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| <b>Title:</b>                 | <b>Waters HPLC Empower Software Use</b>            |                         |                                     |
| <b>Program or Department:</b> | Research   | <b>Document Type:</b>   | PROCEDURE                           |
| <b>Effective Date:</b>        | November 01,2010                                   | <b>Author</b>           | Research Core Facility Co-ordinator |
| <b>Next Review Date:</b>      |  | <b>Reviewing Body:</b>  | Research Core Facility Co-ordinator |
| <b>Emergency Code:</b>        |  | <b>Approving Body:</b>  | Stephen Barker                      |
| <b>Keywords:</b>              | Research ,Guide , Waters HPLC , Software , Empower | <b>Document Number:</b> | 0000001109                          |

## Introduction

Our Waters HPLC system is used Empower software to control the chromatography instrument and to process the data. Empower software is very powerful. It provides a graphical, icon-based user interface to acquire process, manage, and report chromatographic data in the Empower database.

## Associated Procedure

### Guidelines

#### **New Instrument Method and Method Set creation**

1. 1. Open Run Samples and select Project and Chromatographic System
2. 2. Click on Develop Methods
3. 3. Click on desired Instrument method or click new for a new method
4. 4. In instrument editor ensure that entries contain the proper flow rate in the pump section and the proper wavelengths in the detector section.
5. 5. Click File – Save as – Enter name
6. 6. Click next on Method set window
7. 7. Select applicable processing, report and export methods
8. 8. Save method set name, click finish

#### **Making an Injection**

1. Open Run Samples and select Project and Chromatographic System
2. Ensure the 'Single' tab is selected on the bottom of the injection parameters window. Also ensure that 'Run and Process' is selected from the drop down list above the injection parameters window in order for the run to be automatically processed after the injection is complete.
3. Enter Sample or Standard name
4. Set Function to either Inject Samples or Inject Standards
5. Enter desired Method Set
6. Ignore Vial and Injection Volume
7. Enter desired Run Time
8. Press Inject button and load sample (INJECTOR MUST BE IN LOAD POSITION BEFORE PRESSING BUTTON). Ensure that the loop is overfilled by at least two or three times the

sample volume.

9. Turn injection valve to inject when 'Waiting for Injection' is displayed in status bar at bottom of window.

### **Processing Method Creation**

1. Select Browse Project, choose Project
2. In the Channels tab open a chromatogram from a low standard injection
3. Click on Processing Wizard button, click next
4. Select Create a New Processing Method, click next
5. Ensure processing type is LC and algorithm is Apex Track. Ensure Use Processing Method Wizard is selected. Ensure Cross Channel Internal Standard is not selected, click next
6. Highlight desired Integration time, click next
7. Keep default Peak Width and Threshold (do not clear them), click next
8. Select Minimum Area, click next
9. Quantification should be Area, Component is Amount and Calibration Fit is Linear, click next
10. Select No to Cross Channel Processing, click next
11. Select desired peak and enter a name for component , click next
12. Do not enter standard concentration and units, click next
13. Select External Standard Calibration, click next
14. Enter Processing Method name, click finish.

### **Processing Data Manually**

*When standards are processed with a processing method, the standard concentrations are plotted to create*

*a calibration curve. When samples are then processed with that same method, the software automatically*

*calculates the amounts for the unknowns using that calibration curve.*

### **To edit the known concentrations of standards**

1. After selecting 'Browse Project' from the Empower Main Window, open the relevant project.
2. To ensure that proper concentrations for the standards are inputted into the software, select the rows of the desired standard injections in the 'Channels' tab. Then select 'Tools' from the tool bar and select 'Alter Sample'.
3. A new window should open that has a list of the standards selected in the previous step. Select 'Edit' from the tool bar and select 'Amount'.
4. In the new window that opens, ensure the 'All Samples' tab is selected. The window will now display all the components (ie. peaks) in each standard run and their amounts. If the components are incorrect, select 'Edit' and then 'Copy Component from Process Method'. The components saved in the processing method for will now populate the table. Using the 'Next' and 'Previous' buttons you may scroll between each standard injection with it's respective components column highlighted. In the table, enter the proper amount for each component in the column beside its name. Also enter the unit of the entered amount, ensuring that a '<' symbol is inserted before the unit (ie. <mg/mg).
5. Click 'OK' to close the window and then select 'File' and 'Save' in the previous window to save the modifications.

### **Processing Standards to Generate a Calibration Curve**

1. In order to generate a calibration curve, select the 'Channels' tab in 'Browse Project' and highlight the rows of the desired standard injections. Then select 'Tools' from the tool bar and

select 'Process'. In the window that opens, click on 'Use specified processing method' and choose the processing method that you created for the standard. Then select 'Clear Calibration' and ensure 'Calibrate and Quantitate' is selected. Click 'OK'.

2. To review the processed standard chromatogram, click the 'Results' tab and highlight all the standards just processed. Click 'Tools' from the toolbar and select 'Review'. Ensure that the calibration curve is valid by clicking 'Window' from the toolbar and selecting 'Calibration'.

### Processing Samples to Determine Sample Unknown Amounts

1. Highlight the desired sample chromatograms in the 'Channels' tab from 'Browse Project'.
2. Click 'Tools' from the toolbar and select 'Process'.
3. In the window that opens, click on 'Use specified processing method' and choose the processing method that you created for the standard. Then ensure 'Clear Calibration' is not selected and ensure 'Calibrate and Quantitate' is selected. Click 'OK'.
4. To review the processed sample chromatograms, click the 'Results' tab and highlight all the processed samples. Click 'Tools' from the toolbar and select 'Review'. Ensure that the calibration curve is valid by clicking 'Window' from the toolbar and selecting 'Calibration'.
5. To obtain the calculated amounts in the sample, with the result in the review window select 'Window' from the toolbar and select 'Results'. The calculated amount should be listed in the 'Amount' column.

### Generating Reports the Contain Sample Unknown Amounts

1. Click the 'Results' tab from 'Browse Project' and highlight all the processed samples. Click 'Tools' from the toolbar and select 'Preview/Publisher'.
2. In the window that opens, select 'Use the following Report Method' and choose the desired report method that matches your data. If no custom report method has been made, choose 'Injection Summary Report'.
3. In the window that opens a report for each injection selected is made. Click the 'Next Report' icon to switch between reports for all the selected samples. You may also save or print the reports from this window.

**Revision Number**

**Contact**

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