Help - How To" screen displayed". This is a guided help that will guide you through the complete process of basic acquisition and processing.

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- b. To turn off the Empower How to as a preference, select from the menu bar **View – Options**. In the “General Tab” deselect “Show Help at Start”, then click on the Acquisition tab and click to select “Bring Completed Single Inject into Review”. Select **OK**. This will turn off the How To help.
 - c. From the menu bar of the QuickStart window-select File – Save Preferences.
3. **Make the Instrument Method**


- a. Click on the Instrument tab  to make a new acquisition method. The View Method will be boxed with the Instrument highlighted.

- b. Click on the 2690/5 (or other pump icon)  of the Instrument method and select:

1. General Tab: Notice the column position and Equilibration Time. This is for a column switch valve and column equilibration.
2. View the Degas Tab. For most 2695's the degas is checked.
3. Click the Flow tab and enter the desired gradient:

4

	Time	Flow	%A	%B	%C	%D	Curve
1		1.00	90.0	10.0	0.0	0.0	
2	6.00	1.00	10.0	90.0	0.0	0.0	6
3	8.00	1.00	10.0	90.0	0.0	0.0	6

5. Click on the 2996 icon (or other 2D detector) . Select 2D data collection and fill out the table as desired:

Method channel	lambda	Method channel bandwidth
1	254.0	4.0

Sampling Rate: 1.0

Instrument Control


Auto Exposure Exposure Time: 15.00

Digital Filter Filter Response: 1.0


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- c. Select **File – Save** and enter the name of the instrument method as XXXX.

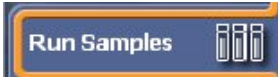

4. _____ **Make a default Processing Method and Method Set**

- a. Click the **Processing Method** icon.  The channels to process will be displayed. This is where chromatogram smoothing, offsets, and math calculations may be accomplished. Select **OK**.
- b. From the menu bar, select **File – Save All**. You can save the processing method for channel 1 as “xxxx1” and the Method Set as “xxxxMS1”, which contains the instrument method and the processing method.

Note: An instrument method and method set must be created before acquiring any chromatographic data.

- c. Click the **Method Set** icon , and in table area of the method set, scroll to the right and select the Report column. Select **default individual report** for each processed channel. Select **File – Save** to save the changes to the Method Set as “xxxxxMS1”.

5. _____ **Make a Sample Set and a Sample Set Method using the wizard.**

- a. Click the  icon. If using the same method all the time, then from the menu bar, select **Customize – Defaults**. For the Instrument Method select the instrument method you just created. Repeat this for the Method Set. Enter in the desired default run time and printer preferences. Click **OK**. Then from the menu bar, select **File- Save Preferences**.
- b. Click on New Sample Set Wizard . This will guide you through the basics of making a sample set including optional bracketing. Leave the Sample Set wizard type to LC PDA/MS and select **Next**.
- c. Select standard injections to occur at the **Start of the Sample Set**. Click **Next**. Leave the calibration options to each standard vial contains a different level. Click **Next**.

- d. In the standard bracket area, enter in the following:


Number of Standards in each group: **x** ; Number of Injections per vial: **x** ;
Injection Volume: **x** ; Run Time: **x** ; Method Set **x** ; Click **Options**: In the **Next Inject Delay** enter in **x** . Click **Next**.


- e. In the describe samples area, enter in the following:

Number of Samples: **x** ; Number of Injections per vial: **x** ; Injection Volume: **x** ;
Run Time: **x** ; Method Set **x** ; Click **Options**: In the **Next Inject Delay** enter in **x**. Click **Next**.

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- f. In the Identification, for the standard name, enter in **Stan**, and in the increment suffix use **1**. For the sample name, enter in **Soda** and in the increment use **a**. Click Next.
- g. Select the Run Mode to **Run and Process**, and select **Next**. Review the summary and click finish. The component editor will be displayed. Enter in the component names in each standard vial. You will identify the components names with the peaks after acquiring the data using the processing method wizard.
- i. Select **OK** after entering all component names and amounts.
- j. From the menu bar select File – Save Sample Set Method and enter in Basic Analysis Set (this will save this table as a template for future analysis) .

- k. Click the Run the Current Sample Icon  and select Run. Select the name the Name for the Sample Set. The sample set will start and the Running tab will displayed with the first sample highlighted in red.

- l.  To see a real time plot on the top right hand area, select the Control Panel tab.

- m. Click the View Acquisition tab  twice to toggle between a real time plot display and turning off this display. This display may be used or not used depending upon the users preferences.

6. _____ Develop a Processing Method in **Review**

- a. Click on the **Browse Project** icon . Click on the Sample Set tab and select the **desired** Sample Set.
- b. Right mouse click on the table and select **View As Channels**. This will display just the sample set as individual channels.
- c. Highlight a standard sample & right mouse and select **Review – Replace Data**. The review section of Empower QuickStart will be displayed with the unprocessed chromatogram.
- d. Click the  **Processing Method Wizard Icon**. The first page of the wizard will be displayed. Select **Create New Processing Method** and click **OK**.
- e. Confirm the Processing type is LC, and the Integration Algorithm is Apex Track. Click the **Use Processing Method Wizard** box, and click **OK**. Apex Track's algorithm will detect peaks with the minimal amount of integration events.






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- f. Use the mouse to box and zoom in on the area of the chromatogram that you want peak detection and integration to occur. Click the **Next**.

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

- g. The chromatogram will be displayed with the peaks integrated. Since this method is being used for samples that are of differing amounts and this is not a regulated company, click **Clear Peak and Threshold** (this will change peak width and threshold for each sample). Click **Next**.
- h. To reject some of the detected peaks, click inside the smallest peak of interest, then click minimum height. There should only be the peaks desired integrated. Click the **Next** button.
- i. Select the Method of **quantitation** drop down box to select **area**. Click in the amount, calibration fit type, and calibration type **linear**. Click **Next**.
- j. For standard LC quantitation, click **No** in the box asking if you want to use channel names for inter-sample calculations


Note: This is for advanced Internal Standards at different masses/wavelengths than the component mass/wavelength.

- k. Since a standard was selected, component names will viewed by clicking on Peak 1 and selecting the proper name from the drop down box. These component names match the names entered in the Standard **Amounts** from **Run Samples**. Click **Next**.
- l. Since multiple standard concentrations were acquired, skip the defaults amounts section and click **Next** (if a single level concentration was used, you may enter in an amount here).
- m. Select the calibration type to External or Internal Standard. If using an Internal standard, click in the drop-down box to select the component name desired for the internal standard. Click **Next**.
- n. Enter the processing method name. Enter in a comment. Click **Finish**. The chromatogram will be displayed with the processing method applied, and integrated.
- o. View the Processing Method,  select the processing parameters icon. This will display the processing method you just created, and can be used to modify and setup advanced processing functions.
- p. To view the Main Chromatogram again, select the Main window icon . Click the  **Integrate** and  **Calibrate** icons to test the processing method. If a sample was selected click the Quantitate icon .

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q. Click the **Peaks tab** at the **bottom** of the Review window and view the result information.



r. Right click on the world icon  to set the table properties. Click on any field desired to not be displayed and then Select **File - Save Preferences**.

7. _____ Adding the Processing Method to the Method Set

a. Select the Methods Set icon



b. If the Method Set you created before is not displayed, then select **File – Open Method Set** and select the desired method set name.

c. Make sure the Processing Method has been changed in the channel table to the desired processing method for each channel. Select **File- Save** to save the changes to the Method Set.

8. _____ Batch Process the Sample Set using the Process and Report Tool for 2D Data.

a. Click the Browse Project icon , then select the Sample Sets tab.

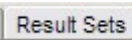

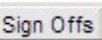
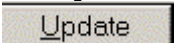
b. Right mouse click on the **desired** Sample Set and select **Process** (you may also select injections or channels).

c. In the Processing area, click **Use Specified Processing Method (or you may select use specified Method Set)**. Click the drop down box & select **the desired processing method (or Method Set)**. Click **Clear Calibration**. In the **How**: select **Calibrate and Quantitate**.

d. In the Report area, make sure that the reporting box is **checked as desired**.



e. Click **OK** and the data will be processed and results will be created.

6. _____ Viewing Results and View Filters



a. Select the Results view.    In the Filter by section, select **Processed Today** from the drop down box. If all the data is not displayed, click the  button to see the results.

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7. _____ Reviewing and calibration curves results

- a. Highlight a sample results (not a standard), and right mouse click to select **Review – Replace Data**.
- b. In the review menu look at the amounts for each peak. Click on the  **calibration icon** and view the calibration curve for each peak. Notice the R^2
- c. Switch to the review main window .

8. _____ Preview the Result and view an existing Report method.

- a. Click the Browse Project icon  to switch back to the result table view.
- b. Highlight the Diet Coke result, and right mouse click and select **Preview**.
- c. In the Preview window, select **Use the Report Generated that was generated to be appropriate for the selected Data**. Select **OK**.
- d. Preview the report and view the data. Save the report as a PDF by select the icon  and saving it to a desired windows file directory.

Congratulations!! – You have now achieved the basic knowledge of how to setup, process, and report chromatographic data with Empower QuickStart interface for basic 2D data.



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