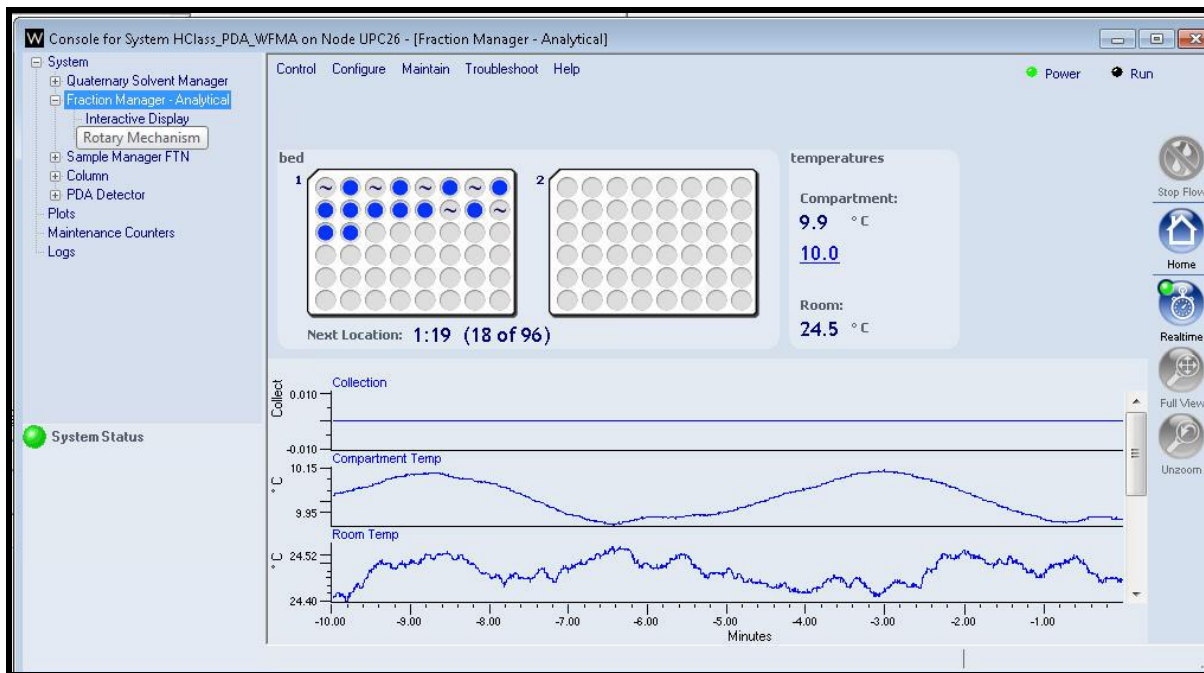


"Waters Fraction Manager-Analytical" with "Empower 3-FR2" Customer Familiarization	
REVISION NUMBER	1.3
DATE	9 June 2019



Waters

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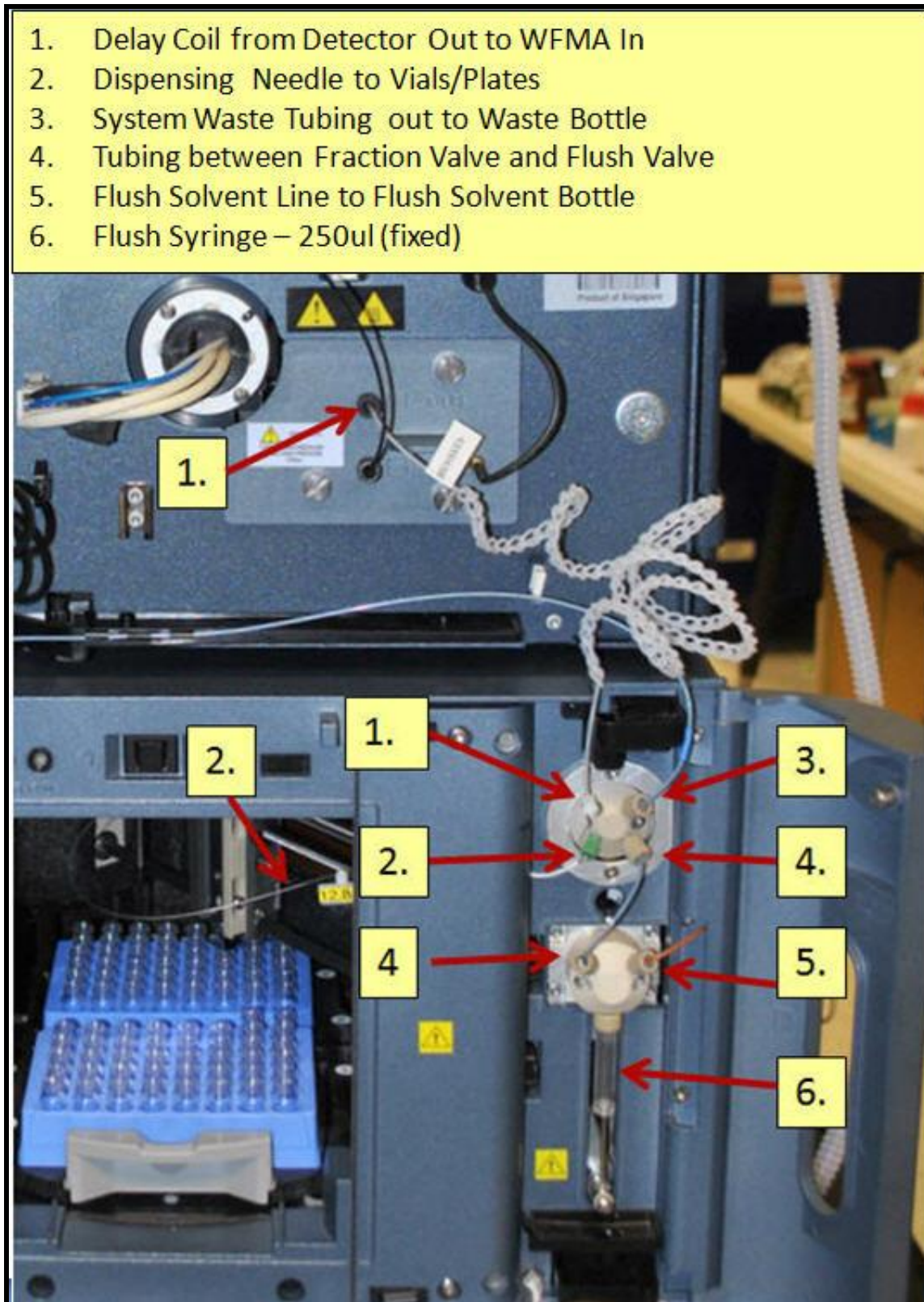
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A: Introduction to the Waters Fraction Manager-Analytical

1. The Waters Fraction Manager Analytical (WFMA) is designed to collect narrow and closely eluting peaks.
2. Collection can be by time, signal, threshold, slope or combinations of these functions. Fraction simulation allows the user to model and optimize collections method with an existing chromatogram.
3. Manual collection, pooling, global waste collection and waste collection are all configure functions that can be performed with the WFMA.
4. The WFMA is supports Empower 2 FR5 (time based), stand alone console (time based), and Empower 3 (FR 1 and 2) using time, threshold, and slope based collection.
5. Below displays typical WFMA and ACQUITY H-Class or Alliance system layouts. Most system configurations are in 2 stacks.

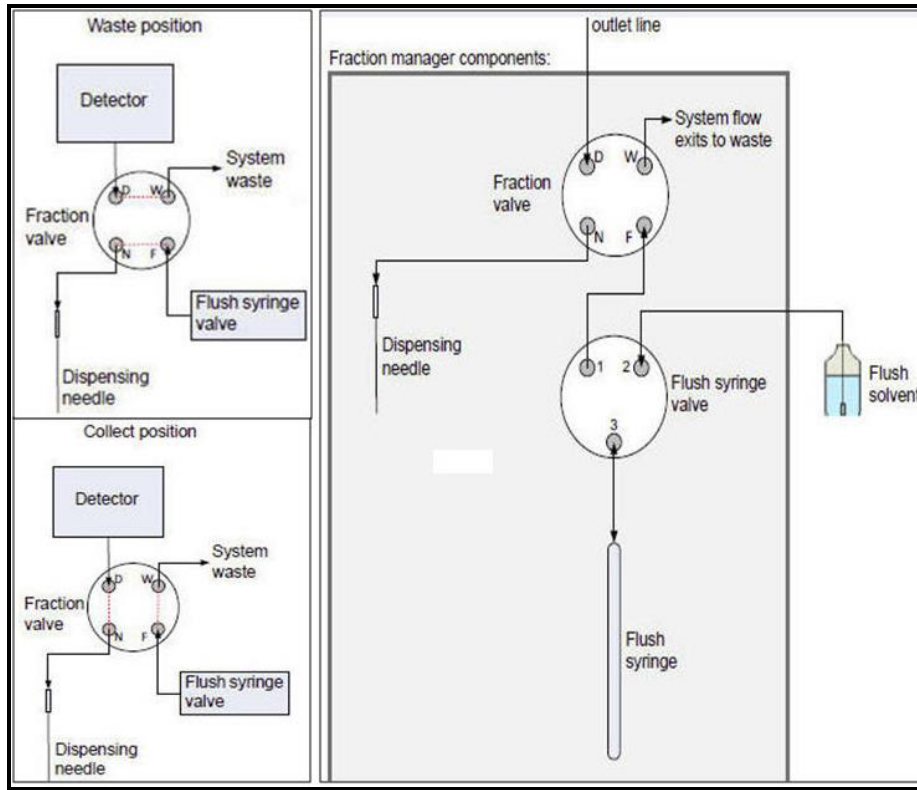


6. WFMA hardware connections to an H-Class or Alliance based systems are straightforward and are as displayed below.



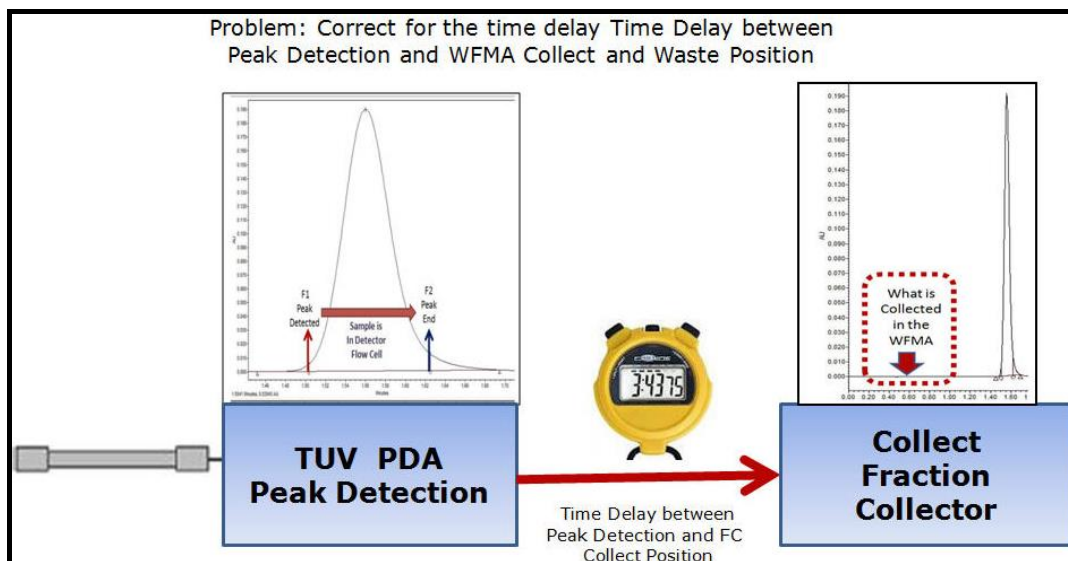
7. The WFMA collection valve has two valves:

- a. The Flush syringe valve for rinsing between injections, peaks, and windows.



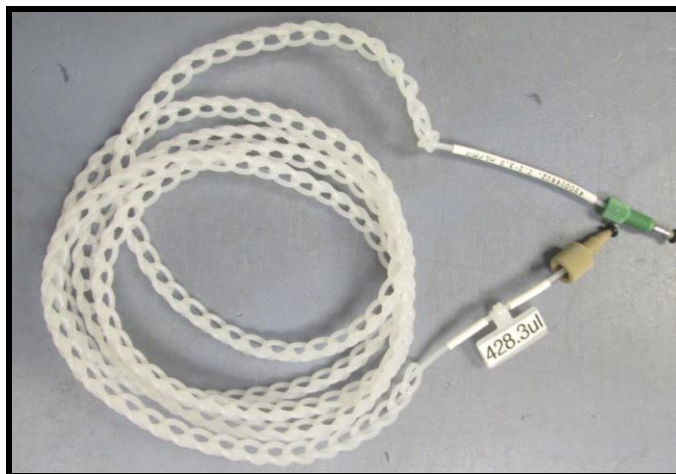
8. Understanding the WFMA Delay Coil:

- a. The Problem with standard fraction collection is that Standard fraction collection operation collects the fraction at the same time the peak is in the UV/PDA flow cell.
- b. The fraction collected at that time will not correspond to the peak, rather at a time before the peak.



9. How the WFMA fixes this issue:

- a. The WFMA uses precise volume delay coils for specified flow rates to account for the timing delay between the peak detection by the UV detector and the peak collection by the WFMA.
- b. The WFMA system ships with the p/n 700010326 delay coil, which is specifically used for flow rate ranges from 0.5-1.0ml/minute.



10. Delay coils are ordered in 3-pack kits. Each Delay coil has an exact volume labeled on the coil itself. Kit part numbers, flow rates and nominal volumes are displayed below

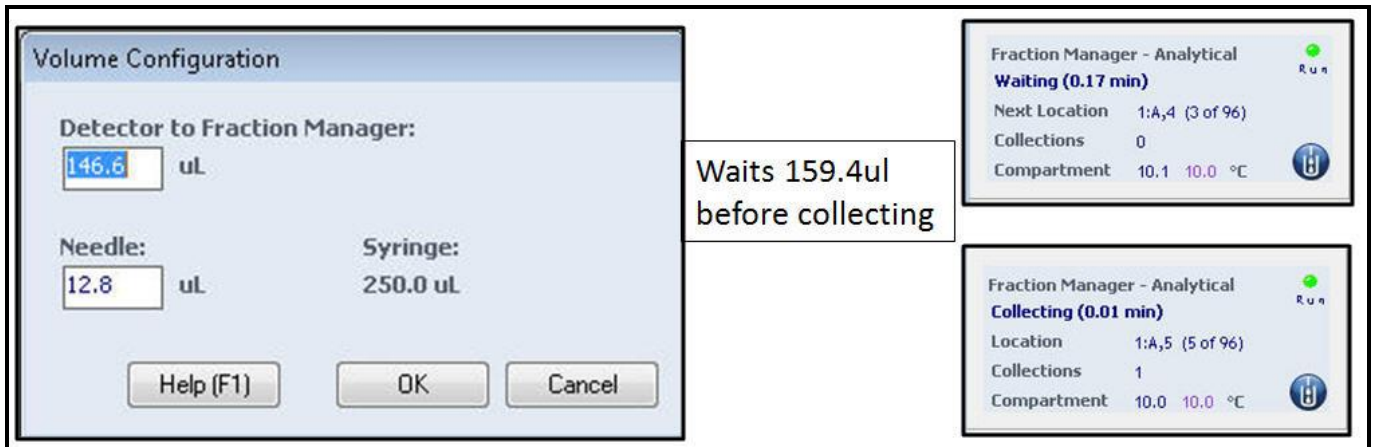
Kit	Flow Rate Range (mL/min)		Coil Dimensions (in)			Nominal Vol (μL)	Delay (s)	
	Low	High	Total Len	Net Len	Diam		Min	Max
p/n 205001416 0.01 - 1.0 ml Flow Kit for ACQUITY H-Class and Alliance Systems with the WFMA								
1	0.1	0.2	51	16	0.007	32.2	7.1	25.3
1	0.2	0.5	126	33	0.007	79.5	7	31.2
1	0.5	1	112	30	0.01	144.1	7	21
p/n 205001417 0.5 - 2.2 ml Flow Kit for ACQUITY H-Class and Alliance Systems with the WFMA								
1	0.5	1	112	30	0.01	144.1	7	21
2	1	1.5	168	42	0.01	216.2	7	15.7
2	1.5	2.2	168	42	0.012	311.4	7.8	14.6
p/n 205001418 2.2 - 5.0 ml Flow Kit for Alliance Systems Only with the WFMA								
3	2.2	3	225	55	0.012	417	7	14.7
3	3	4	168	42	0.016	553.5	7.3	12.5
3	4	5	202	50	0.016	665.6	7	11.3
p/n 205001419 0.1 - 1.0 ml Flow Kit for ACQUITY H-Class and Alliance Systems with the WFMA and QDa*								
4	0.1	0.2	51	42	0.007	32.2	7.1	25.3
4	0.2	0.5	126	42	0.007	79.5	7	31.2
4	0.5	1	112	42	0.01	144.1	7	21

* Delay Coil is longer to attach the QDa to the WFMA

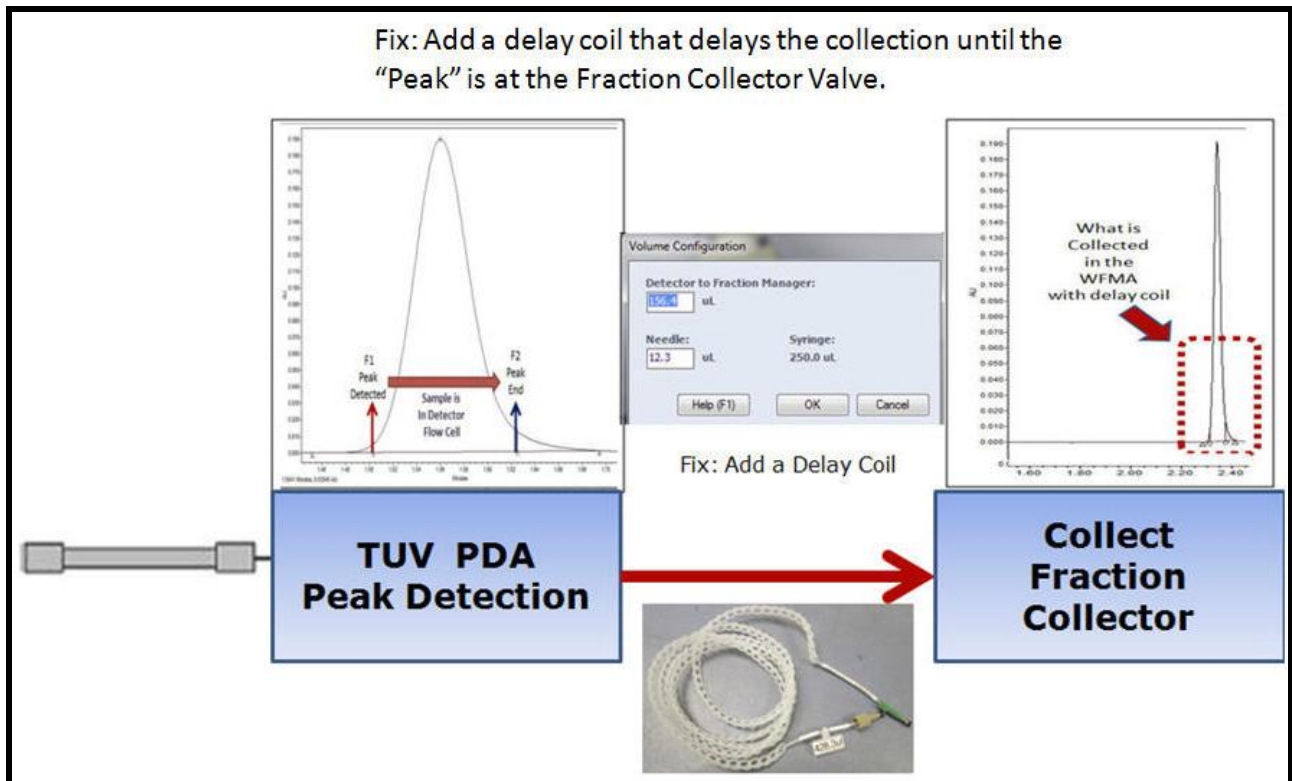
** For Alliance Systems with a 2489 or 2998 add these flow cell volumes to the delay:

2489 Analytical flowcell: 18 uL ----- 2998 Analytical flowcell: 4.8 uL

- The delay coil volume is entered in the WFMA's console in the "Volume Configuration" area. The WFMA's collection needle volume is added to the "delay coil volume" to calculate the time delay between the detector and the WMA collector so the peak detected is properly aligned with the peak collection.



- This illustration depicts how the delay coil and the "Volume Configuration" work together to align the detected peak with the collected peak by delaying the collection time.



13. If you are using Empower 2 or standalone control, then WFMA collection is time based only. The timed based tubing kits to connect the detector the WFMA are listed below:

Time Based Collection Only Interconnection Tubing Kits		
205001427	Low Flow Detector - WFMA Tubing Kit	ACQUITY H-Class (Flow rate to 2.2mL)
205001428	High Flow Detector - WFMA Tubing Kit	Alliance Only (Flow rate to 5mL)

14. For detectors that require a split flow, (QDa and ELSD), the flowing part numbers are required for the WFMA.

Flow Splitting Options		
Part Number	Description	System
176003560	ISM and WFMA-QDa Splitter Kit	H-Class
205001413	ISM Splitter Kit for WFMA-QDa	H-Class
205001415	ELSD Splitter Kit for WFMA	H-Class or Alliance
176003246	ISM and Alliance Splitter Kit	Alliance

15. WFMA important module specifications are listed below. See the WFMA specification sheet for all specifications.

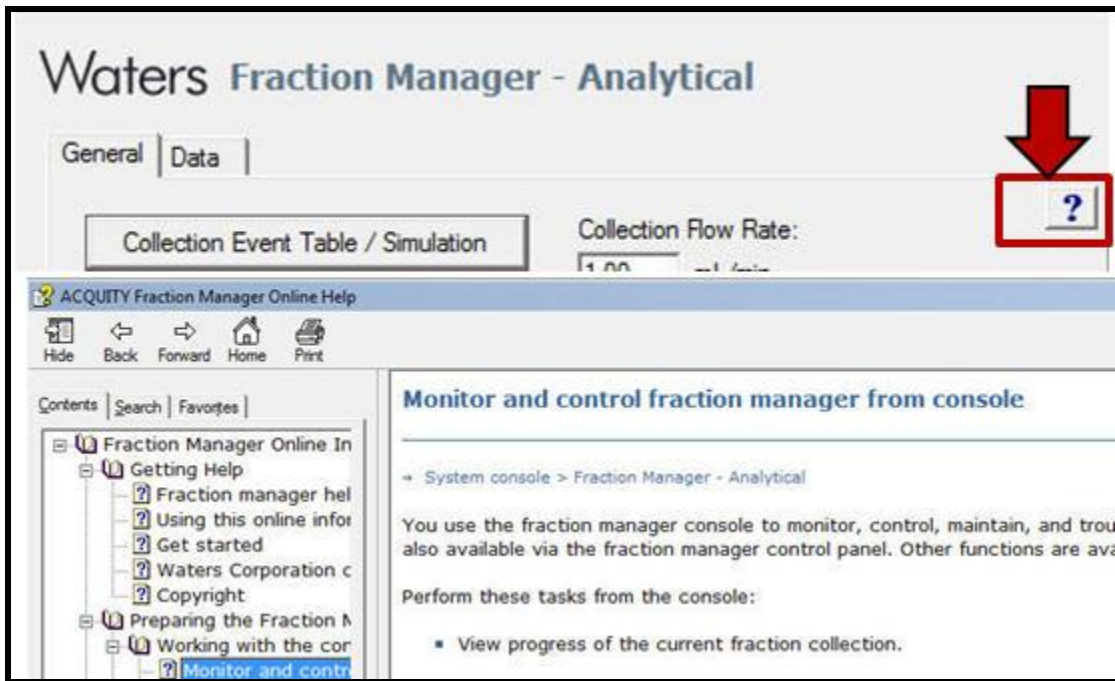
System Setup	Alliance + WFMA	ACQUITY H-Class + WFMA
Flow Rate	0.1-5ml/minute	0.1-2ml/minute
pH Range	2-12	2-12
Temperature	4-40	4-40
Inert	WFMA is Bio-Inert and compatible with H-Class Bio	
Fraction Collector Volume	25ul-10000ul	
Backpressure	<300 psi	

16. WFMA options include a variety of different plate types and flow needles. Below are the part numbers for the various options.

Part Number	Description	Fractions	Total # Fractions (2 Plates)	Comments
205001426	High Flow WFMA Collection Needle Kit*			Required for flow rates from 2.2mL- 5mL/min or viscosity >1.8 (ex: 50/50 MeOH/H ₂ O)
205001042	Kit, 10 mL Vial Holder (2 PK)	15	30	Use with 186001420 -10mL Vials (100 PK)
186002643	96 Well 350µL ACQUITY Collection Plate	96	192	
186005837	Plate, 96-Well, 700 µL Round Well 25/PK	96	96	
186002481	1ml Round Collection Plate 50/Pk			
186002482	2ml Square Collection Plate 50/Pk			
405003743	Holder, 48 Well, 2mL Vial	48	96	Use with 18600273- 2mL Vials (100 PK)
405003742	Holder, 24 Well, 4mL Vial	24	48	Use with 186000840- 4mL Vials (100 PK)
405003741	Holder, 48 Well, .65 mL Tube (Eppendorf)	48	96	
405003740	Holder, 24 Well, 1.5mL Tube (Eppendorf)	24	48	

17. Waters support for the WFMA are located in the following locations:

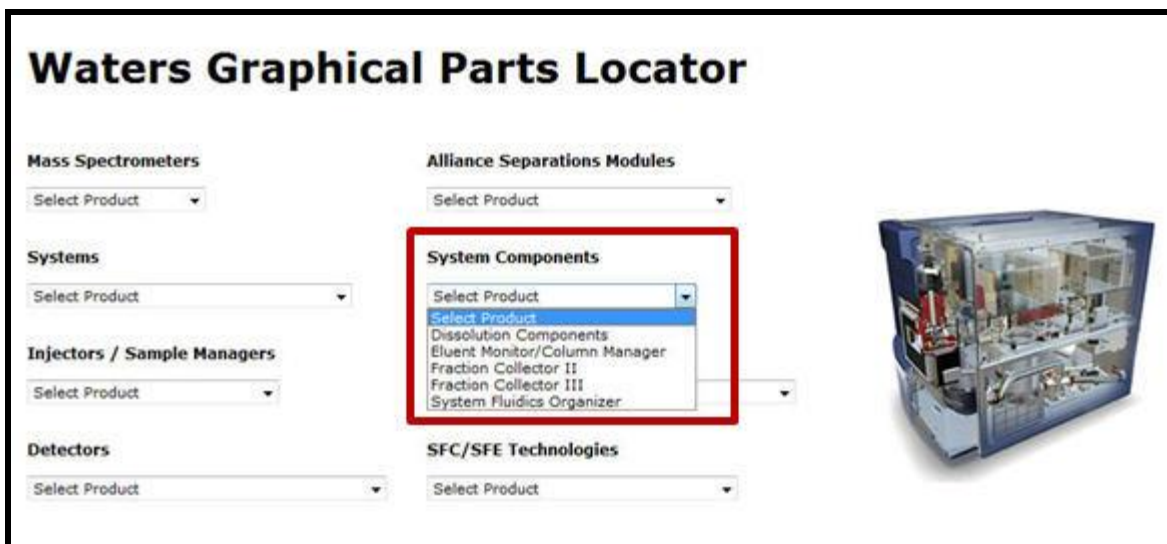
- a. Direct Fraction Manager – Analytical Online Help. Select the “?” or click “F1” in the instrument method or Console for help.



- b. Spare Parts for the Waters Fraction Manager – Analytical are located at:

- a. WWW.Waters.com\services

- b. in the Waters web site select “support-spare parts-graphical parts locator-system components- Waters Fraction Manager - Analytical.



c. Waters iRequest Technical Service / Support through the waters.com support section.

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For Waters LC, MS instruments, Empower & MassLynx software. (Requires instrument serial number or support Plan ID.)

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For maintenance, repair, qualification, and relocation. (Requires instrument serial number or support Plan ID.)

IREQUEST ONSITE TRAINING
Based on your group size, budget and schedule. (Requires login to www.waters.com.)

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C: System Workflow for Waters Fraction Collection - Analytical

The WFMA System workflow summary to complete analytical sample collection is described below.

The general steps are detailed below, while the specific instructions for each are detailed in this manual.

1	Access Run Samples using the WFMA Training Project
2	Access the ACQUITY Console and Configure the WFMA
3	Enter the WFMA Volume Configuration
4	Setup Bed Configuration and layout
5	Teach the Needle Position for Fraction Collection
6	Configure the WFMA Collection Types in the ACQUITY Console
7	Setup an Instrument Method (no Collection)
8	Process Chanel Data for Integration and Peak Detection and get the results
9	Access the Instrument Method and access the processed result in the WFMA instrument panel
10	Add Peak Collection by Time, Threshold, Slope, Boolean or Peak using Auto fill from Results
11	Optionally, use the "Fraction Simulation" function
12	Collect the Fractions for each peak into the WFMA
13	Overlay the "UV-PDA wavelength" and "Collection" Channels in Review. Ensure that peak collection matched peak detection
14	Take a vial from one of the peak collection and put it in a Sample Injector vial location
15	Re-inject the collected sample vial and determine if only the one "Collected Peak" is displayed in Review
16	Take the collected peaks from the Results View and create a Fraction Collector Report
17	Save the Report and Preview it
18	Print or Save the Report as a PDF

D: Access Run Samples and configure the WFMA in the Console

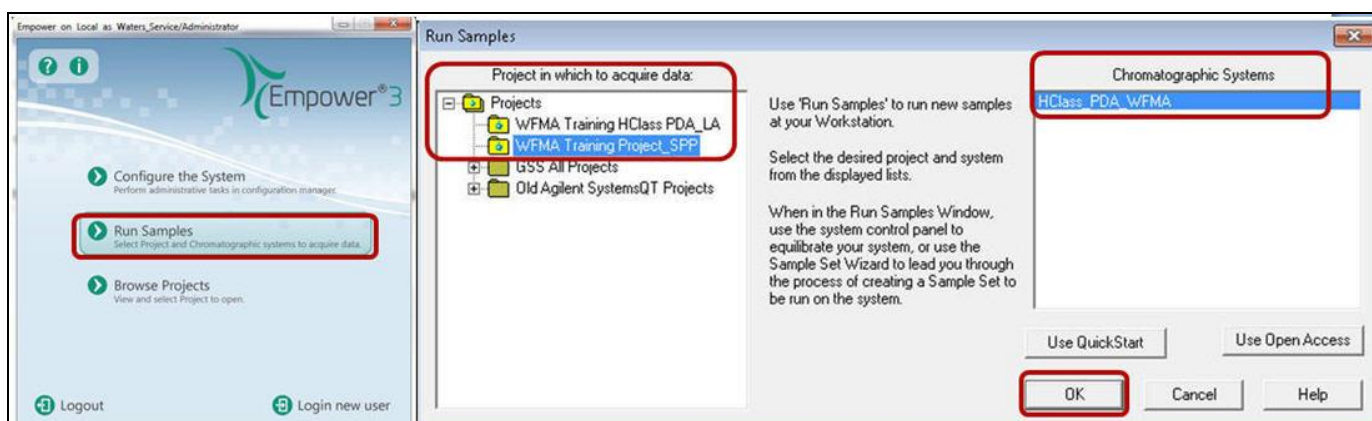
Log into Empower and create an ACQUITY HClass system with the Waters Analytical Fraction Manager in the configuration. Restore the WFMA training Project.



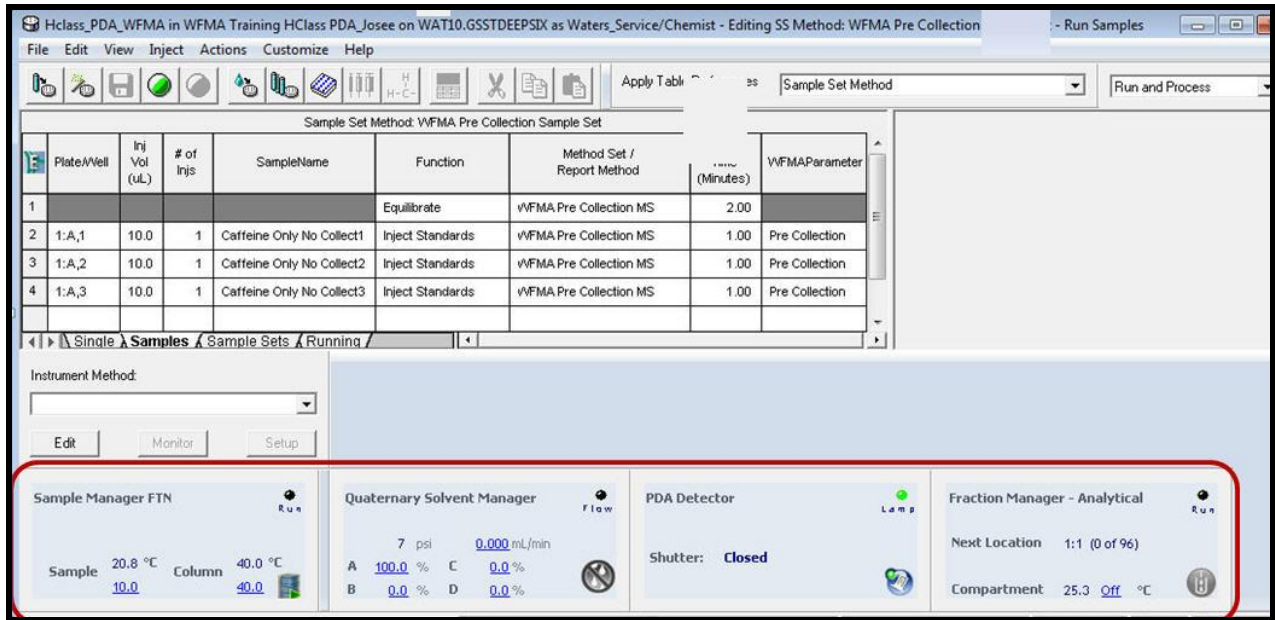
1. Double click on the Empower **icon**.
2. Enter in your user name and your Empower password.



3. From the Empower login window select "Run Samples".
4. Select a project and the chromatographic system that contains the WFMA. Select OK.

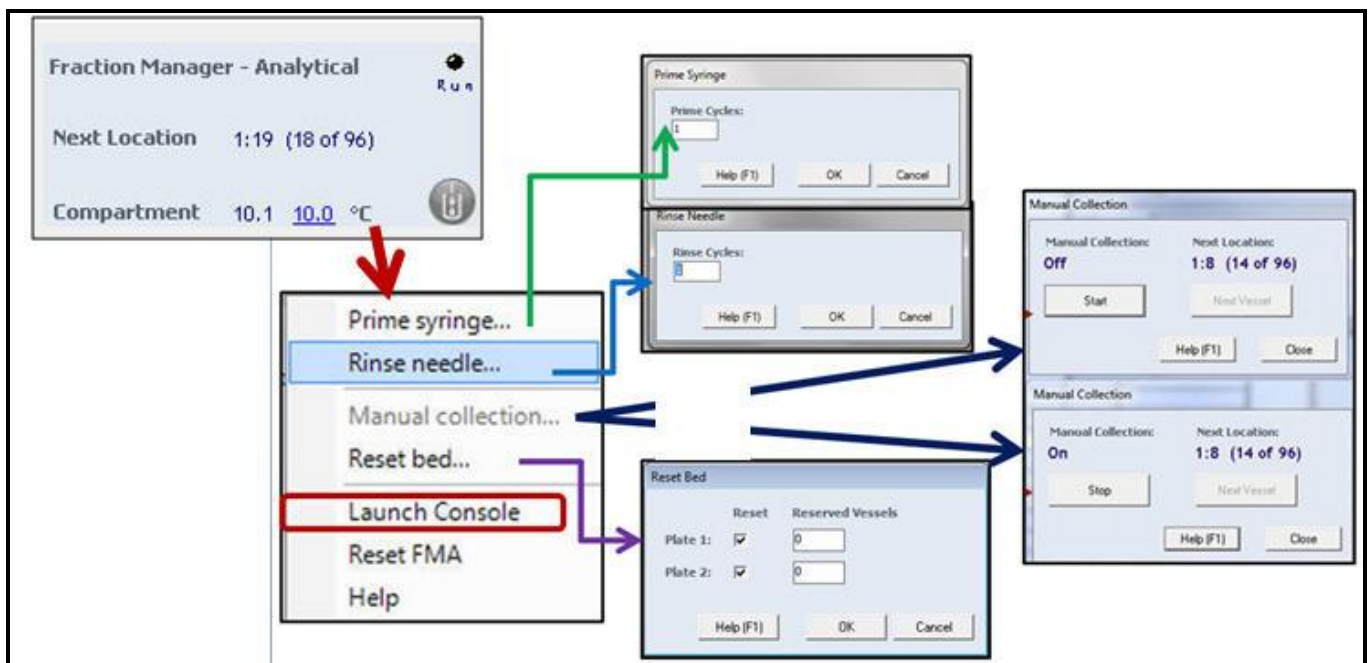


5. The Fraction Manager Analytical control panel is displayed at the bottom of the Run Samples window.

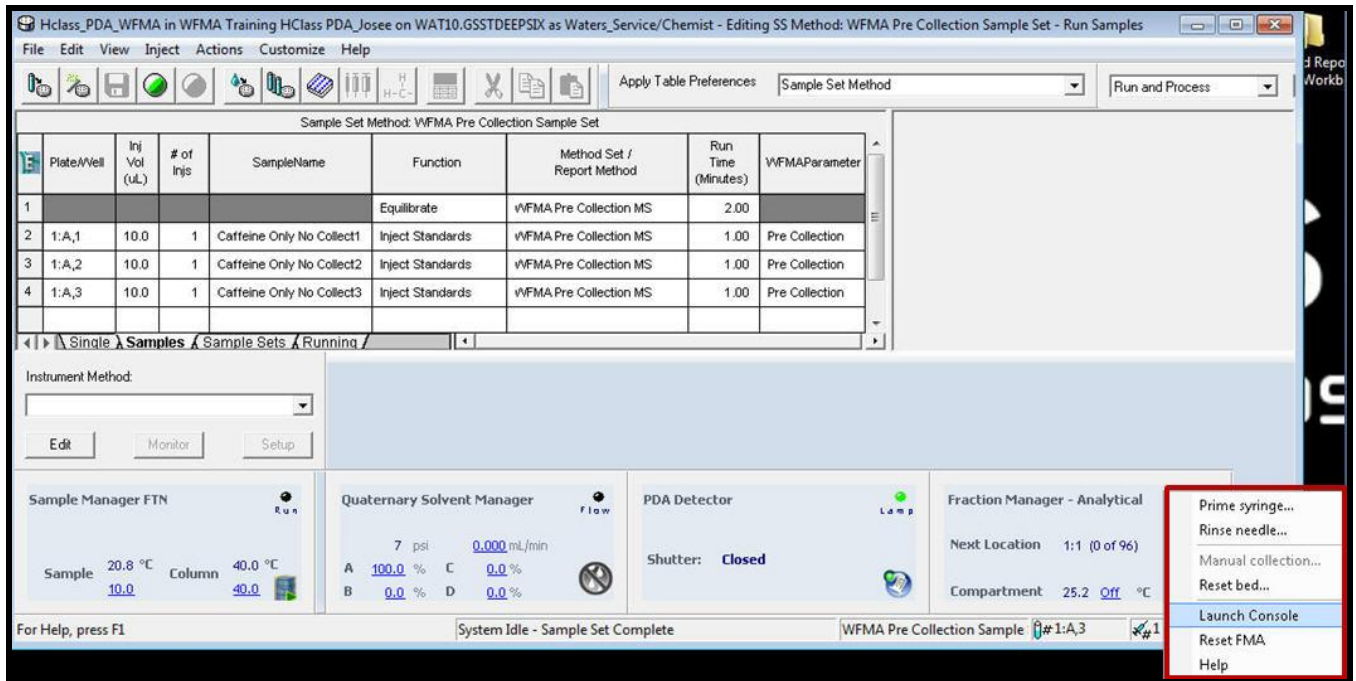


6. Right click inside the WFMA Instrument panel to access the "Prime Syringe", "Rinse Needle", "Reset Bed", "Manual Collect", "Reset FMA" and "Launch Console" functions. Note the recommended prime and rinse needle setting below:

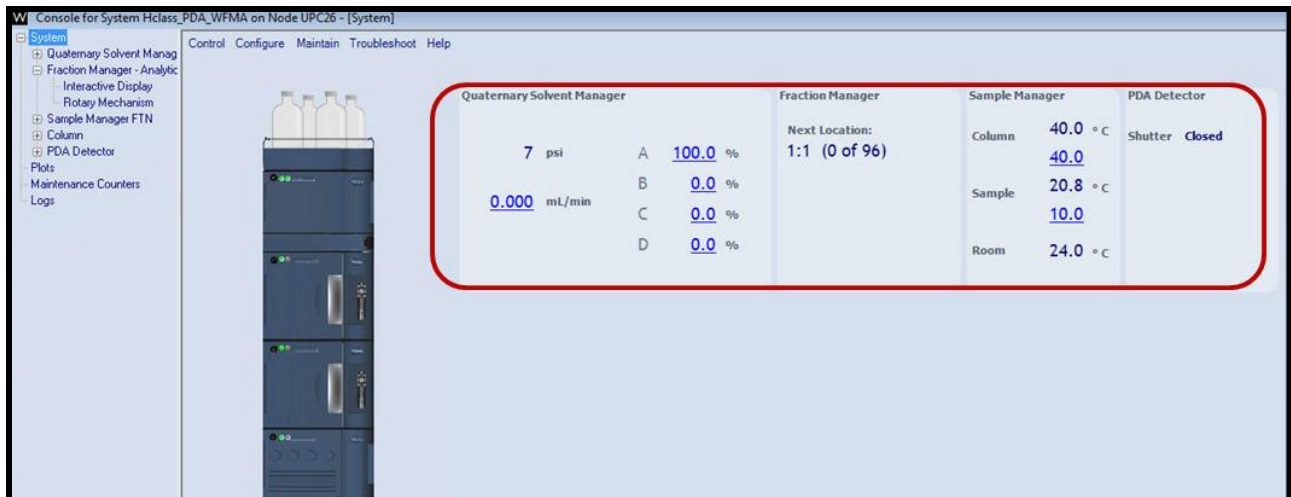
Recommendation	Prime Syringe	Rinse Needle
System Startup	25 cycles	25 Cycles
Daily	2-3 cycles	2-3 cycles



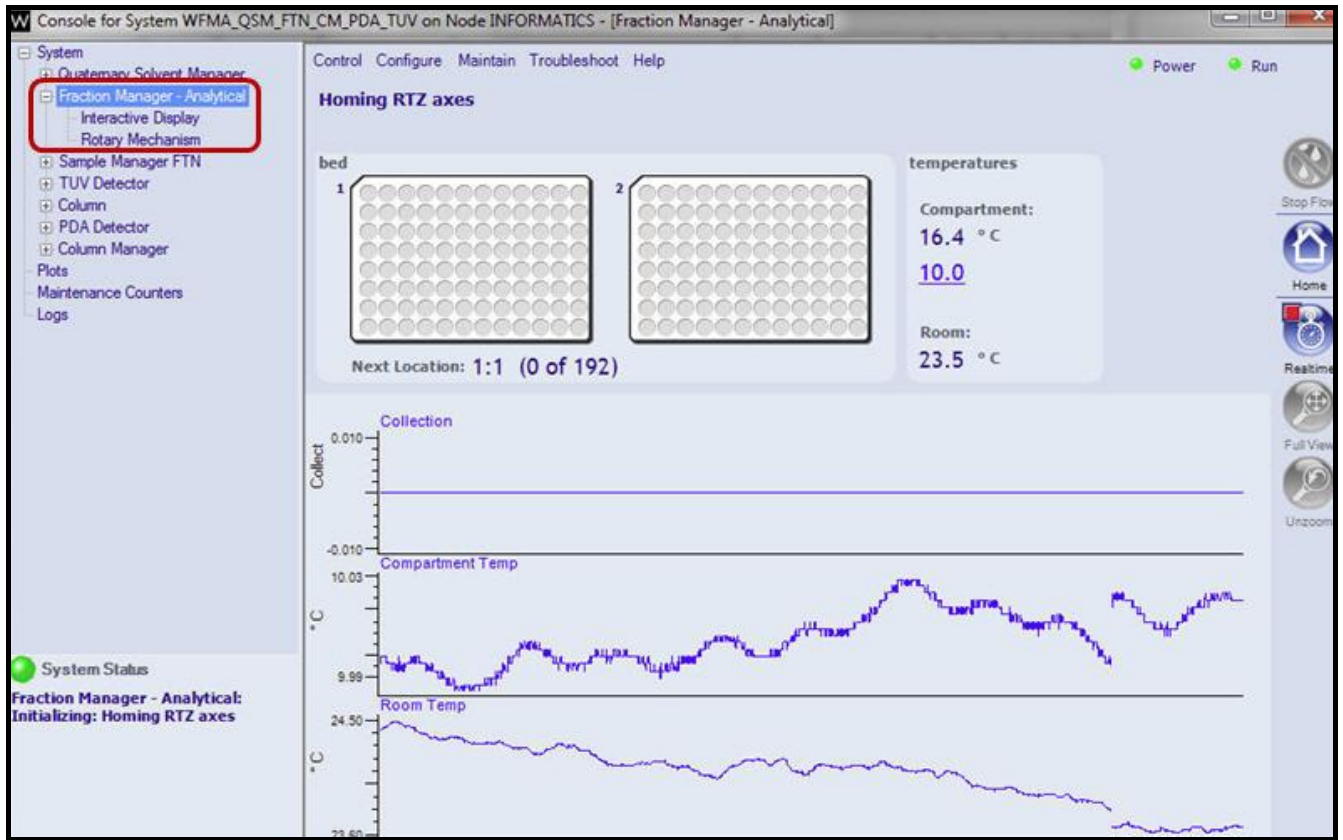
7. Right click on the Fraction Manager and select "Launch Console".



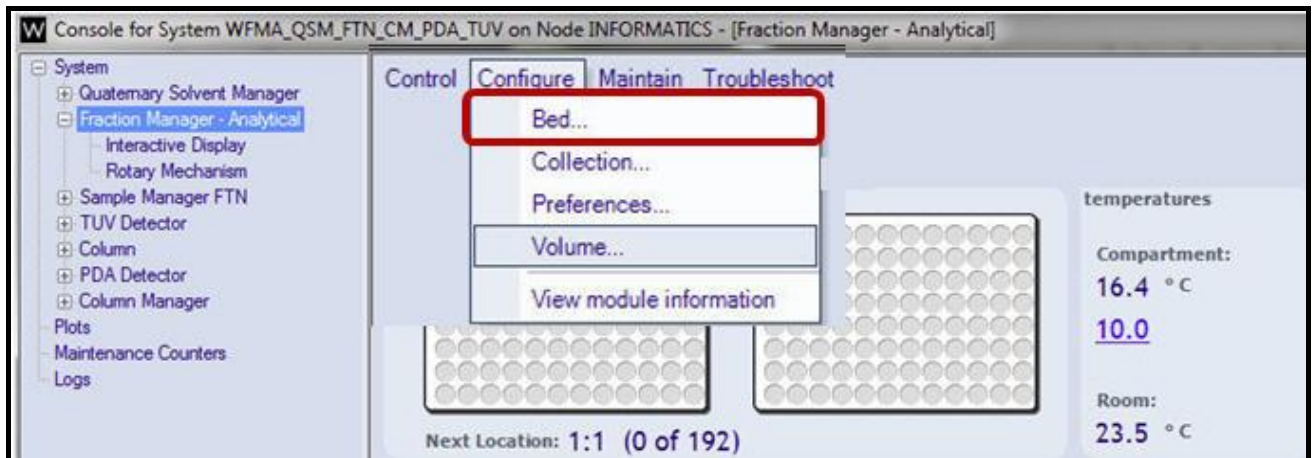
8. In the Console System display, the "Fraction Manager" area displays the next location for collection or if collecting the actual location of collection.



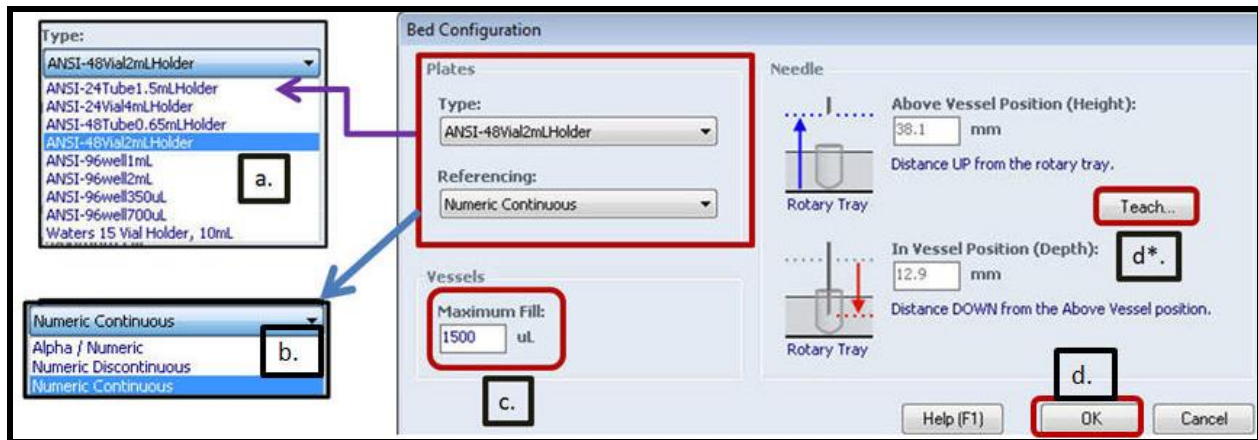
9. In the Console, select the "Fraction Manager – Analytical" component in the system.



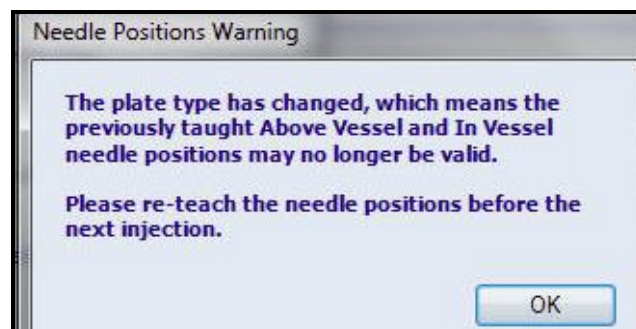
10. In the "Fraction Manager – Analytical" select "Configure – Bed...."



11. The Bed Configuration is to select the plate height, referencing positions, vessel fill amount, and teaching the needle position for above the vial and inside the vial.



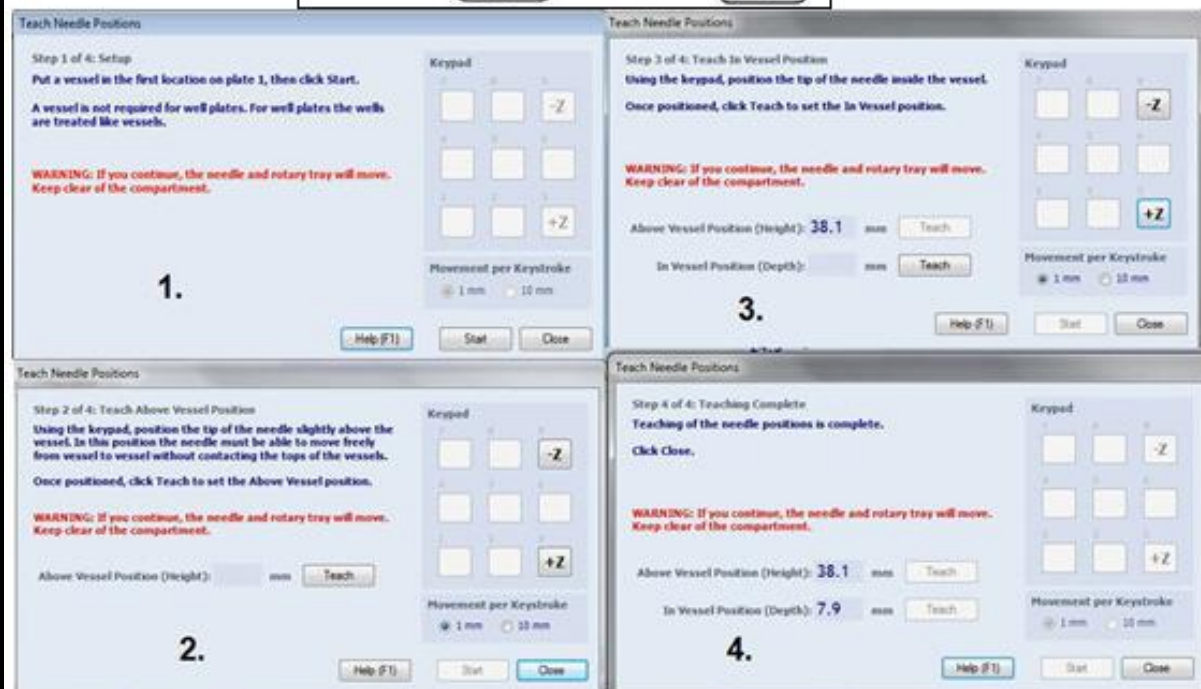
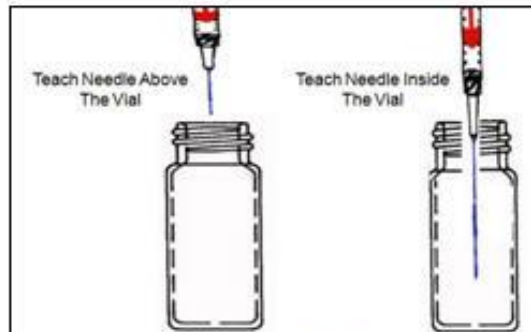
- a. Select the Plate Type. ANSI holders and plates can be transferred directly into the H-Class auto-injector after collection.
- Note: Exceptions are the "24 - 4 ml vial" and the "15 - 10 ml vial" holders, which will need to be transferred to other injection vials for Alliance or H-Class injections.
 - Selecting a different Plate Type will require you Teach the needle the height for "above the vial" and "in the vial" positions (this is described on the next page).



- b. Select the Referencing style for number and order selection.
- c. Select the Maximum Vessel Fill. Once the volume is exceeded, the collector will continue to the next vessel or vial.

- d. Select the Teach button. This must be completed every time you switch "plate configurations". This is to make sure the needle height is set properly for each type of plate.

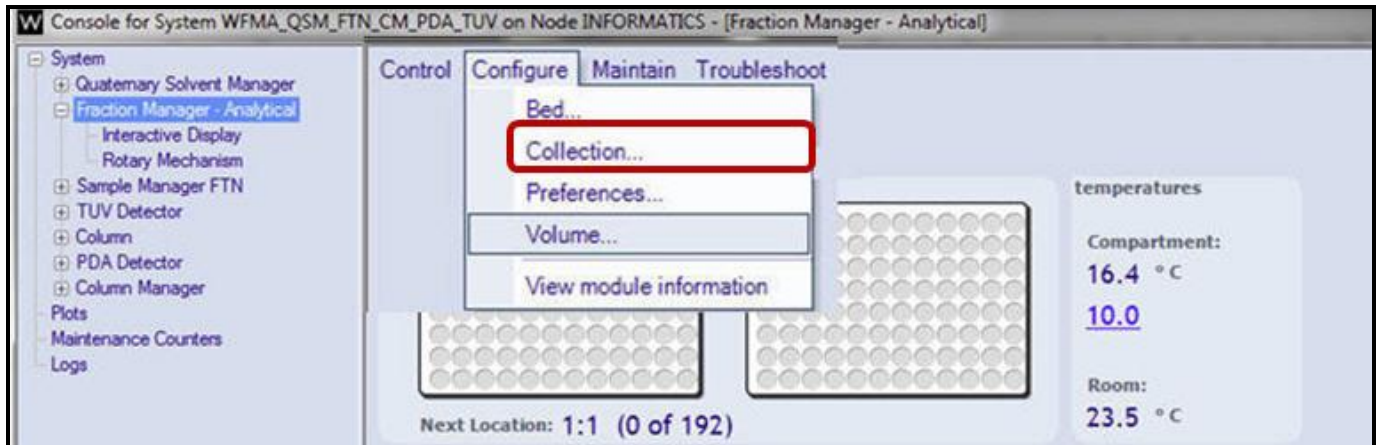
The Teach position sets up the needle height position for "above the vial" and "inside the vial" during sample fraction collection.



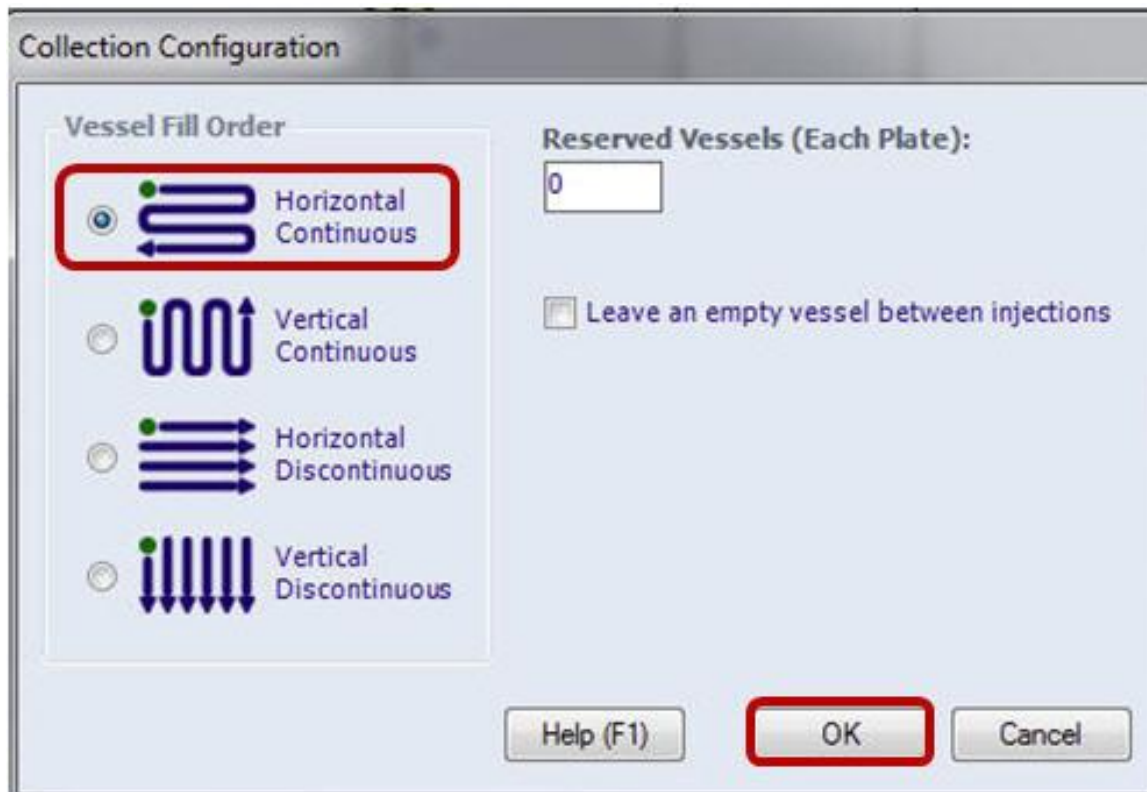
The procedure below details the steps to configure the needle position.

1. Place a vial in position 1 and click "Start".
2. For "Teach Above Vessel Position",
 - a. Select "1 mm" or "10 mm" movements.
 - b. Use "Page Up" or "Page Down" to move the needle just "Above the Collection Vessel".
 - c. Click "Teach".
3. For "Teach Inside the Vessel Position",
 - a. Select "1 mm" or "10 mm" movements.
 - b. Use "Page Up" or "Page Down" to move the dispensing needle "inside the Collection Vessel".
 - c. Click "Teach".
4. Click Close - You have completed the teach function.

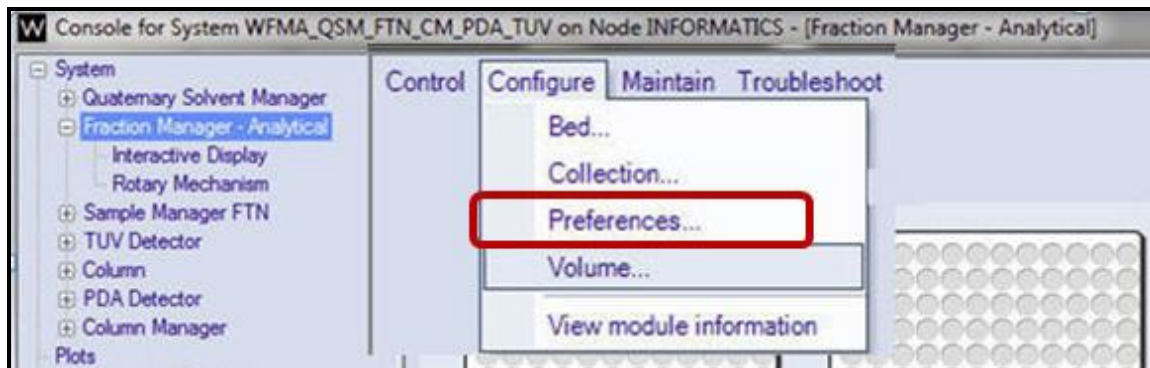
12. In the "Fraction Manager – Analytical" select "Configure – Collection...."



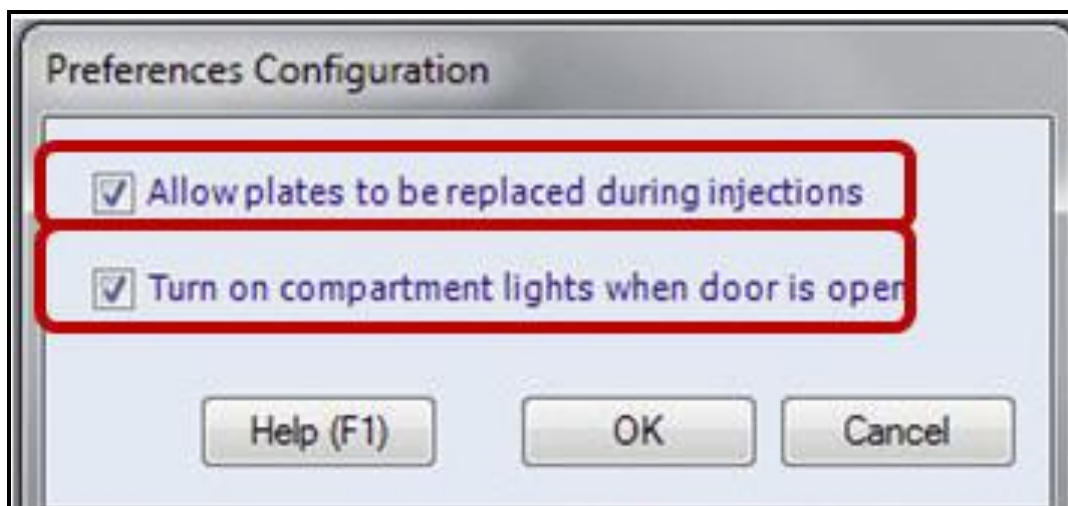
- In the "Collection Configuration", select the Vessel Fill Order to the mode desired. Horizontal Continuous is the default.
- The "Reserved Vessels" is used to allow for blanks or standards in the WFMA collector.
- The "Leave an Empty Vessel between injections can be used to get better separation between collections.
- Select OK.



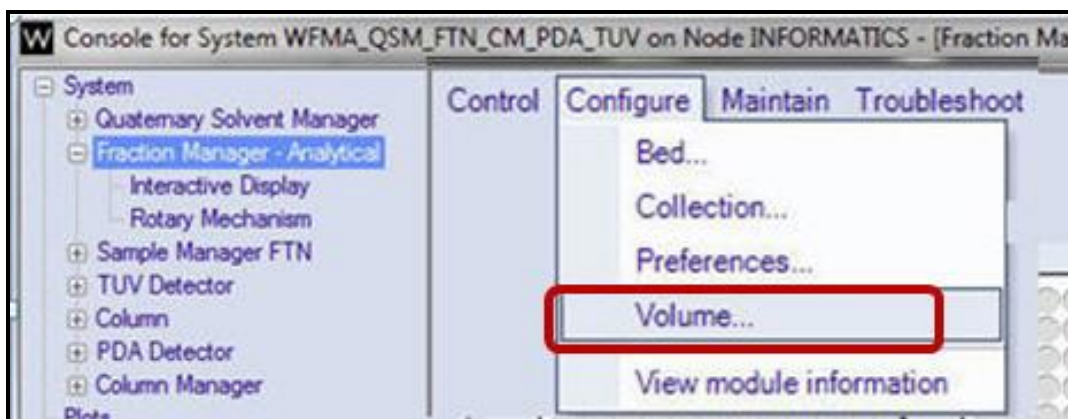
13. In the "Fraction Manager – Analytical" select "Configure – Preferences....."



- In the "Preferences Configuration" dialog box select as desired (defaults are checked as on).
- Allow Plates to be replaced during injection (not covered by Empower Security).
- Turn on Compartment Lights when door is open.



14. In the "Fraction Manager – Analytical" select "Configure – Volume....."



15. In the "Volume Configuration" use the following information to determine the delay volume:

- a. Identify the flow rate you will be operating for fraction collection.
- b. Identify what delay coil you will be using for Fraction Collection (based upon flow rate).
- c. Place the desired coil in between the PDA/UV detector and the Fraction collector.
- d. Enter the exact volume listed directly on the delay coil.
- e. For the Alliance Systems and a 2489 or 2998 detector add these additional volumes to account for the flow cell volume for the detector.

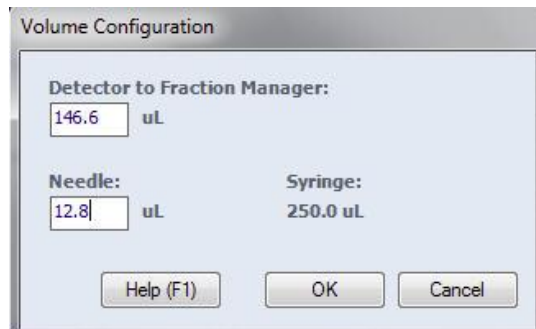
2489 Analytical flowcell: 18 uL

2998 Analytical flowcell: 4.8 uL



Kit	Range		Nominal Vol (µL)
	Low	High	
p/n 205001416 0.01 - 1.0 ml Flow Kit			
1	0.1	0.2	32.2
1	0.2	0.5	79.5
1	0.5	1	144.1
p/n 205001417 0.5 - 2.2 ml Flow Kit			
1	0.5	1	144.1
2	1	1.5	216.2
2	1.5	2.2	311.4
p/n 205001418 2.2 - 5.0 ml Flow Kit			
3	2.2	3	417
3	3	4	553.5
3	4	5	665.6
p/n 205001419 0.1 - 1.0 ml Flow Kit - QDa*			
4	0.1	0.2	32.2
4	0.2	0.5	79.5
4	0.5	1	144.1

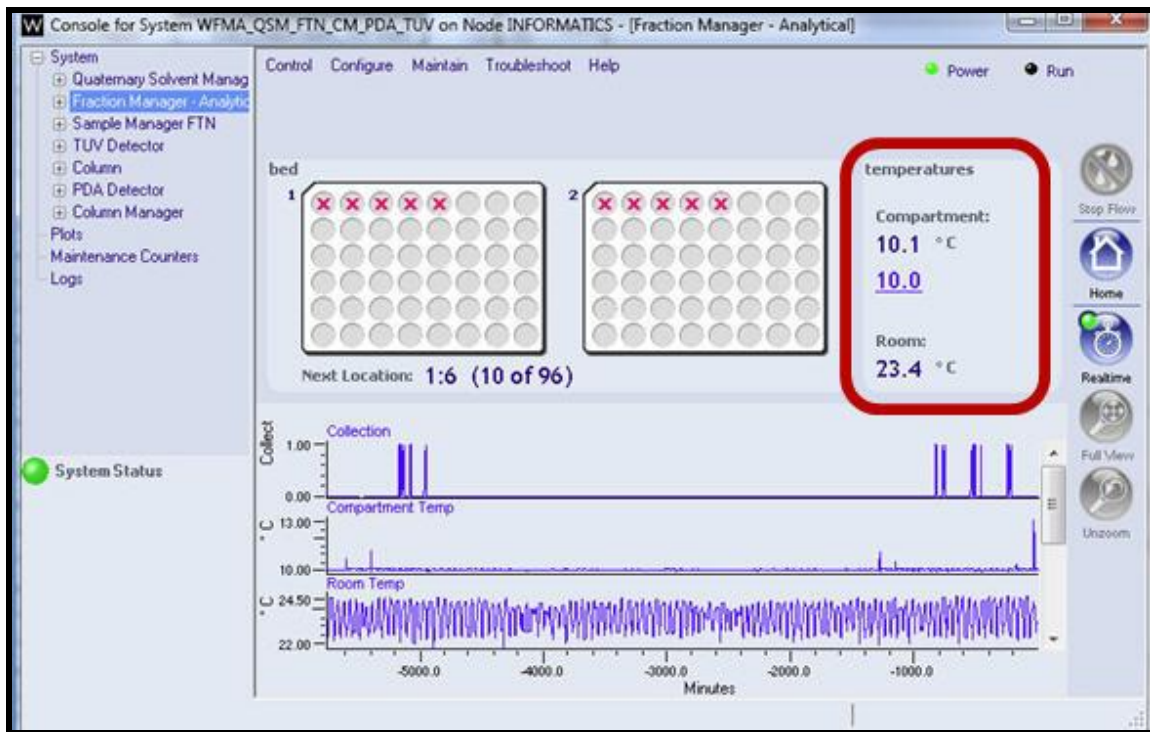
- f. Enter the needle volume. The needle volume is listed on the needle itself.
- g. Syringe is always 250ul (Fraction Manager Syringe).
- h. Make sure the enter the true volume on the delay coil and needle, then select OK.



16. In the Console, select Fraction Manager – Analytical – Interactive Display.



17. Click on the Fraction Manager area to View the Collection – Compartment Temp and Room Temp plots.



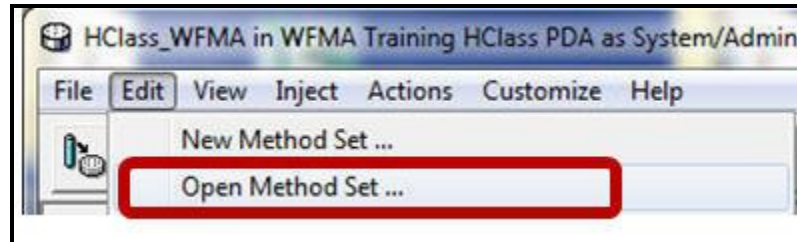
18. Minimize the console and return to the Run Samples window.

D. Instrument Method and WFMA No Collection Acquisition

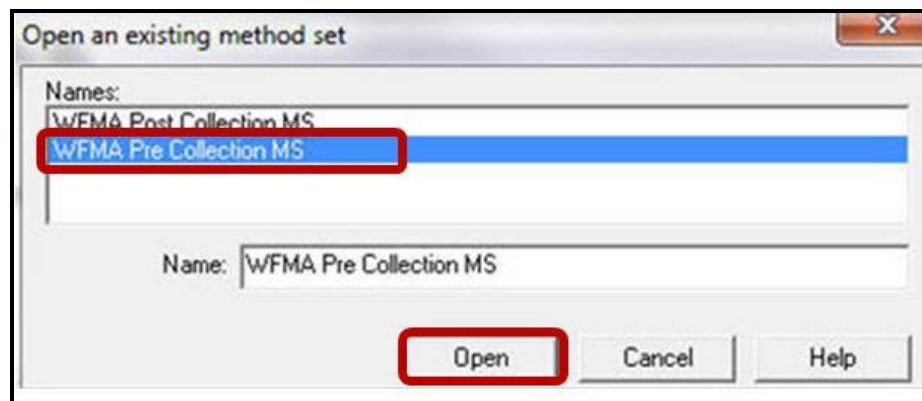
Note: The first acquisition does not include Fraction Collection. This is to establish collection times. Fraction Collection will be added after the peak retention time is identified.

This section details fraction collection by retention time. See "I -J" sections for details about slope, threshold, and fraction simulation.

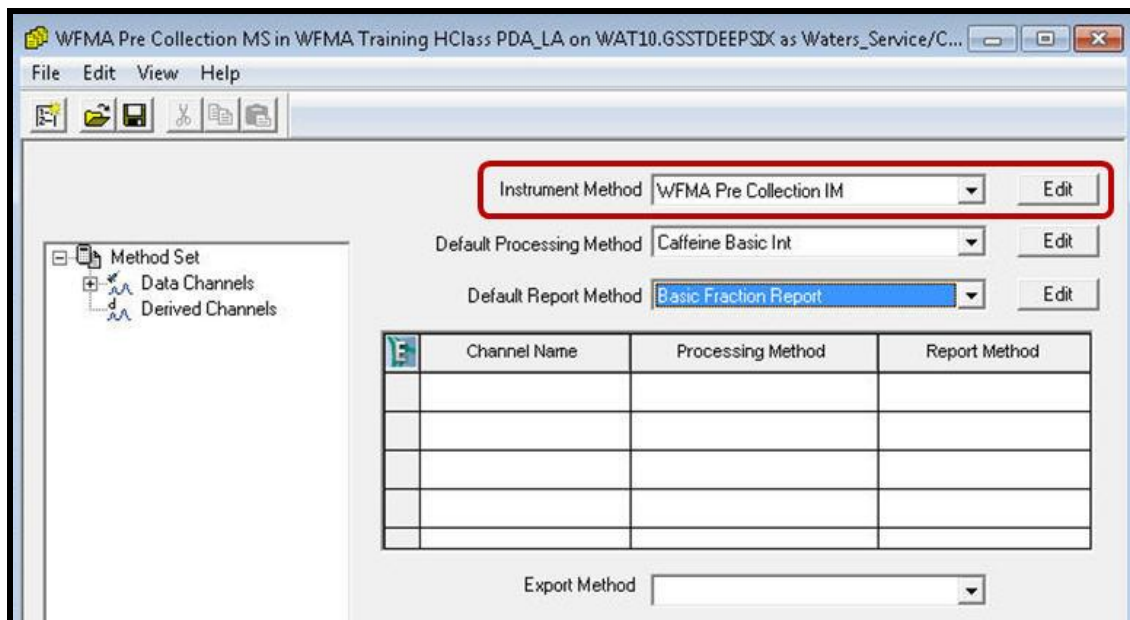
1. In the Run Samples window, select "Edit - Open Method Set...." .



2. In the Open and existing method set or create a new method set.



3. In the "Method Set" select "Instrument Method - Edit" to create or open an Instrument method.



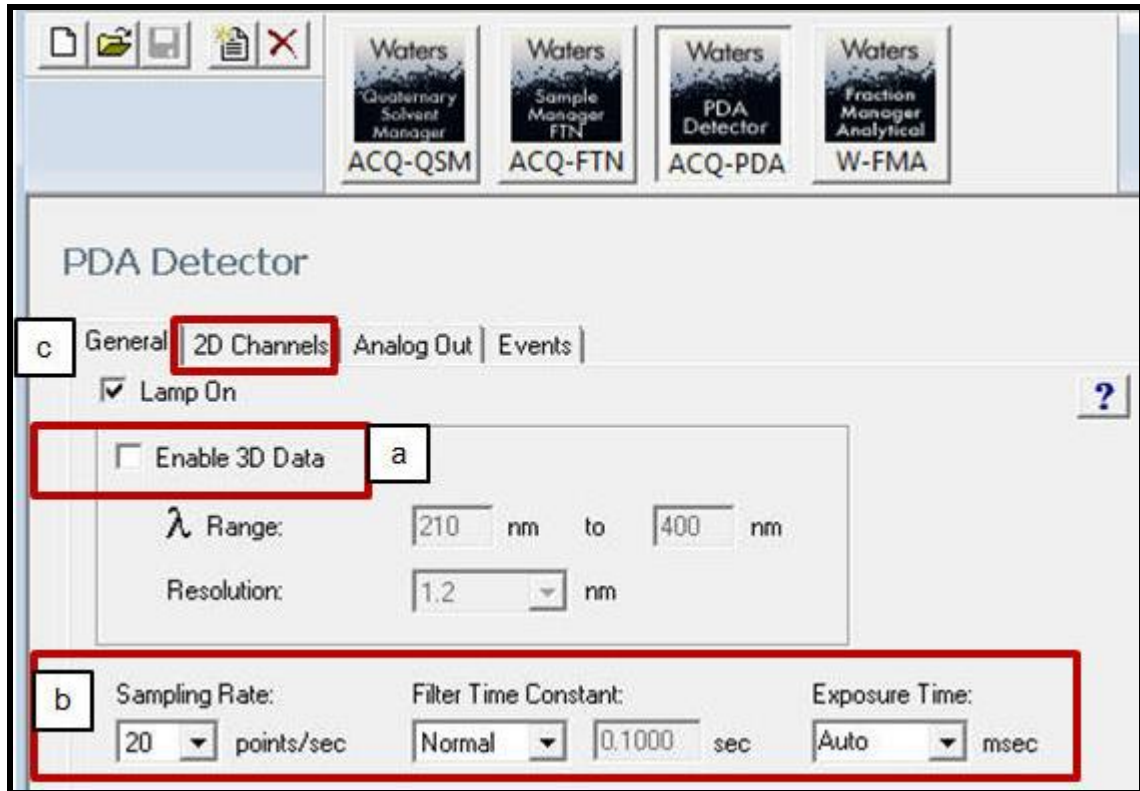
4. Create an instrument method for determining collection times without collection.
 - a. The method should be reproducible for retention time when using time based collection.
 - b. This instrument method will disable WFMA operation and collection.
 - c. Select the pump (ACQ-QSM in this example) and enter in a flow rate and gradient.

	Time	Flow (mL/min)	%A	%B	%C	%D	Curve
1	Initial	0.600	90.0	10.0	0.0	0.0	Initial
2	10.00	0.600	90.0	10.0	0.0	0.0	6
3	30.00	0.000	90.0	10.0	0.0	0.0	11

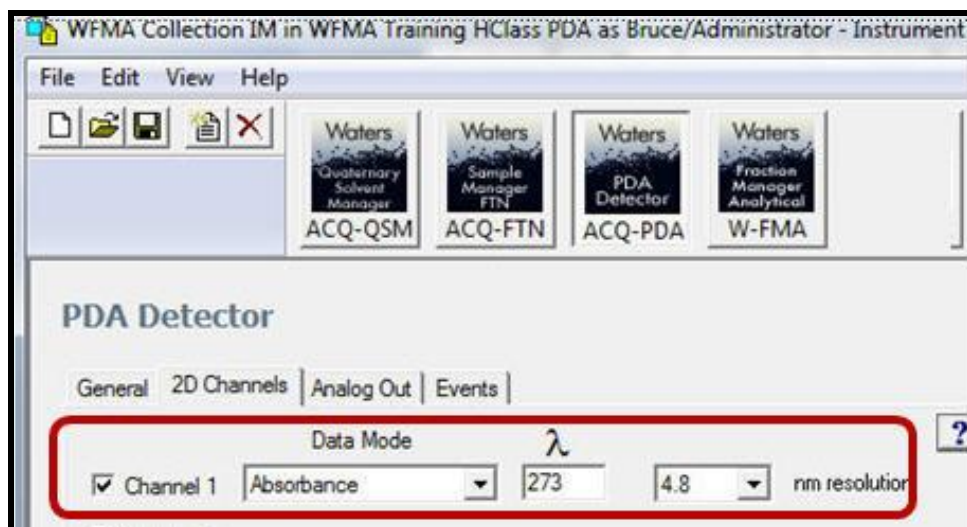
5. Select the Injector (ACQ-FTN in this example) and enter in the injection parameters.

6. Setup the Detector (ACQ-PDA in this example) settings. Notes:

- Only TUV and PDA (2489, 2998, ACQUITY PDA, ACQUITY TUV) are supported for peak based detection.
- For the ACQUITY PDA and 2998 PDA detectors, only 2D Channels are supported for peak based detection.
- QDa and other detectors are only for time based collection.

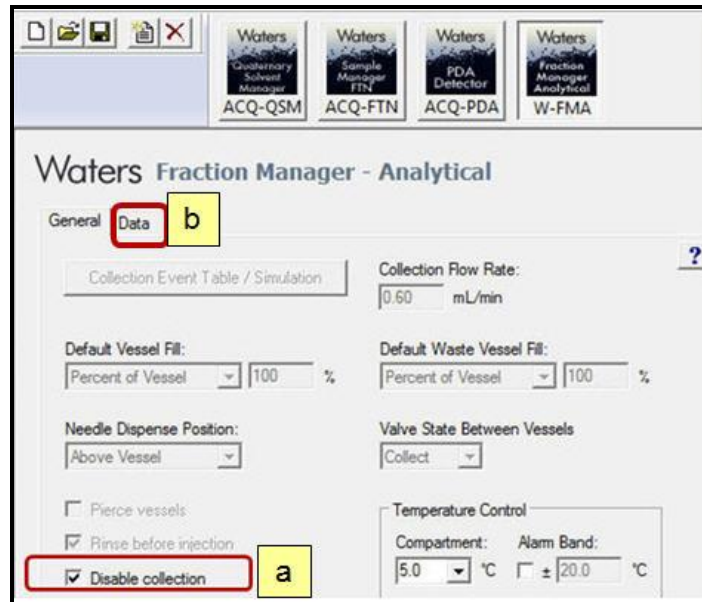


- Select the 2D Channels tab use the data mode of "Absorbance" and select a wavelength. This can be used for "timed" or "peak" based collection.

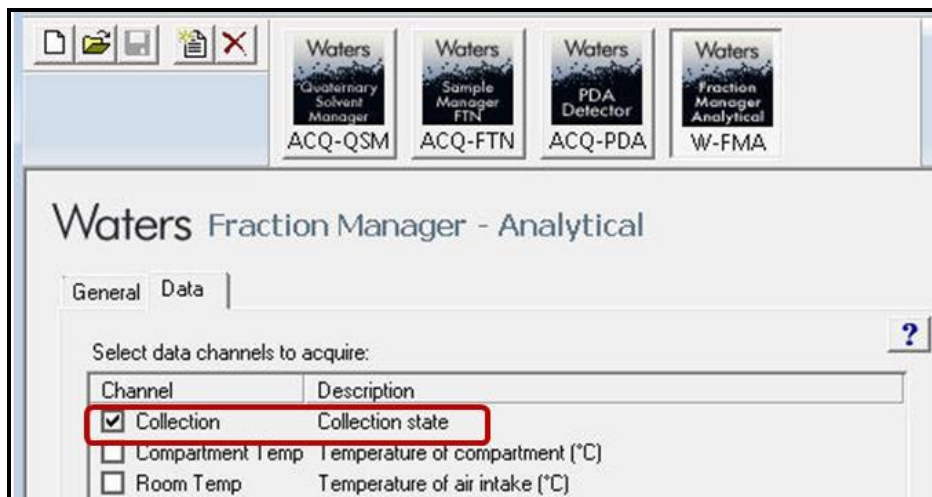


7. Select the Waters Fraction Manager –Analytical icon.
 - a. Select the “**Disable Collection**” button.
 - b. Click on the “**Data**” tab.

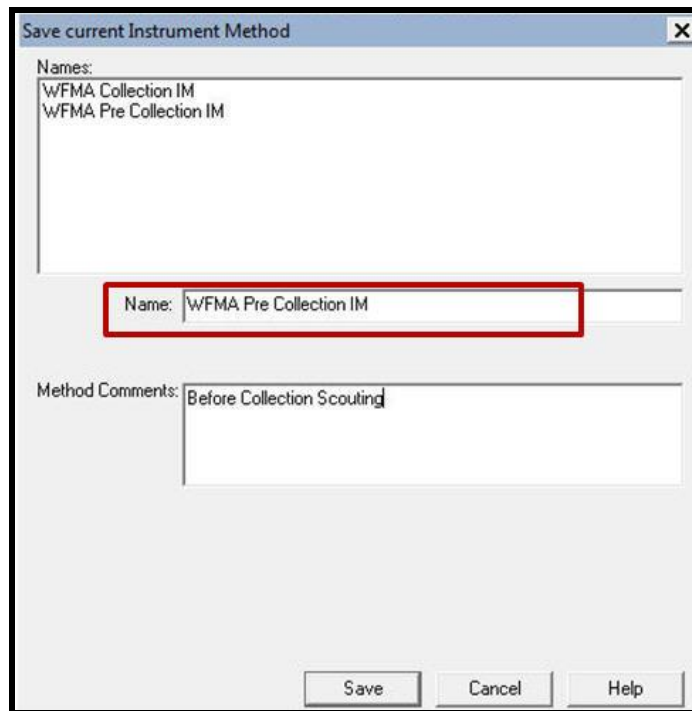
Note: All the details of Fraction Collection will be covered after acquisition and processing of the first sample to identify fraction collection times.



8. In the WFMA Data tab, select the Channel for “Collection State”. This will be used to display when the fraction collector occurs.

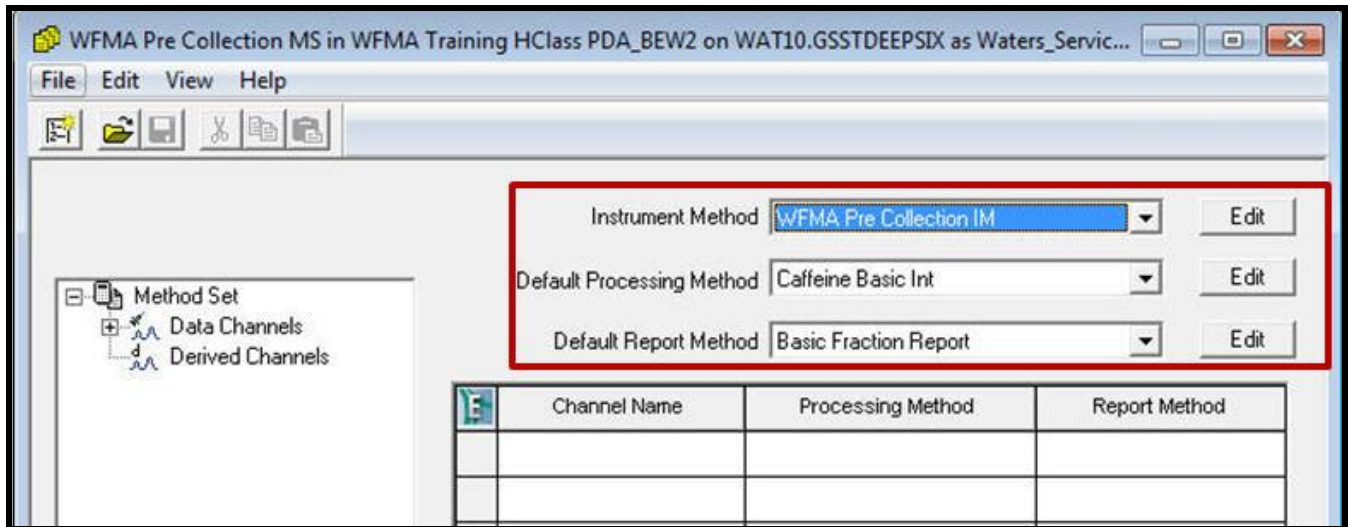


9. Select File – Save As and Save the Instrument Method as “...Pre Collection IM”.



10. Add the Instrument method to the Method Set. Select “File – Save” or “File- Save As”. This will save the Instrument Method to the “... Pre Collection MS”.

Note: A basic Processing Method and Fraction Report Method are included with the Fraction Manager Familiarization project.



11. Make a Pre-Collection Sample Set with an Equilibration then 3-6 replicate injections. This sample set is used to establish collection retention times.

The screenshot shows the Waters Fraction Manager software interface. The title bar indicates the user is editing a sample set method named 'WFMA Pre Collect'. The menu bar includes File, Edit, View, Inject, Actions, Customize, and Help. The toolbar contains various icons for file operations and data management. Below the toolbar, there are dropdown menus for 'Run Only' and 'Continue on Fault', along with an 'Apply Table Preferences' button and a 'Sample Set Method' dropdown. The main area displays a table for the sample set method 'WFMA Pre Collection Sample Set'.

	Plate/Well	Inj Vol (uL)	# of Injs	SampleName	Function	Method Set / Report Method	Run Time (Minutes)	WFMAParameter
1					Equilibrate	WFMA Pre Collection MS	2.00	
2	1:A,1	10.0	1	Caffeine Only No Collect1	Inject Standards	WFMA Pre Collection MS	1.00	Pre Collection
3	1:A,2	10.0	1	Caffeine Only No Collect2	Inject Standards	WFMA Pre Collection MS	1.00	Pre Collection
4	1:A,3	10.0	1	Caffeine Only No Collect3	Inject Standards	WFMA Pre Collection MS	1.00	Pre Collection

12. Click the Green Start button and use the "Run Only" function.

This close-up screenshot shows a portion of the software toolbar. A red box highlights the Green Start button (a green circle with a white play symbol). Another red box highlights the 'Run Only' dropdown menu, which is currently set to 'Run Only'.

13. Name the Sample Set ".....Pre Collection....", then click Run.

The screenshot shows the 'Run Sample Set' dialog box. The 'Name for this sample set' field is highlighted with a red box and contains the text 'WFMA Pre Collection BEW'. The 'Sample set method name' field contains 'WFMA Pre Collection Sample Set'. Under 'Settings for this Sample Set', the 'Run Mode' is set to 'Run Only' and the 'Suitability Mode' is set to 'Continue on Fault'. The 'Run' button at the bottom is also highlighted with a red box.

14. During the acquisition, setup the display preferences for acquisition by:

- Right mouse click in the chromatogram display and select "Customize Channels".
- Select Separate Plots, and check Display Status for "PDA 273nm@ 4.8" and "Collection".
- Select Ok.
- From the menu bar of the Run Samples window select "File – Save Preferences.

Note: The Collection channel will be a straight line for the "No Collection" injections.

The screenshot displays the Waters Fraction Manager software interface. The main window shows a table of sample runs and two chromatograms. The 'Customize Channels' dialog box is open, showing the '2D Channels' tab. The dialog box has three radio buttons: 'Separate Plots' (selected), 'Overlay in Single Plot', and 'Stack Plot'. Below these are three rows of channel settings:

	Plot Type	Display Status	Wavelength (nm)
1	PDA Spectrum	<input type="checkbox"/>	254.0
2	PDA Ch1 273nm@4.8nm	<input checked="" type="checkbox"/>	
3	Collection	<input checked="" type="checkbox"/>	

The 'OK' button is highlighted with a red box and labeled 'c'. The 'Customize Channels' option in the right-click context menu is highlighted with a red box and labeled 'a'. The chromatogram on the right shows a single sharp peak at approximately 0.20 minutes, labeled 'PDA Ch1 273nm@4.8nm'. The chromatogram on the left shows a flat line at 0.00 AU, labeled 'Collection'.

15. After the Sample Set has completed, exit out of Run Samples.

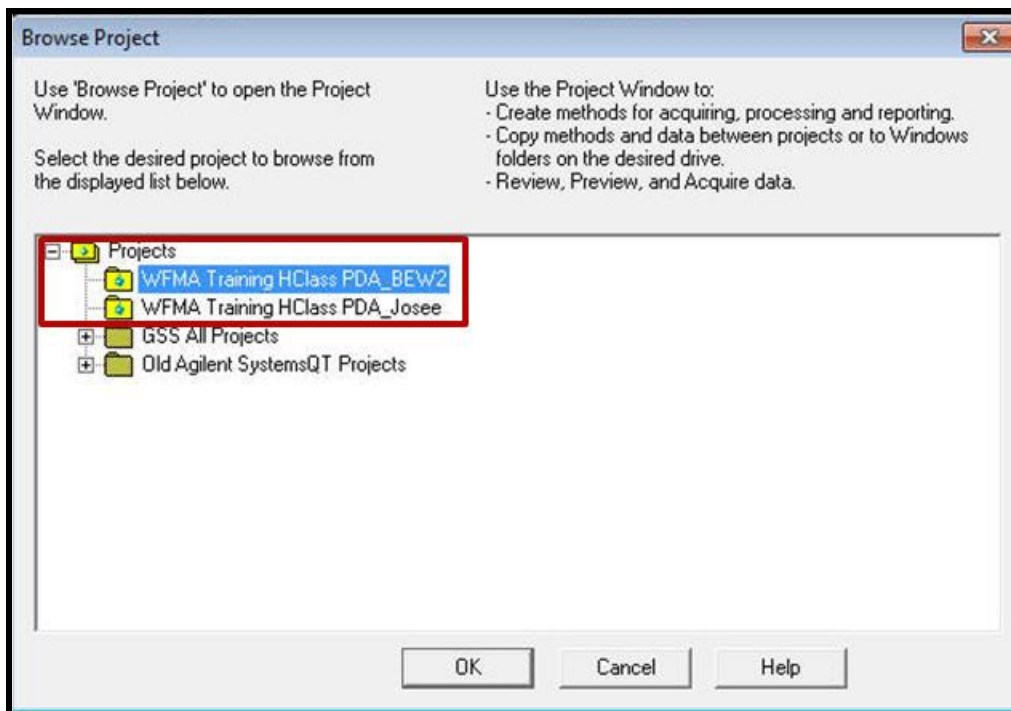
E: Processing Data for Time Based Collection

Note: After acquisition is complete, use Review to determine integration parameters and retention time(s). Save the processed chromatogram result.

The processed chromatogram result can be used to automatically insert the peak start and end times for peak collection in the WFMA instrument method settings.

This section details the steps for timed based collection.

1. In the Empower menu, select Browse Projects and select your project, then select "OK".

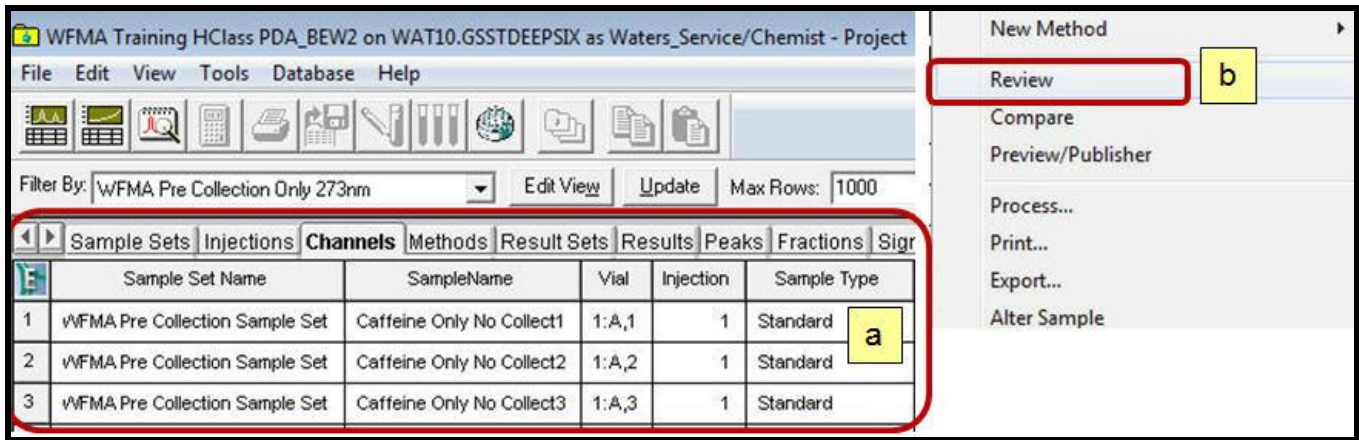


2. In the Project view, select the Channels tab.

The screenshot shows the 'Channels' tab selected in the 'Project view'. The table below represents the data displayed in the 'Channels' tab:

SampleName	Vial	Injection	Sample Type	Date Acquired	Channel	Channel Description
1 Caffeine Only No Collect3	1:A,3	1	Standard	Tuesday, September 02, 2014 10:46:33 AM EDT	PDA Ch1 273nm@4.8nm	PDA Ch1 273nm@4.8nm
2 Caffeine Only No Collect3	1:A,3	1	Standard	Tuesday, September 02, 2014 10:46:33 AM EDT	Collection	Collection
3 Caffeine Only No Collect2	1:A,2	1	Standard	Tuesday, September 02, 2014 10:44:09 AM EDT	PDA Ch1 273nm@4.8nm	PDA Ch1 273nm@4.8nm
4 Caffeine Only No Collect2	1:A,2	1	Standard	Tuesday, September 02, 2014 10:44:09 AM EDT	Collection	Collection
5 Caffeine Only No Collect1	1:A,1	1	Standard	Tuesday, September 02, 2014 10:41:44 AM EDT	PDA Ch1 273nm@4.8nm	PDA Ch1 273nm@4.8nm
6 Caffeine Only No Collect1	1:A,1	1	Standard	Tuesday, September 02, 2014 10:41:44 AM EDT	Collection	Collection

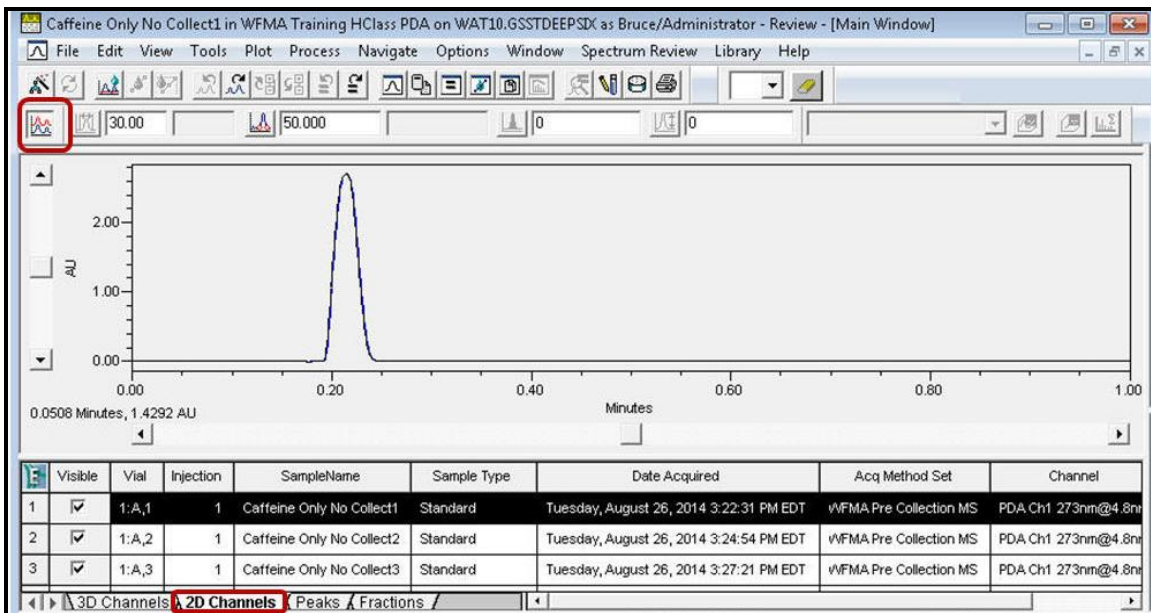
3. Highlight all your data from your sample set in the channels tab.
 - a. Right click
 - b. Select "Review".



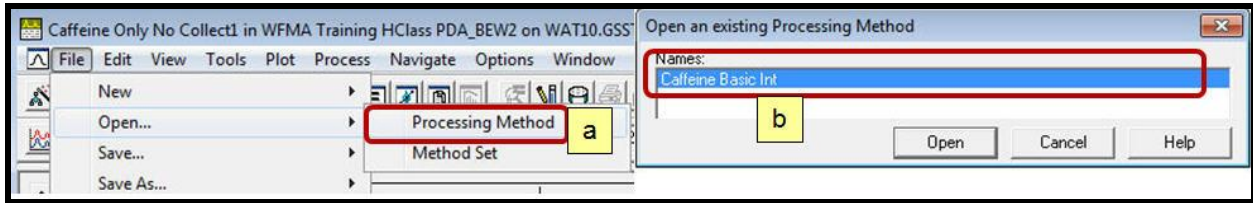
4. In the Review window, select the "2D Channels" tab below the tables and click the Overlay icon



- a. Makes sure all channels visually overlay.
- b. Toggle the "Overlay" icon again to revert back to single chromatogram display.



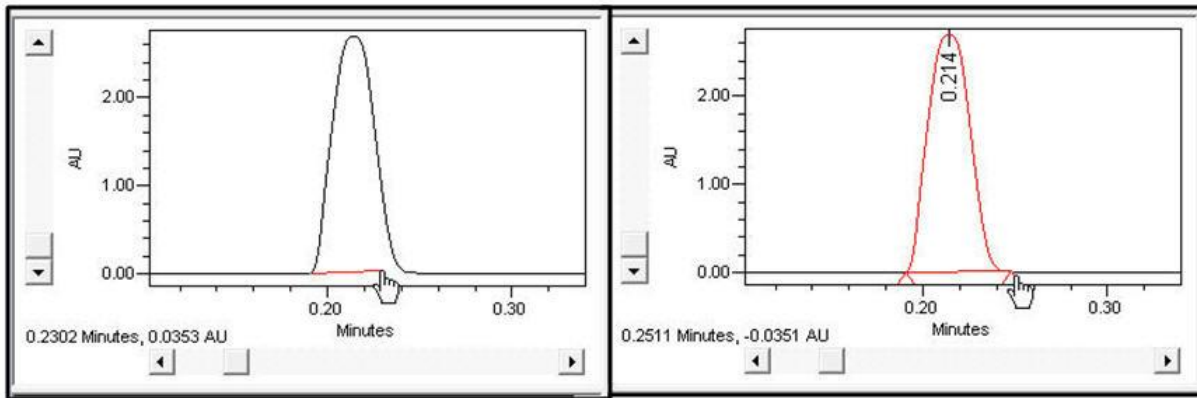
- From the menu bar select File – Open and select the “Basic Processing Method” or create a new processing method.



- Click the Auto Integrate icon  and the Quantitate icon .

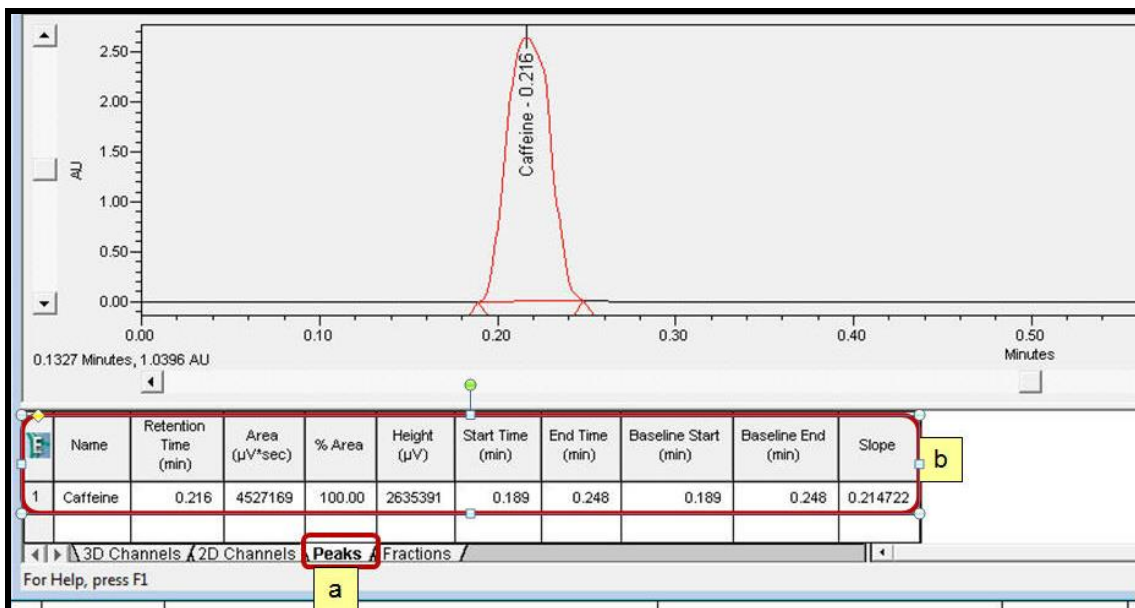
OR:

- Manually integrate the peak by clicking and dragging across the peak.

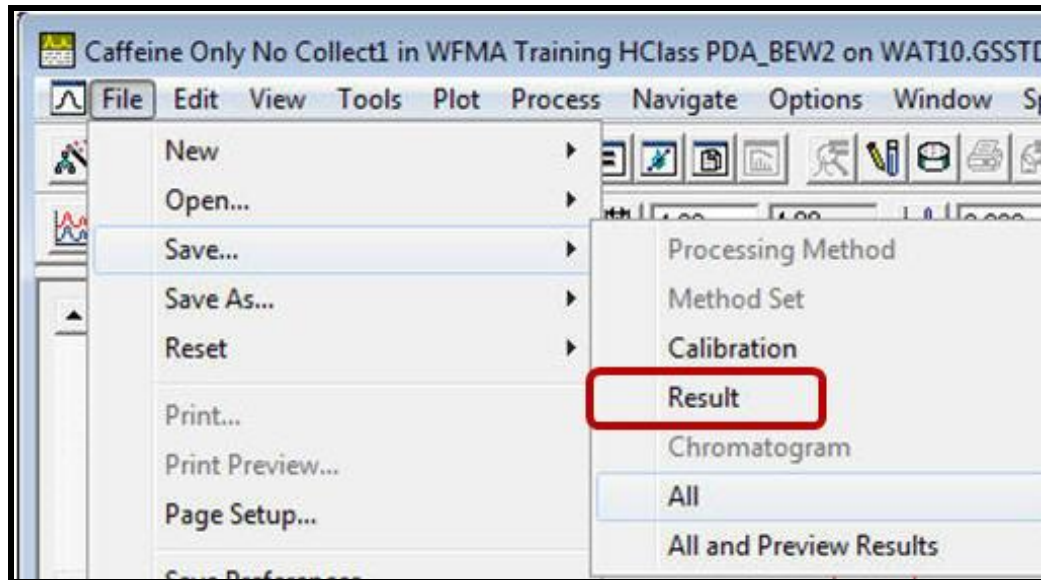


- Click the Peaks tab at the bottom of the table and view the Start Time and End Time of the peak. These will be automatically inserted into the Fraction Manager’s instrument method.

Note: The Slope and Threshold values can be used for Peak based fraction collection.



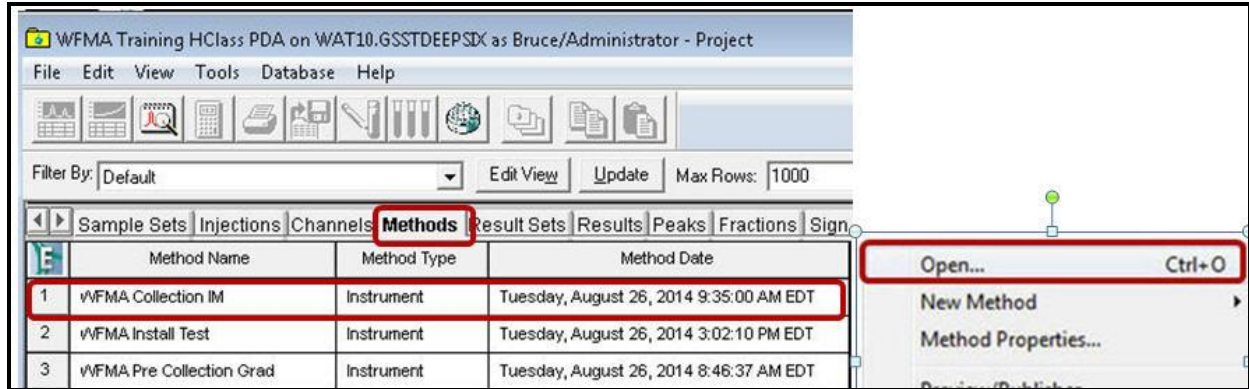
- From the Review window select File – Save Result. This will save the processed result. This processed result will be used for optimizing the Fraction Collection Instrument Method settings for time based peak collection.



- Exit the Review window and return to the Project.

F: Edit the WFMA Method Editor for Time Based Collection

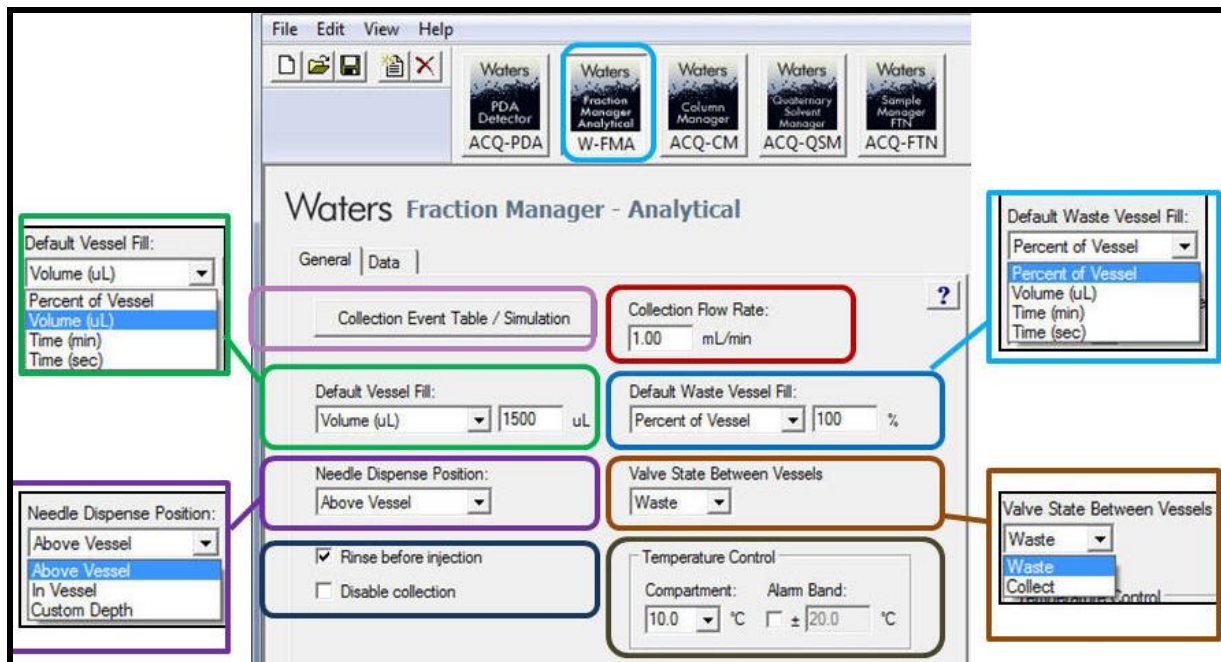
1. From the Project view select the Methods tab.
2. Select the "pre-collection" Instrument Method, right click and select "Open".



3. In Instrument Method, click on the Fraction Manager icon.



4. Below are the selection choices for the "Fraction Manager – Analytical" instrument settings. Specific details about each setting are in Section I: Advanced Fraction Manager Functions.



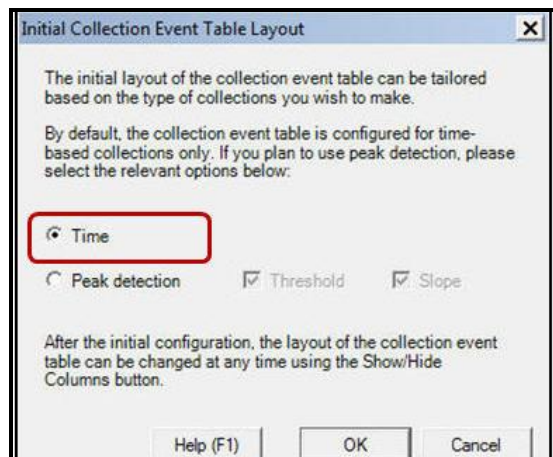
5. Good default settings for 48 vial settings for the Fraction Manager are:

Settings (Left Side)	Value to Enter	Settings (Right Side)	Value to Enter
Default Vessel Fill	Volume:	Collection Flow Rate	0.60
Needle Dispense Position	Above Vessel	Default Waste Vessel Fill	75%
Rinse Before Injection	Checked On	Valve State Between Vessels	Waste
Disable Collection	Checked Off	Temperature Control	10

6. Click on the Collection Event Table /Simulation button.

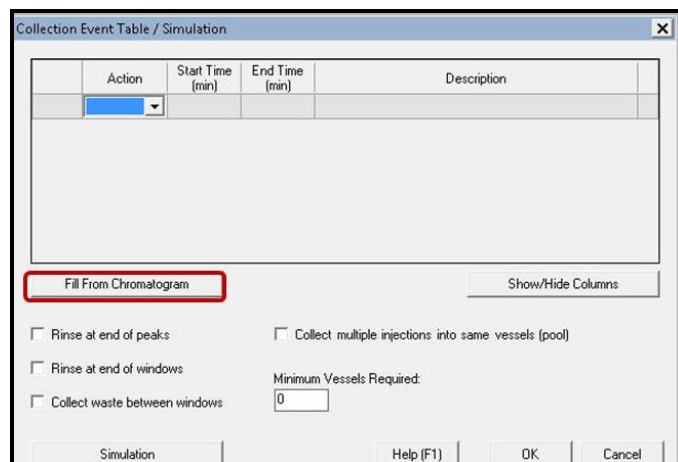


7. The Collection Event Table can be configured for table layout upon first time selection to show or not show peak slope or threshold based collection (this can be re-configured using the show hide column feature in the event table).

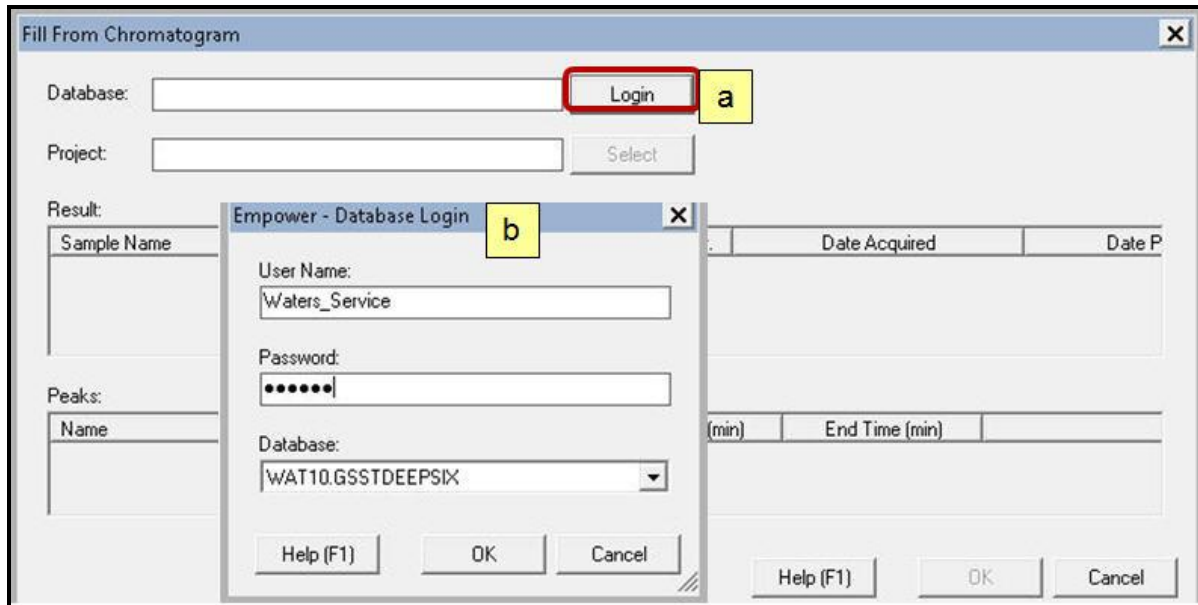


8. Time Based collection can be manually entered into the event table or automatically entered by using the "Fill from Chromatogram" button.

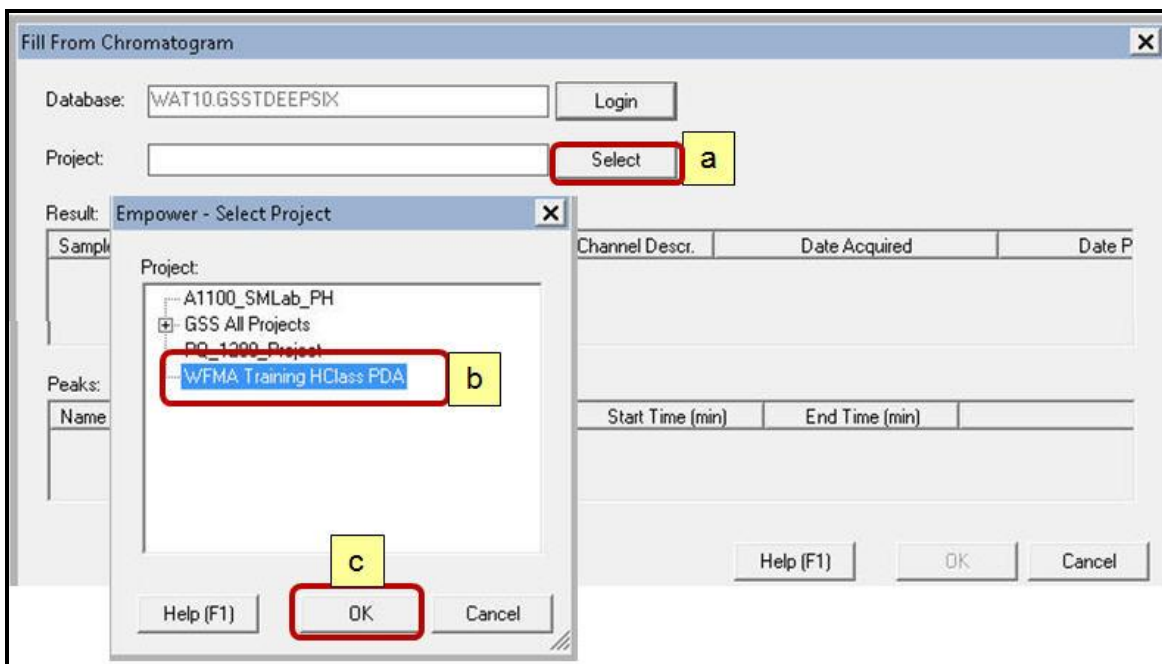
Note: For this training and installation tests, click on the "Fill from Chromatogram" button.



9. The "Fill from Chromatogram" function automatically fills the Fraction Manager's collection table from a processed result using time based collection. Below is the sequence of steps to use this function.
- Click the Login button. To utilize this function you must re-log into the Empower database.
 - Enter in your user name and password. Click Ok to Log in.

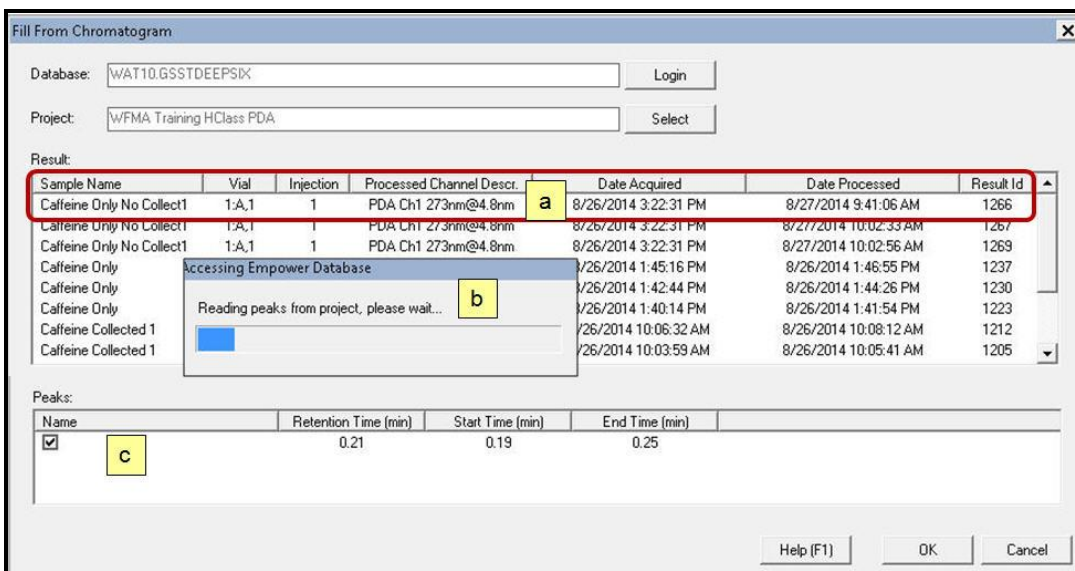


10. Click "Select" to access the Empower project that has the pre-collection results.
11. Select the desired project and select "OK".



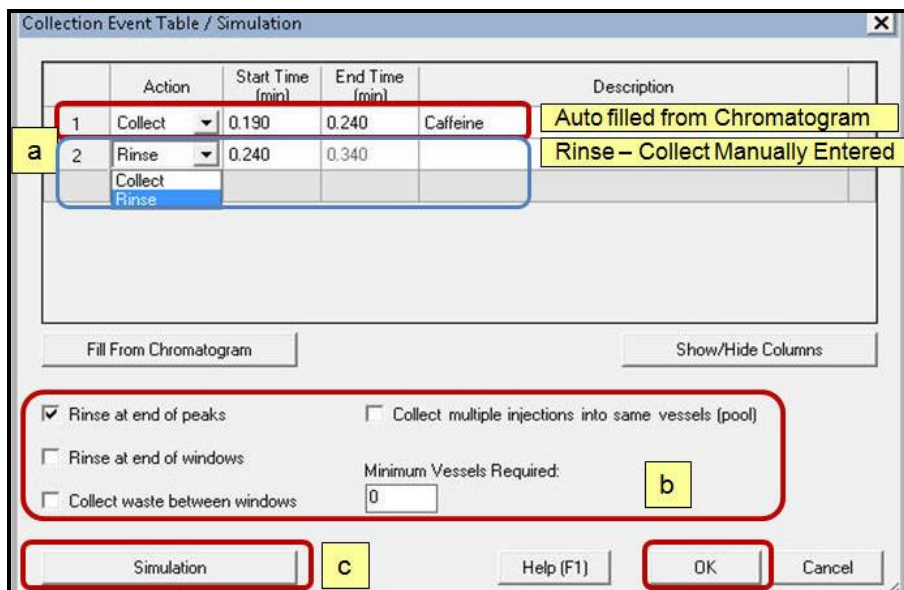
12. Fill the peak collection table:

- a. Highlight a processed result from the specified project (this is from the Projects Results view).
- b. The result must have peak integration for the auto-fill collection table to work.
- c. The Peaks table displays a component name (if quantitated), retention time (apex), and start time and end time. Select Ok and each integrated peak will be added to the collection table.



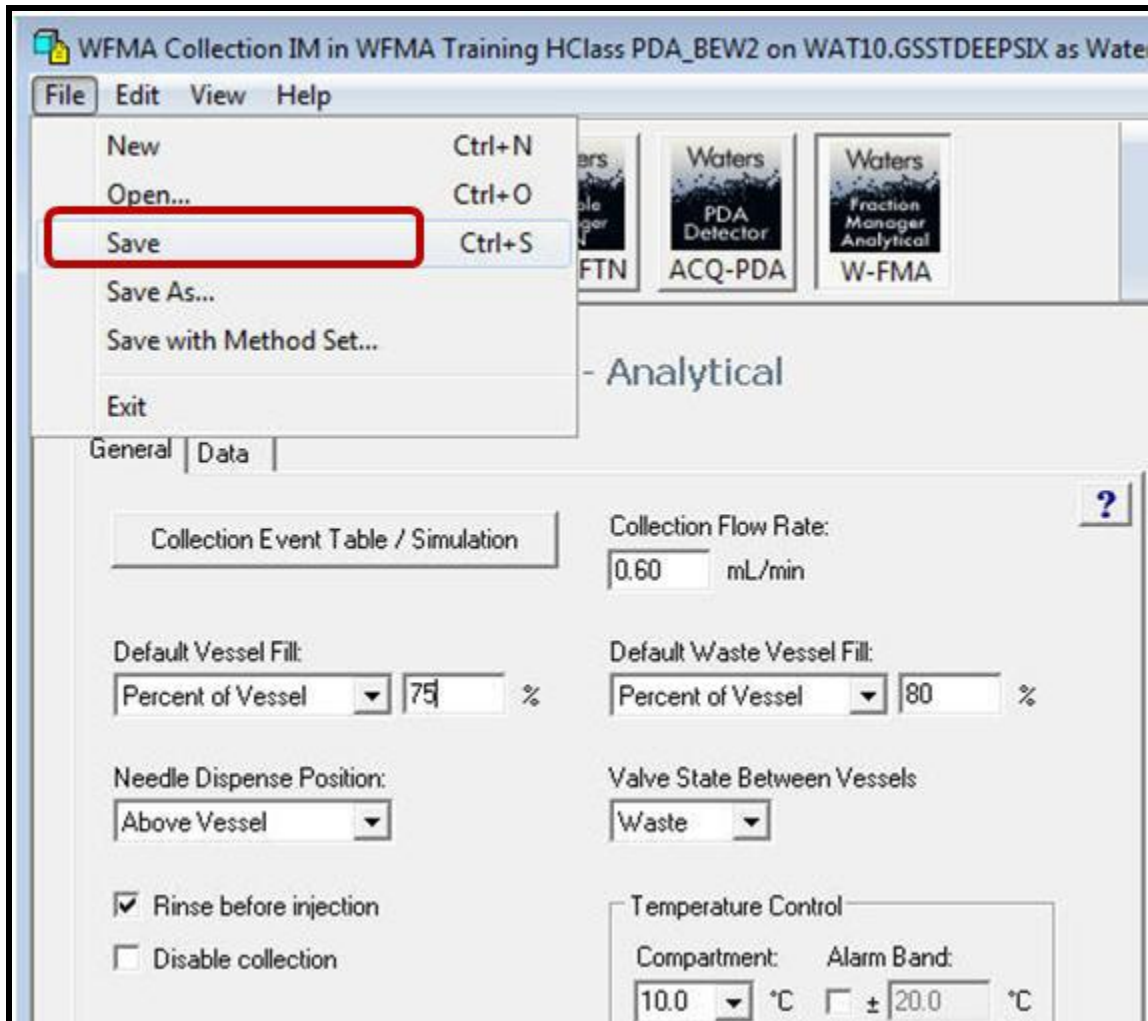
13. The Collection table is filled out from the Peaks table. A basic collection methods is displayed below. Select OK.

- a. The event table can be filled out by "Fill from Chromatogram" or manual entry.
- b. Select the Rinse/Waste/Pooling options. This will be discussed in chapter I - collection method overview.
- c. Simulation is for Peak Detection using Slope and Threshold. This will be discussed in section J.



14. Select OK to exit out of the "Collection Event Table".

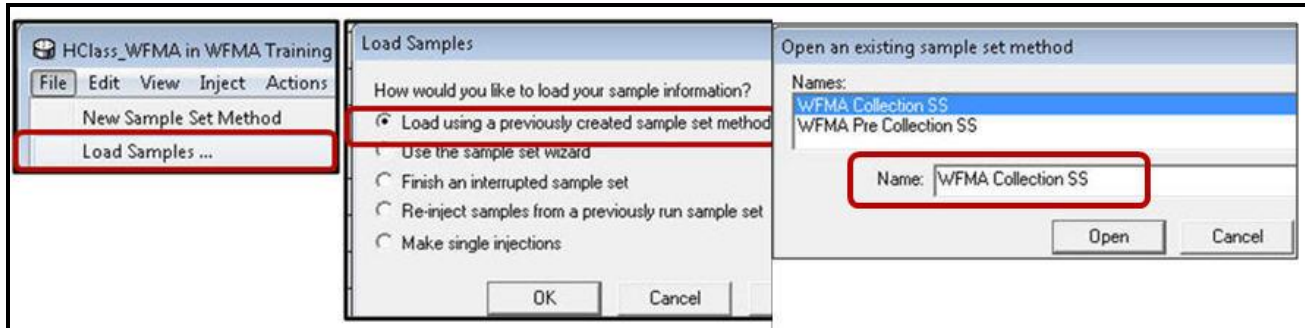
15. Select File - Save to save the changes to the "PreCollection" instrument method and save it as the "Collection IM".



16. Select File - Exit to leave the instrument Method editor.

G: Acquire Data using the WFMA with Time Based Collection

1. Select File – Load Samples, Load using a previously created sample set method, and select a sample set that has the new collection instrument method included and click "Open".

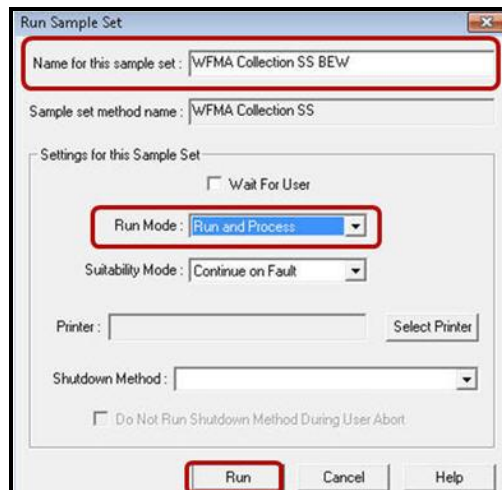


2. View the Sample Set Method Table. Verify that the Method Set has the collection instrument method enabled.

Plate/Well	Inj Vol (uL)	# of Injs	SampleName	Function	Method Set / Report Method	Run Time (Minutes)	WFMAParameter	
1				Equilibrate	WFMA Collection MS	2.00		
2	1:A,1	10.0	1	Caffeine Collected 1	Inject Standards	WFMA Collection MS	1.00	Collected Caffeine Time Based
3	1:A,2	10.0	1	Caffeine Collected 2	Inject Standards	WFMA Collection MS	1.00	Collected Caffeine Time Based
4	1:A,3	10.0	1	Caffeine Collected 3	Inject Standards	WFMA Collection MS	1.00	Collected Caffeine Time Based

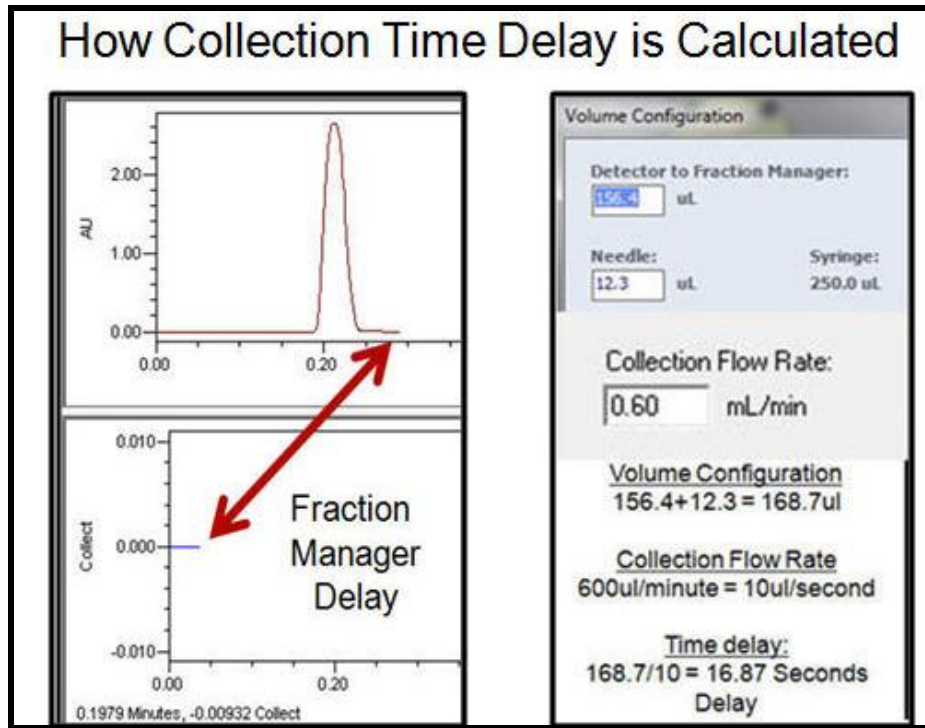


3. Click on the Run Samples icon and change the name to "Collection ...", Click "Run and Process" and click "Run".



- During the acquisition, the detector chromatogram real time plot will be displayed before the collection display. This is to account for the delay time differences between peak detection by the PDA/UV detector and the peak Collection by time.

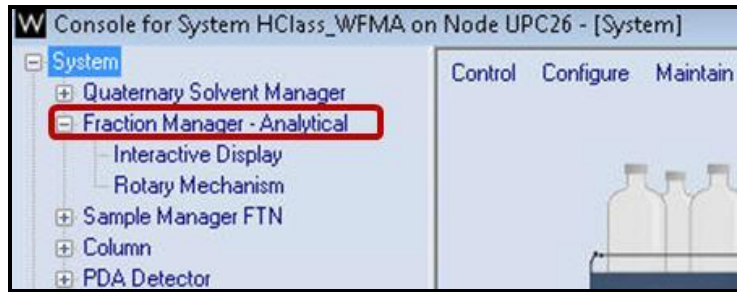
Note: If you do not see Fraction Collection, right click on the Fraction Manager panel, and select "Reset WFMA", then right click, and select "Reset Beds".



- During acquisition and fraction collection, the Fraction Manager icon displays real time information including:

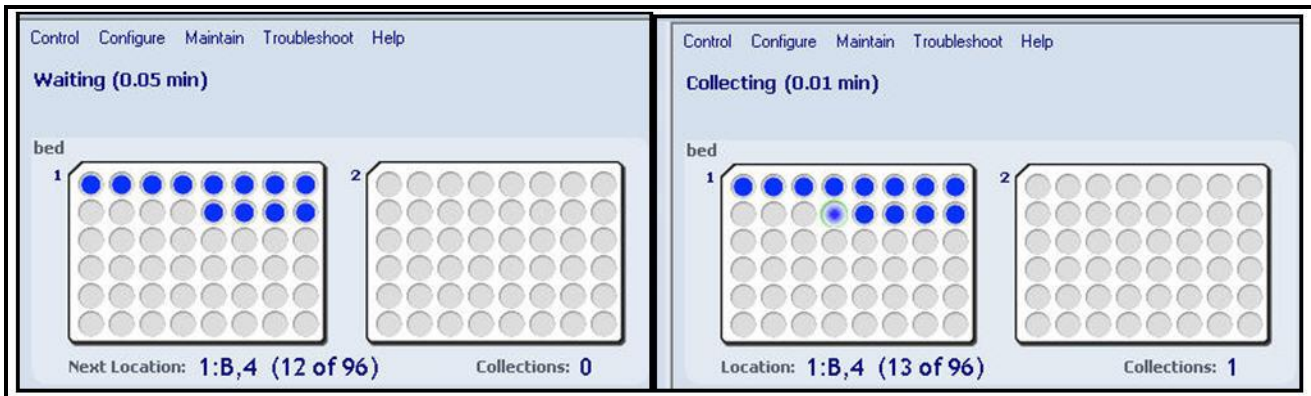
<p>Bed Full - Running</p> <p>Fraction Manager - Analytical ● Run</p> <p>Running</p> <p>Next Location Bed Full</p> <p>Collections 0</p> <p>Compartment 12.1 10.0 °C</p>	<p>Homing RTZ Axis</p> <p>Fraction Manager - Analytical ● Run</p> <p>Homing RTZ axes</p> <p>Next Location 1:6 (10 of 96)</p> <p>Compartment 10.1 10.0 °C</p>	<p>Door Open - Close</p> <p>Fraction Manager - Analytical ● Run</p> <p>Please close the door</p> <p>Next Location 1:1 (0 of 192)</p> <p>Compartment 18.0 10.0 °C</p>
<p>Fraction Delay</p> <p>Fraction Manager - Analytical ● Run</p> <p>Waiting (0.17 min)</p> <p>Next Location 1:A,4 (3 of 96)</p> <p>Collections 0</p> <p>Compartment 10.1 10.0 °C</p>	<p>Collecting</p> <p>Fraction Manager - Analytical ● Run</p> <p>Collecting (0.32 min)</p> <p>Location 1:8 (15 of 96)</p> <p>Collections 1</p> <p>Compartment 10.1 10.0 °C</p>	<p>Next Location</p> <p>Fraction Manager - Analytical ● Run</p> <p>Next Location 1:8 (14 of 96)</p> <p>Compartment 10.1 10.0 °C</p>

- Open the ACQUITY console, and then click on the Fraction Manager – Analytical display.

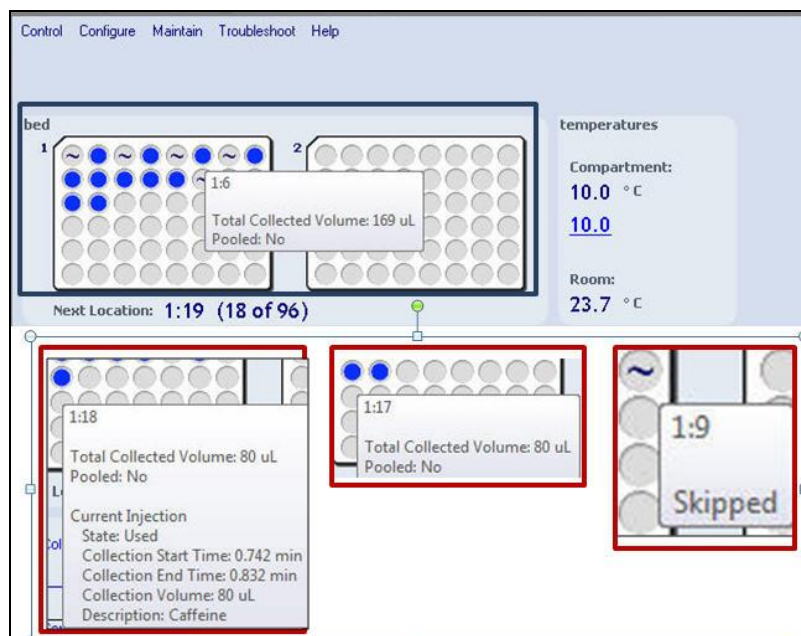


- View the bed collection layout. This displays the fractions that have been collected and the next collection location.

Note: By default the collection goes to the next collection location that was not used. Use the "Reset Sample Bed" to start collection at position 1.



- Click on each collected Fraction to display additional information about the sample collection.



9. Complete the Acquisition Sample Set, and then exit out of the Run Samples Window.

The screenshot displays the 'Run Samples' window in Waters Empower 3. The window title is 'HClass_WFMA in WFMA Training HClass PDA on WAT10.GSSTDEEPSDX as Waters_Service/Chemist - Editing SS Method: WFMA Collection Sample Set - Run Samples'. The interface includes a menu bar (File, Edit, View, Inject, Actions, Customize, Help), a toolbar, and a main control area. A table lists sample set details, and two graphs show AU and Collect vs. Minutes. The bottom section contains status panels for Solvent Manager, Sample Manager, PDA Detector, and Fraction Manager.

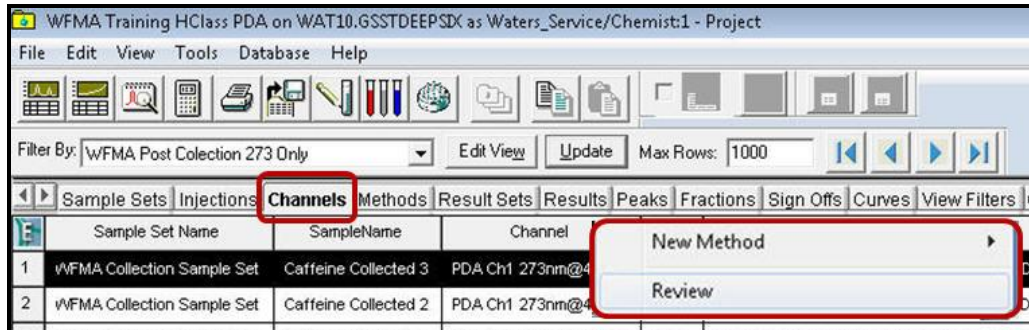
Plate/Well	Inj Vol (uL)	# of Injs	SampleName	Function	Method Set / Report Method	
1				Equilibrate	WFMA Collection MS	
2	1:A,1	10.0	1	Caffeine Collected 1	Inject Standards	WFMA Collection MS
3	1:A,2	10.0	1	Caffeine Collected 2	Inject Standards	WFMA Collection MS
4	1:A,3	10.0	1	Caffeine Collected 3	Inject Standards	WFMA Collection MS

The graphs show AU (Absorbance Units) and Collect vs. Minutes. The top graph is titled 'PDA Ctr1 273nm@4.8nm' and shows a peak at approximately 0.25 minutes. The bottom graph is titled 'Collection' and shows a pulse at approximately 0.25 minutes.

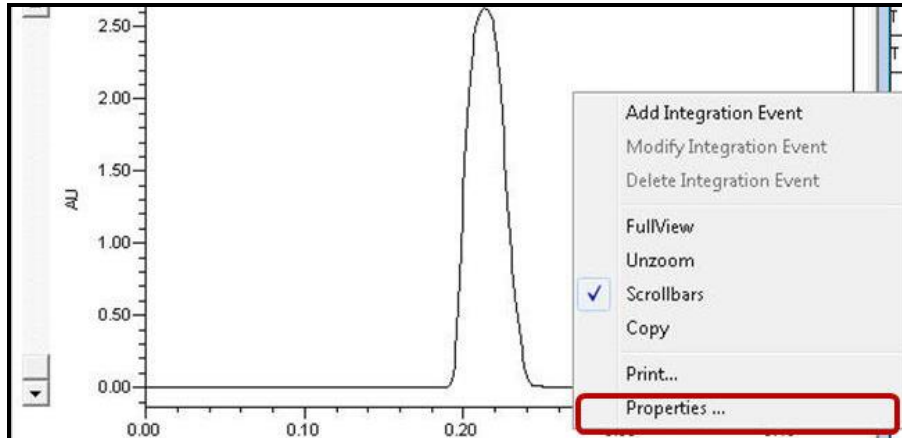
The bottom section contains status panels for Solvent Manager, Sample Manager, PDA Detector, and Fraction Manager. The Solvent Manager shows 7297 psi and 0.600 mL/min. The Sample Manager shows Sample 21.6 °C, Column 40.0 °C, and 10.0. The PDA Detector shows Shutter: Closed. The Fraction Manager shows Next Location 1:B,4 (12 of 96) and Compartment 10.0 10.0 °C.

H: View WFMA Results and Reports

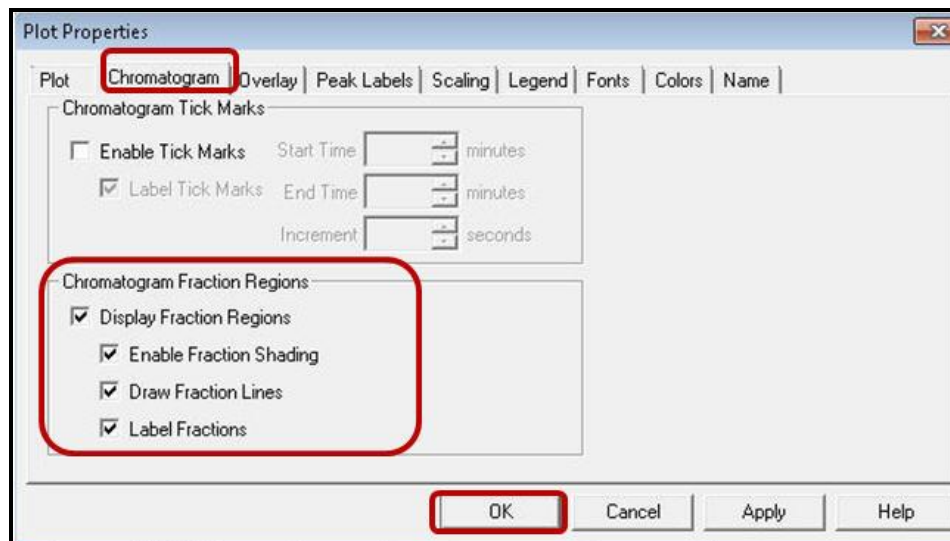
1. Select Browse Projects, then in the Fraction Collector project window select the Channels view.
2. Select a Sample from the Collection Sample Set, right click and select Review.



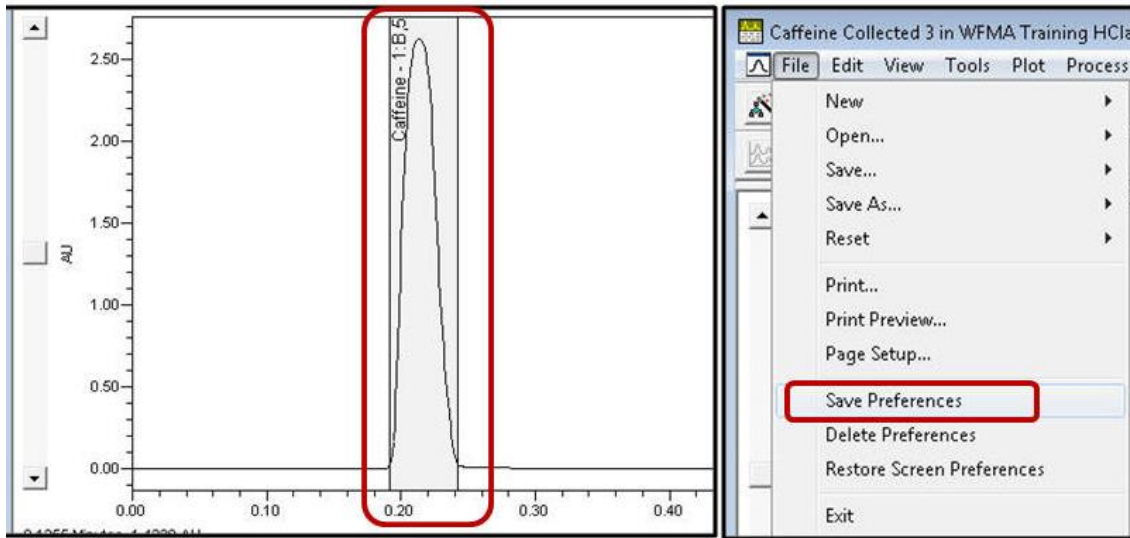
3. In the Review window, right mouse click inside the Chromatogram display and select "Properties".



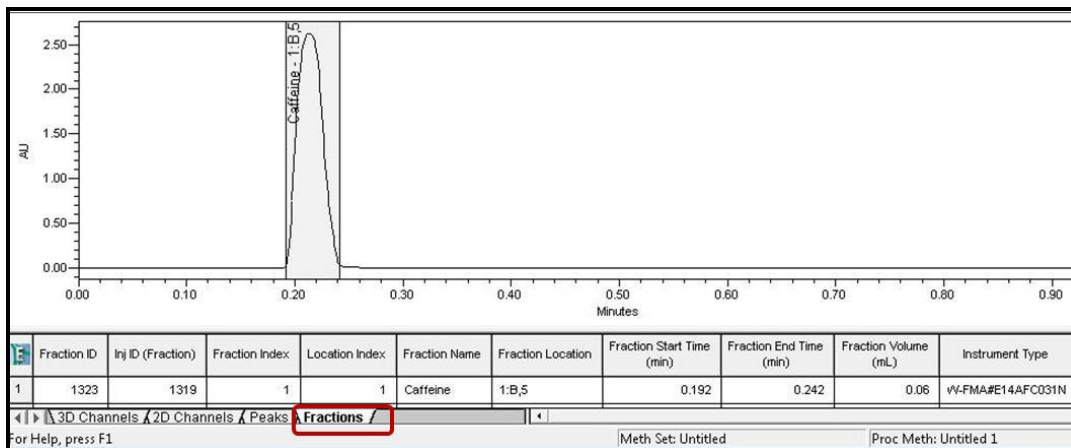
4. In the Plot Properties, select the Chromatogram Tab. Check all the "Chromatogram Fraction Regions" options to display the Chromatogram with the collected Fraction overlaid. Select OK.



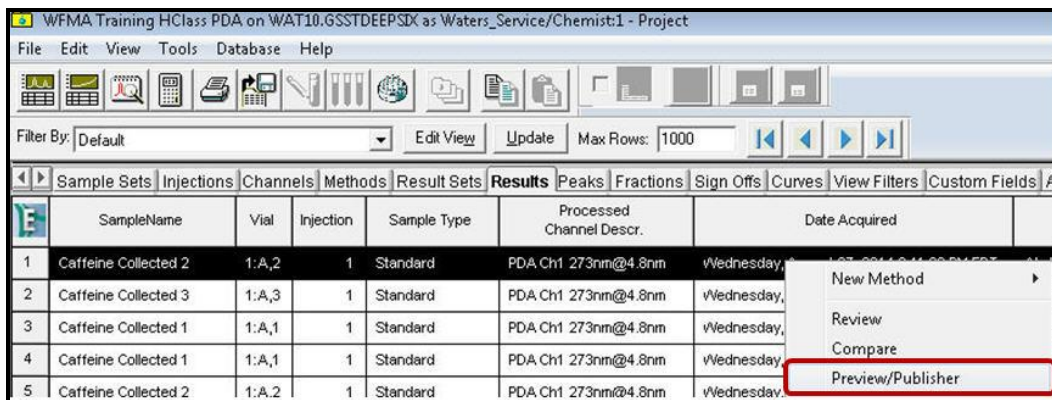
- View the Chromatogram, and note the overlaid Fraction with the chromatogram. This fraction plot display can be saved as a preference. By selecting File – Save Preferences.



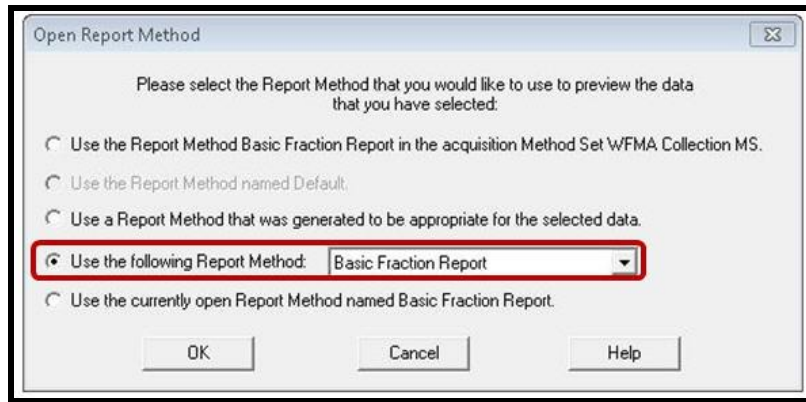
- In the Tables area, select the Fractions tab. View the details listed in the table. This fraction information is included for each collected Fraction.



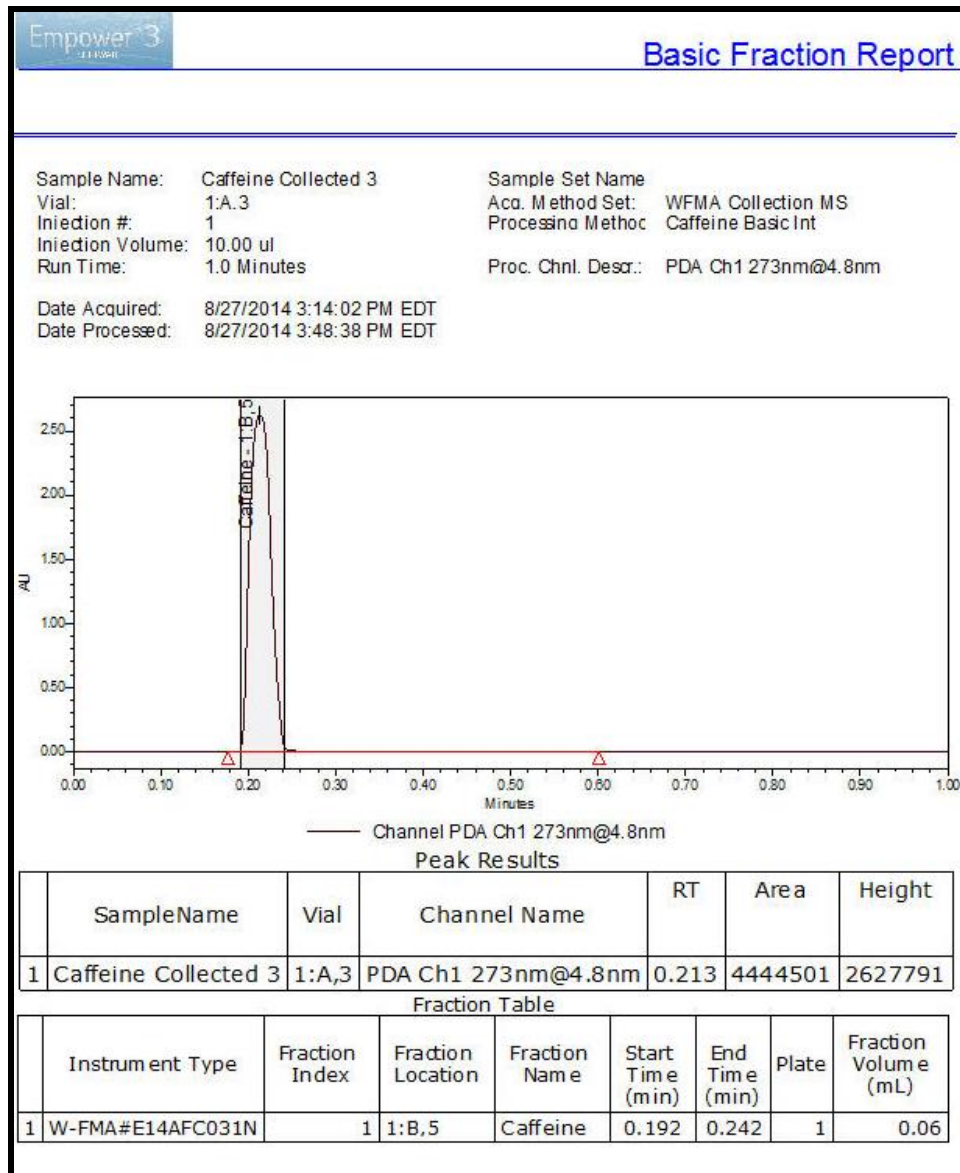
- Exit out of the Review window. In the Project select the Results tab, highlight a collected result, right mouse click and select "Preview/Publisher".



8. Select "Use the following Report Method" and select "Basic Fraction Report", then select Ok.

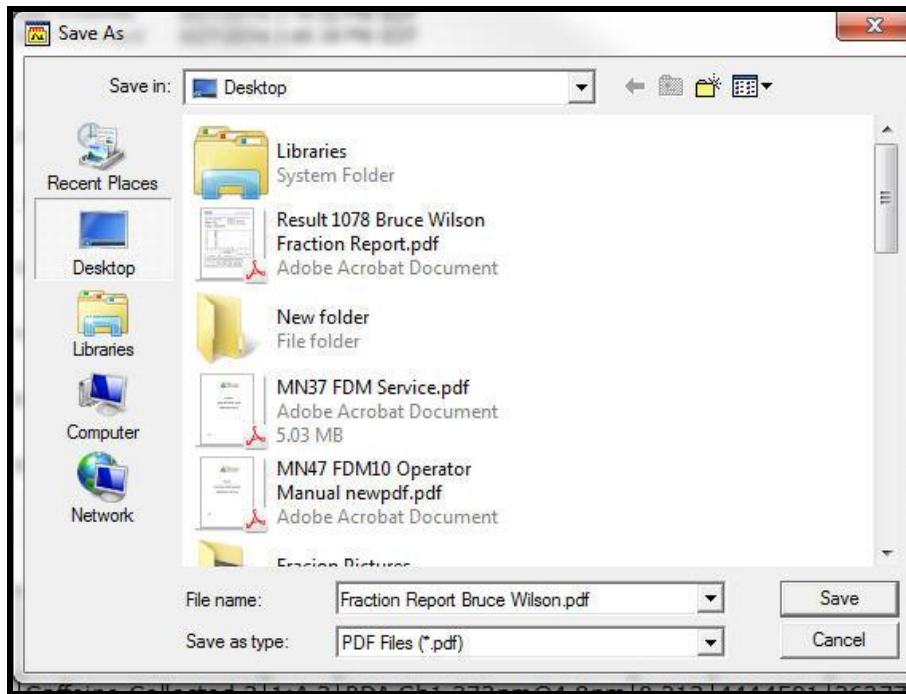


9. Preview the Basic Fraction Report.





10. Click on the "Print" icon or "Save Report" icon in the preview report.



11. You have now completed the basic overview of how to use the "Waters Fraction Manager – Analytical" for retention time based collection.

Note:

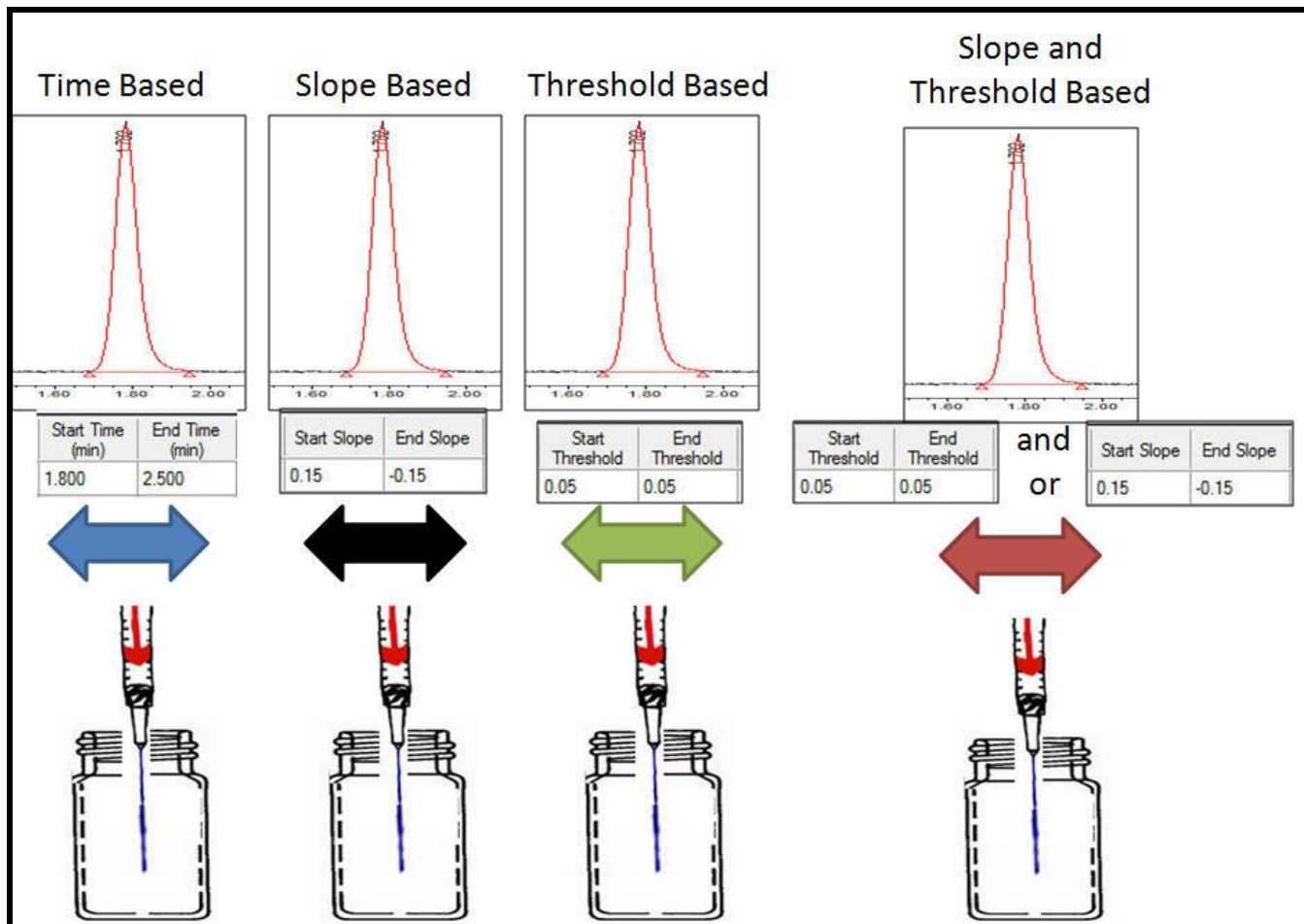
- **Section "I" covers the Fraction Manager - Analytical instrument method in greater detail.**
- **Section "J" covers how to use the collection simulation function of the WFMA instrument method.**
- **Application assistance can be purchased by contacting your local Waters Support Specialist.**

I: Fraction Manager Instrument Method Overview

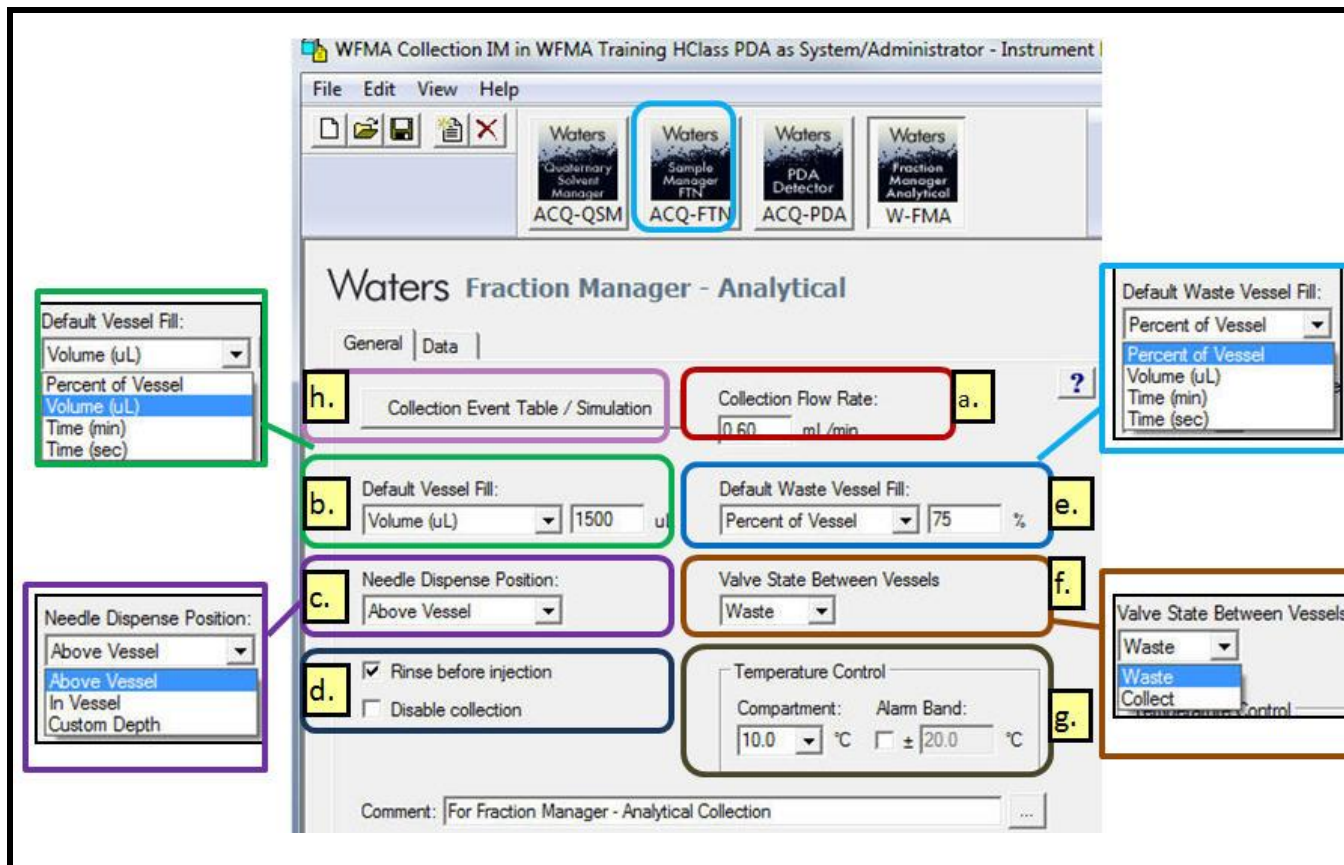
This section provides a more in-depth overview of the "Waters Fraction Manager – Analytical" Instrument Method. Fraction collection simulation will be covered in section J.

1. The WFMA can collect fractions using time (all detectors), or "slope", "threshold", or "slope and threshold" (PDA and TUV detectors only).

Peak Collection by Time	Peak Collection by Slope - Threshold
Manually Enter Time Collections	Manually Enter Slope and Threshold
Fill From Chromatogram Function	Fraction Simulation Function
Fraction Simulation Function	Copy and Paste Slope/Threshold from Simulation
Copy and Paste Time from Simulation	

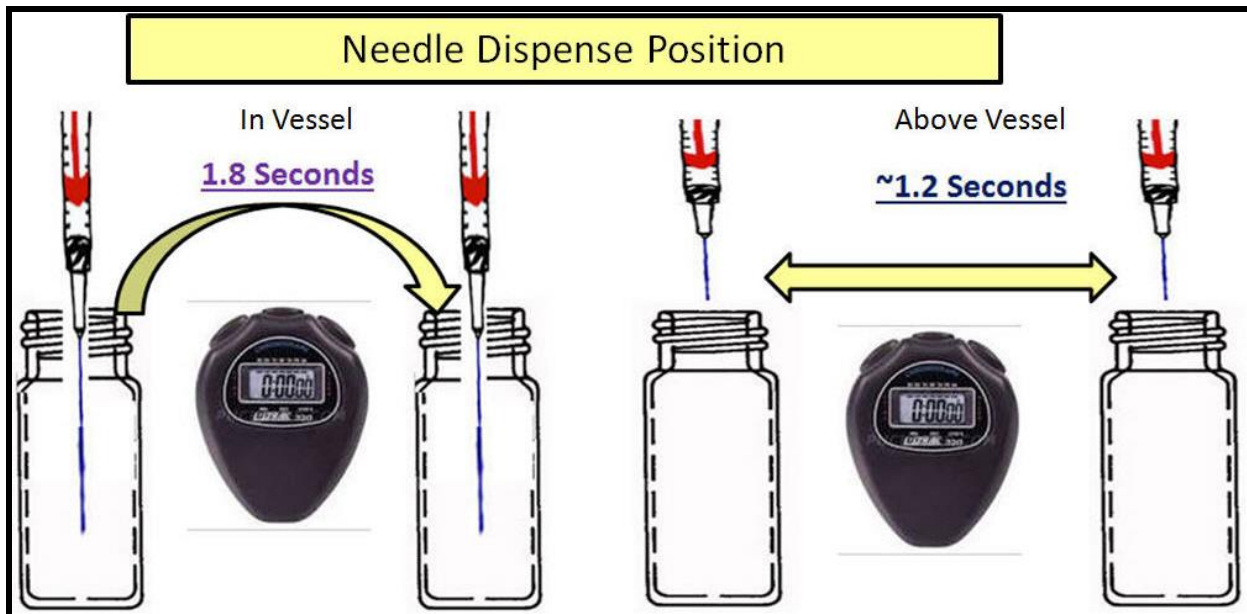


2. Basic Method selections for the Fraction Manager – Analytical are displayed below. A description of each function is discussed below this picture.



- a. Collection Flow Rate: This is the flow rate to the collector from the detector.
- For non-split systems (PDA, TUV) enter the flow rate that is pumped.
 - For split flow systems. The collection flow rate must be calculated by using a “to-contain graduated cylinder” and collect the flow with the splitter in line for 10 minutes.
- b. Default Vessel Fill: Select the maximum volume, percent of vessel or time permitted for each vial or collector well.
- The Default is Percent of Vessel (100%). It is advised to change this to 90%.
 - Optionally use the volume based calculation for the specific vial.
 - Time based values will need calculated based upon flow rate and vessel size.

- c. Needle Dispense Position: Select "In Vessel" to reduce carryover but speed from collect to collect decreases from ~ 1.2 seconds to ~ 1.8 seconds.



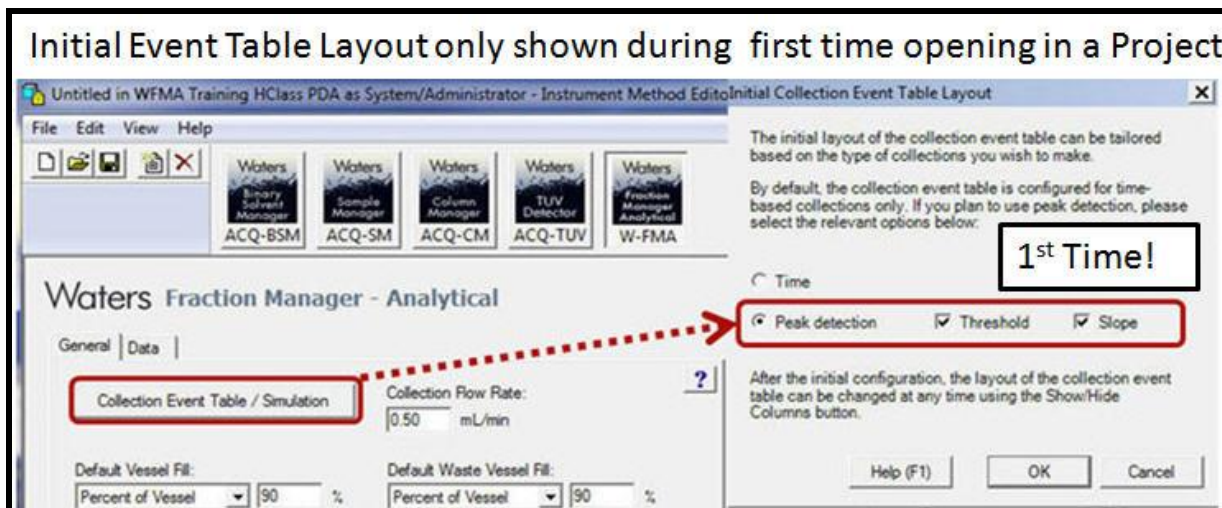
- d. Rinse before injection: Reduces carryover between sample injections.
- e. Default Waste Vessel Fill: Select the maximum volume of waste vessel, percent of waste vessel or time permitted the Waste Vessel. It is recommended to change the waste vessel from default 100% to 75-90% of fill.
- f. Valve State between Vessels: the fraction valve's position when the needle moves between vessels during collection. The fraction valve position during travel between vessels can be closed (Waste position) or open (Collect position).
- Eluent continues to flow regardless of the valve's position.
 - It is advised to use the Waste position for all collection vials except for well plates.
 - For maximum recovery of sample, use well plates, set the valve state to collect, and specify that the needle dispense in the vessel.
- g. Temperature Settings: 4 – 40- degrees.

3. Select the Collect Even Table\Simulation button.

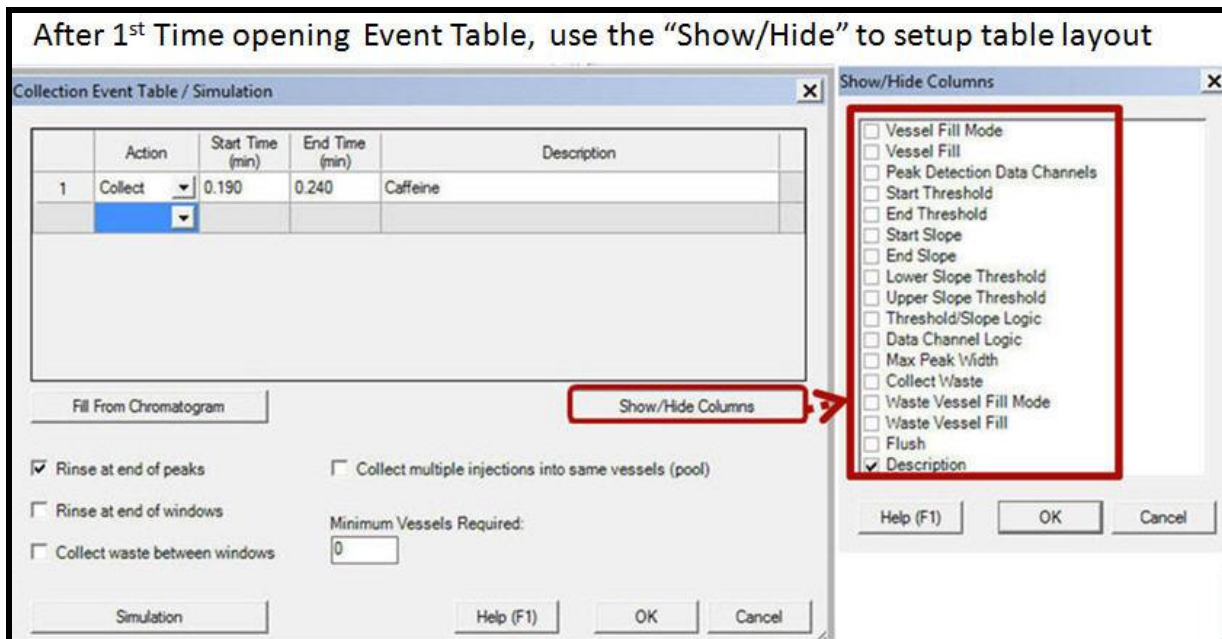


Note: The Collection Event Table layout dialog box will only be displayed upon first time opening the Collection Event Table in a specific project (picture 1).

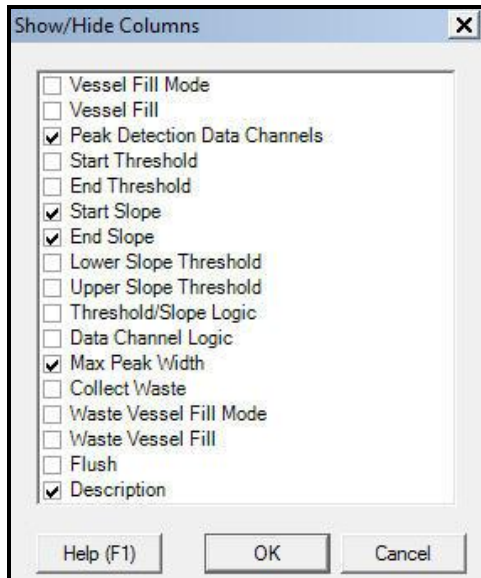
4. If you do not see this dialog box displayed, then select show / hide button below the event table (picture 2). For this training, select the Show Hide button to select specific event columns.



Or when opening the Event Table the 2nd Time:

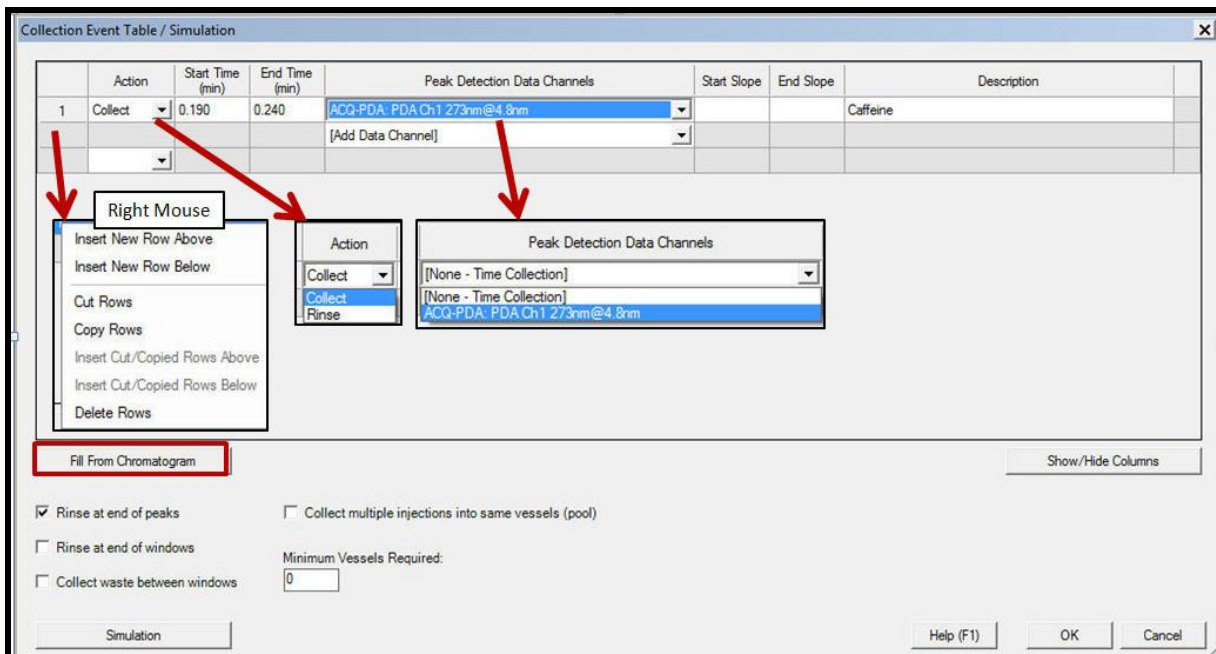


5. For this overview, select "Peak Detection Data Channels", "Start Slope", "End Slope" "Max Peak Width" and "Description" for the columns to be displayed in the Peak Collection table.



6. The Collection Event Table can be filled out by "Fill from Chromatogram" or "Manual" entry. Some of the actions that can be selected in the table are:

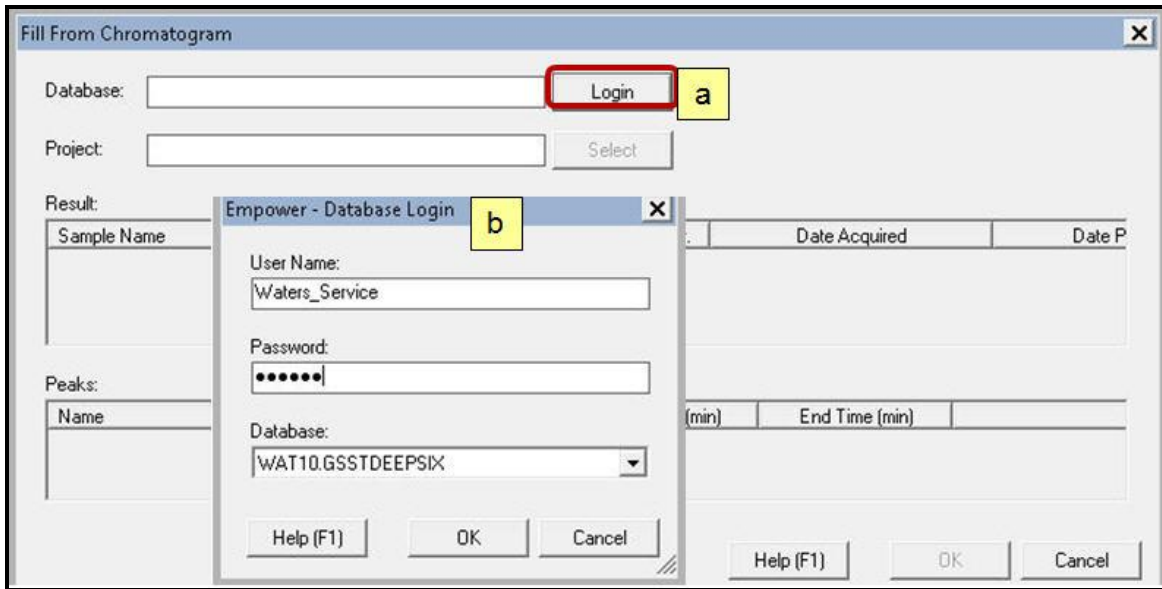
- a. Right click in the "Number" column to Insert, Copy, or Cur Rows.
- a. Collect or Rinse: Rinse will automatically add a 0.1 minute delay time between collections
- c. Peak Detection Data Channels: PDA 2D or TUV channels (QDA, ELSD, cannot be used).



7. The "Fill from Chromatogram" function uses a processed result for time based collection. Below is the sequence of steps to use this function.

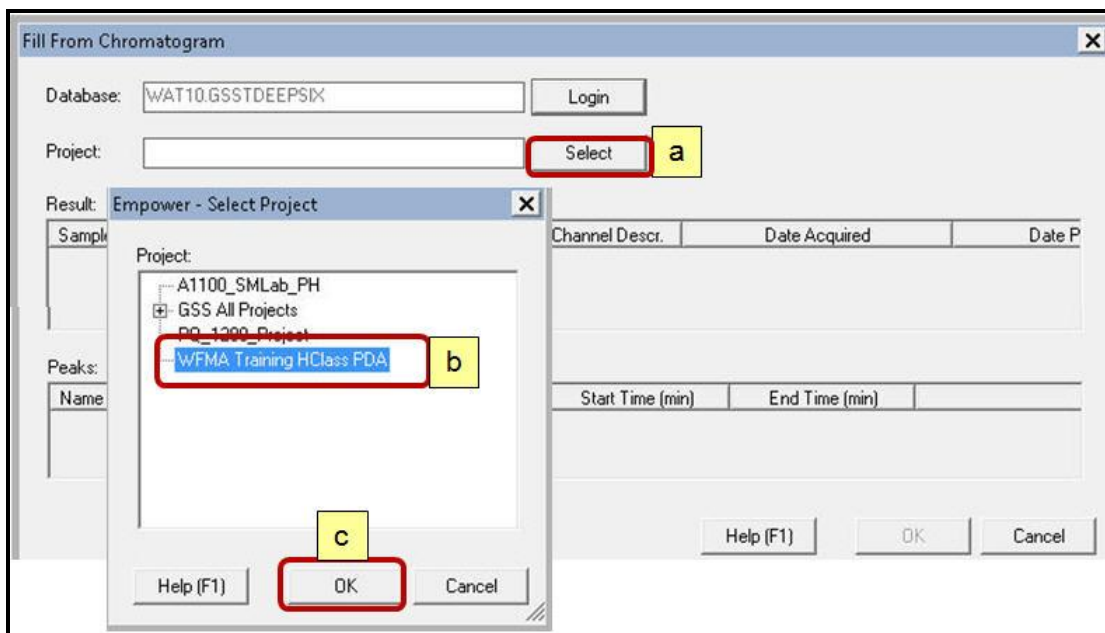


- a. Click the "Fill from Chromatogram" button.
- b. Click the Login button. To utilize this function you must re-log into the Empower database.
- c. Enter in your user name and password. Click "Ok" to Log in.



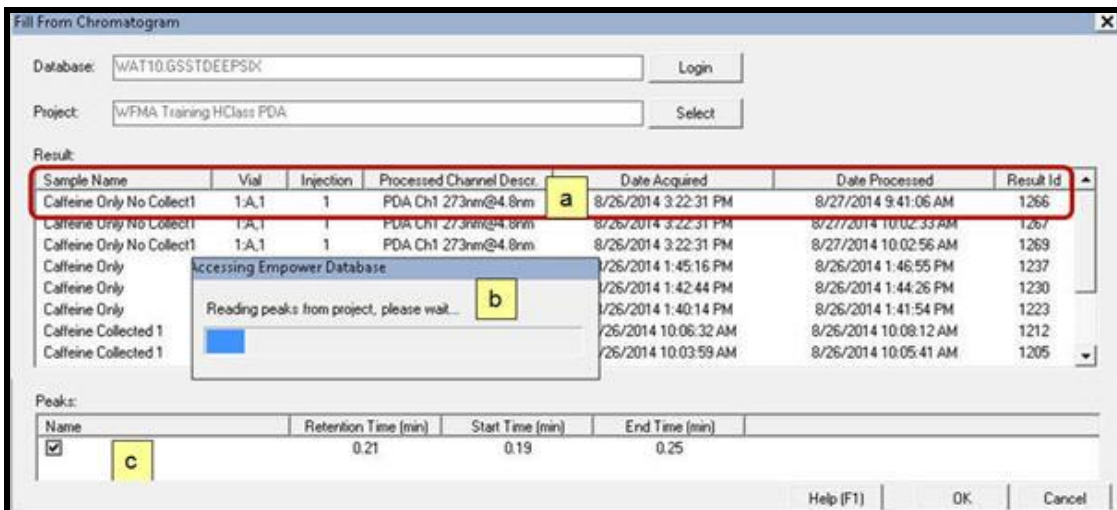
8. Click "Select" to access the Empower project.

9. For this training, select your collection project and select "OK".



10. Fill the peak collection table:

- a. Highlight a processed result from the specified project (from the Projects Results view).
- b. The result must have peak integration for the auto-fill collection table to work.
- c. The Peaks table displays a component name (if quantitated), retention time (apex), and start time and end time. Select Ok and integrated peaks are added to the collection table.

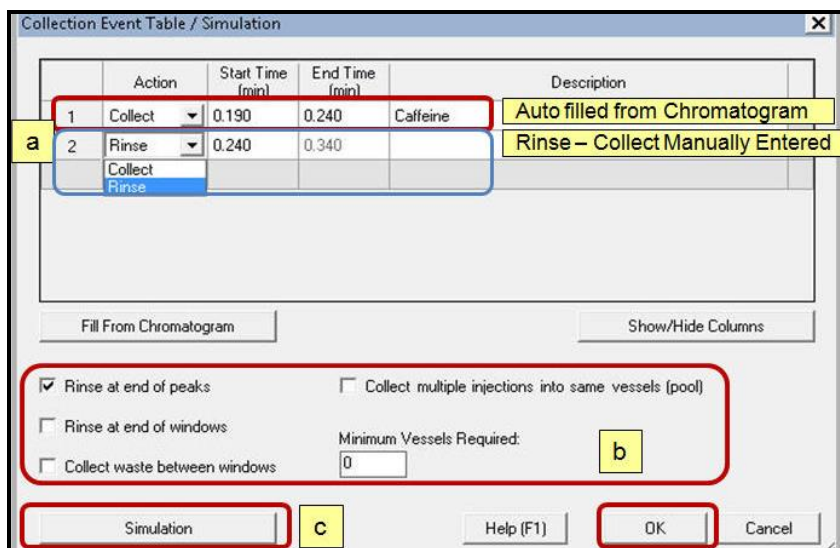


11. The Collection table is filled out from the Peaks table. Select OK.

- a. The event table can be filled out by "Fill from Chromatogram" or manual entry.
- b. Select the Rinse/Waste/Pooling options. Sample pooling (Collected samples into one vessel) only works when selecting multiple injections from the same injection line in the sample set.

Plate/Well	Inj Vol (uL)	# of Injs	SampleName	Function	Method Set / Report Method	Run Time (Minutes)	WFMAParameter
1: A,3	10.0	10	1.0 mg/ml	Inject Standards	WFMA Collection Pooling	3.00	WFMA Sample Pooling

- c. Simulation is for Peak Detection using Slope and Threshold. This is discussed in section "J".

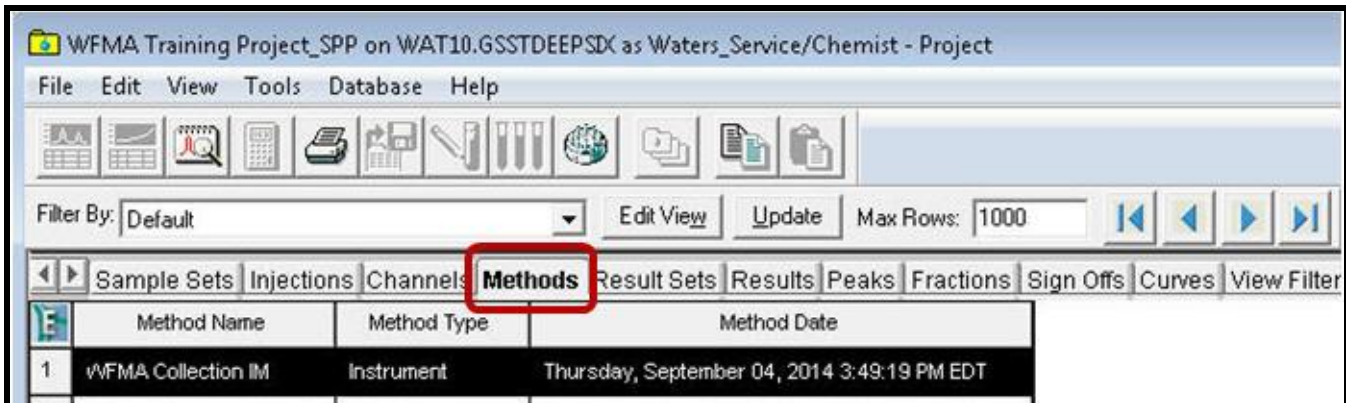


J: Fraction Manager Simulation

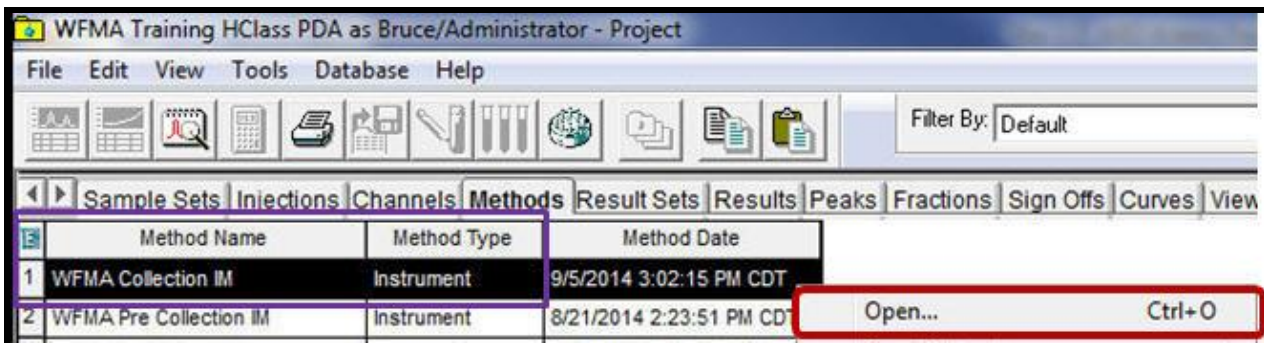
The "Simulation" function uses the Empower Toolkit and a collected chromatographic channel to simulate timed based or peak based collection. Slope, Threshold, or Retention Time values may be copied from the "Simulation" and pasted into the "Collection Table".

Below is the sequence of steps to utilize the "Simulation" function.

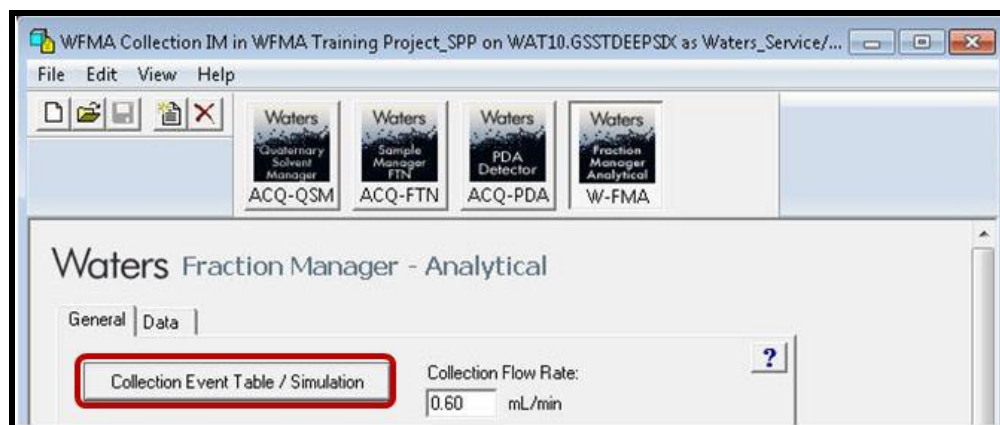
1. Select the an Empower pre-collation Project, then select the "Methods" tab.



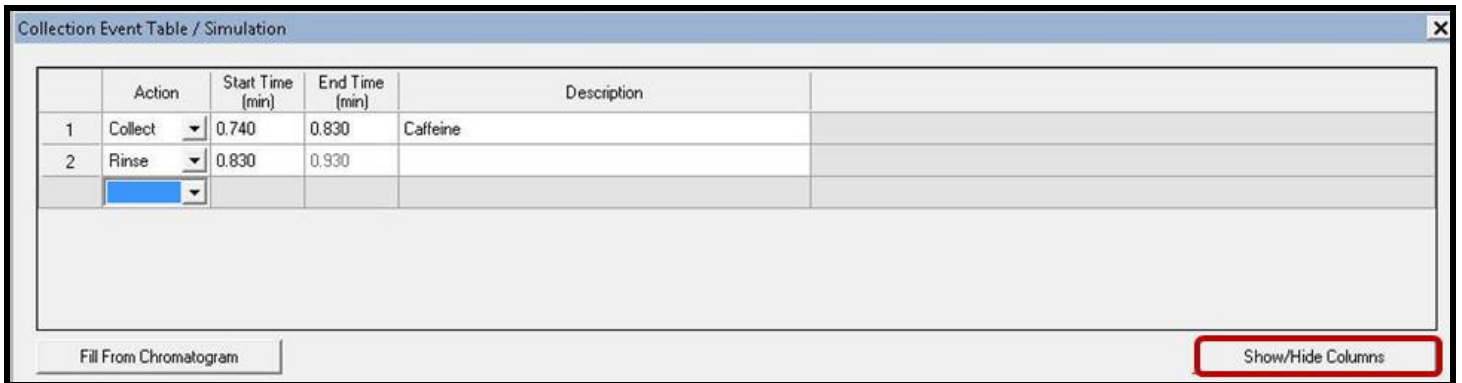
2. Select the "Pre-Collection" Instrument Method and select "Open".



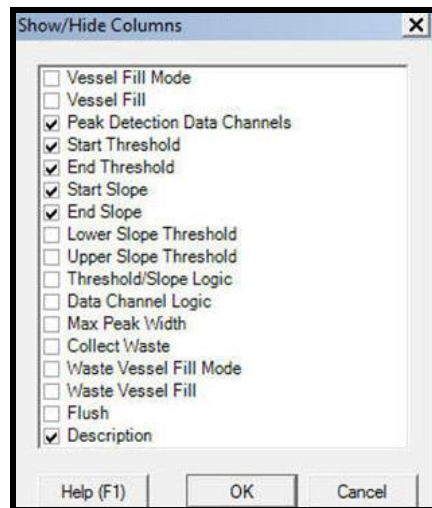
3. In the Waters Fraction Manager – Analytical instrument panel select the "Collection Event Table\Simulation" button.



4. Click the "Show Hide" button.

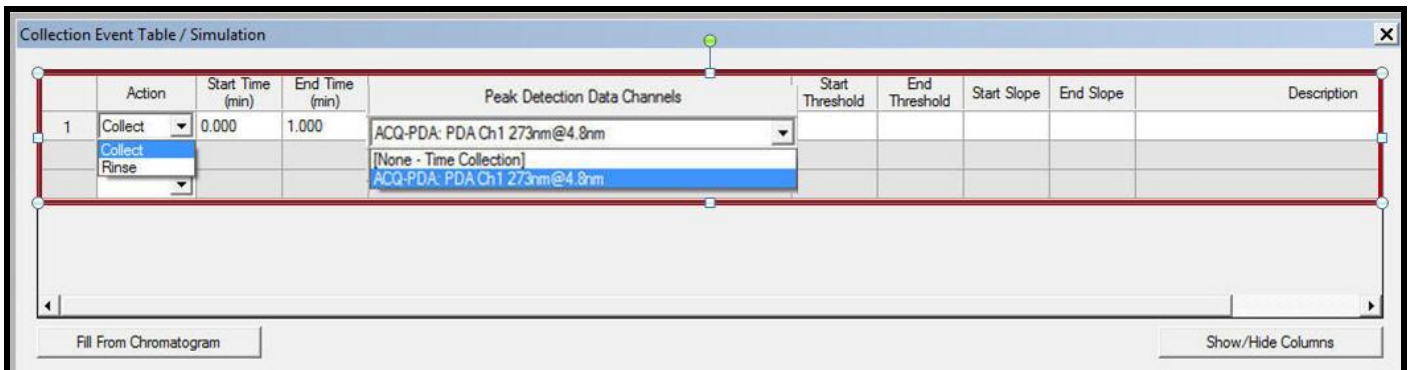


5. In the Show/Hide function make sure to select "Peak Detection Data Channels". Also select "Start Threshold", "End Threshold", "Start Slope", and "End Slope" for display.

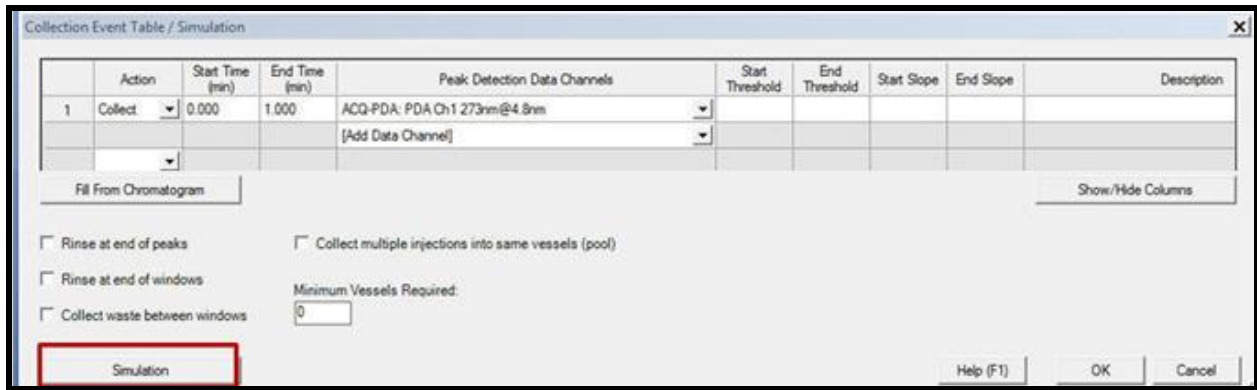


6. Select an Action of "Collect" and select a "Peak Detection Data Channel" from a PDA or TUV channel.

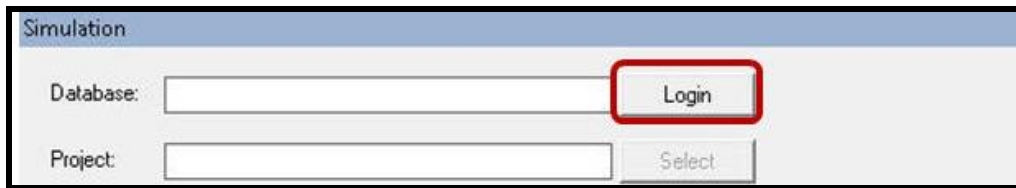
Note: This example uses the "ACQ-PDA: PDA CH1 273nm@4.8nm" wavelength.



7. Click on the Simulation button



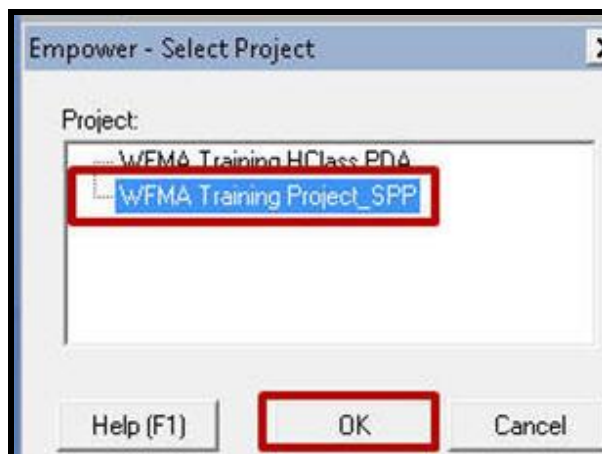
8. Click the Login button. To utilize this function you must re-log into the Empower database.



9. Enter your User Name and password for the Empower Database Login and select OK.

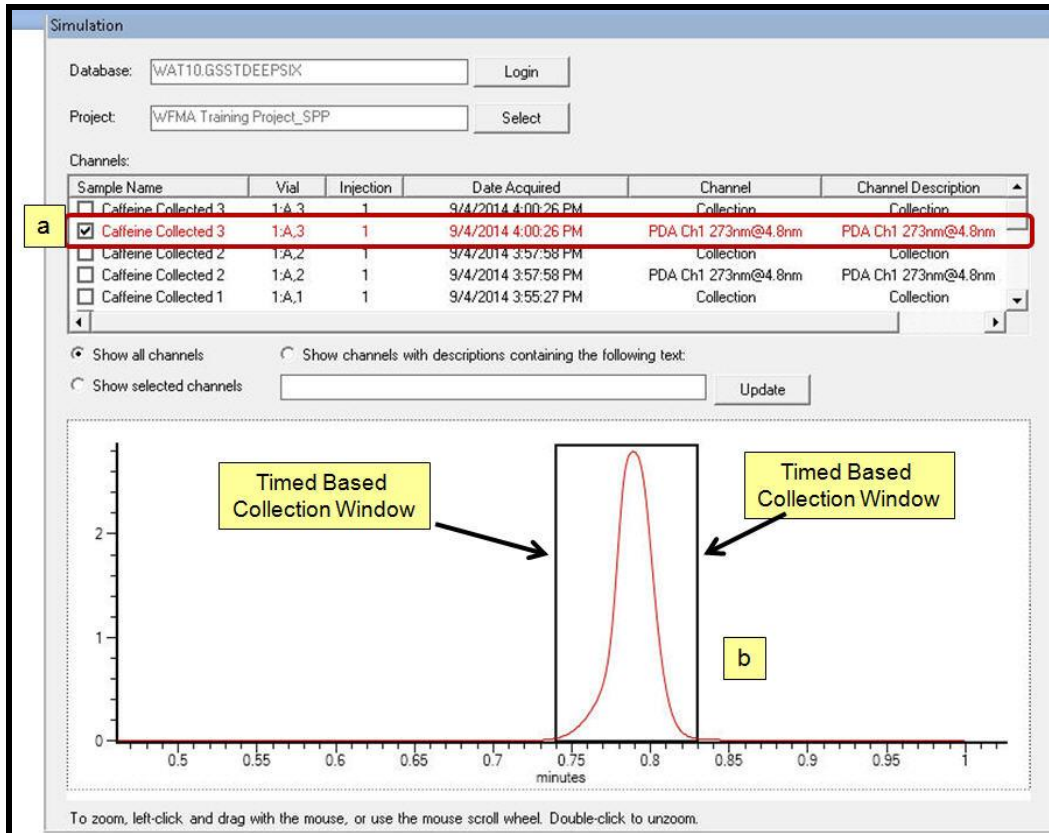


10. Click "Select" to access an Empower project and select OK.

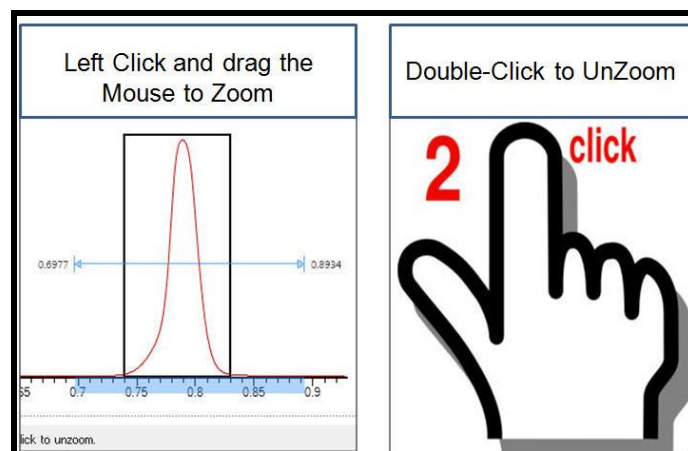


11. The Simulation displays channels for peak selection.
12. Select the desired TUV or PDA channel for use with peak simulation.
13. The chromatogram is displayed with a box around the peak showing the location for time or peak based collection. These boxes are from the "Collection Events Table".

Note: Slope and Threshold based detection can only use 2D PDA or TUV data.



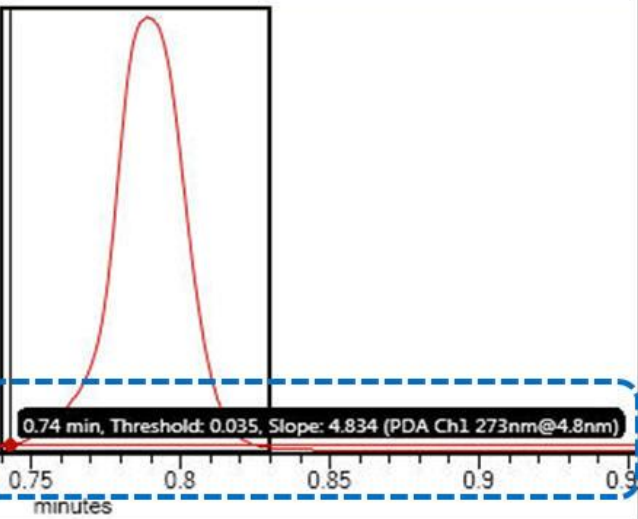
14. To zoom in on the simulation chromatogram, left click and drag across the chromatograms baseline.
15. To unzoom, double mouse click inside the simulation window.



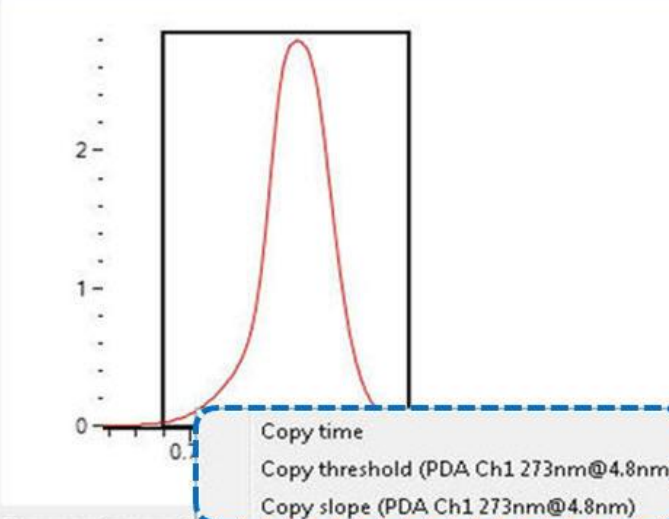
16. Follow the steps as displayed below to copy and paste a Time, Threshold, or Slope value into the Collection Event Table.

Note: You can only copy one peak detection variable at a time into the Collection table.

1. Move the cursor along the peak.
2. Time, Threshold and Slope are displayed for each time point.

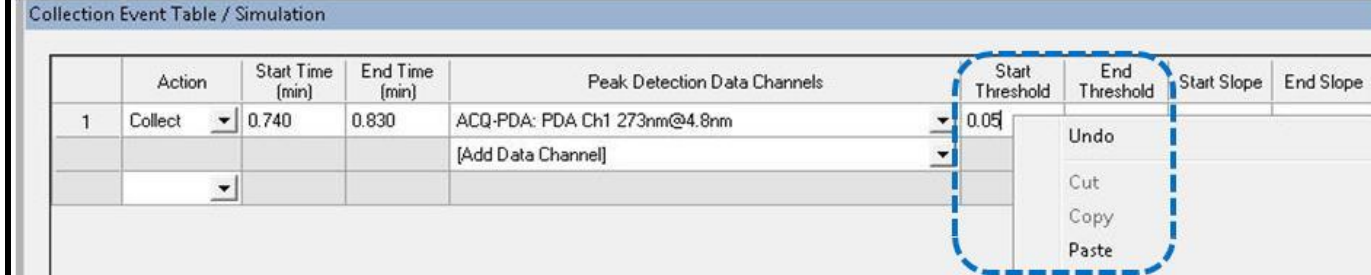


3. Right mouse click and select "Copy time", "Copy threshold" or "Copy slope".



4. Move the mouse to the Collection Event Table's "Start Threshold" column, right mouse click and select Paste.

Collection Event Table / Simulation					
	Action	Start Time (min)	End Time (min)	Peak Detection Data Channels	
1	Collect	0.740	0.830	ACQ-PDA: PDA Ch1 273nm@4.8nm	0.05
				[Add Data Channel]	

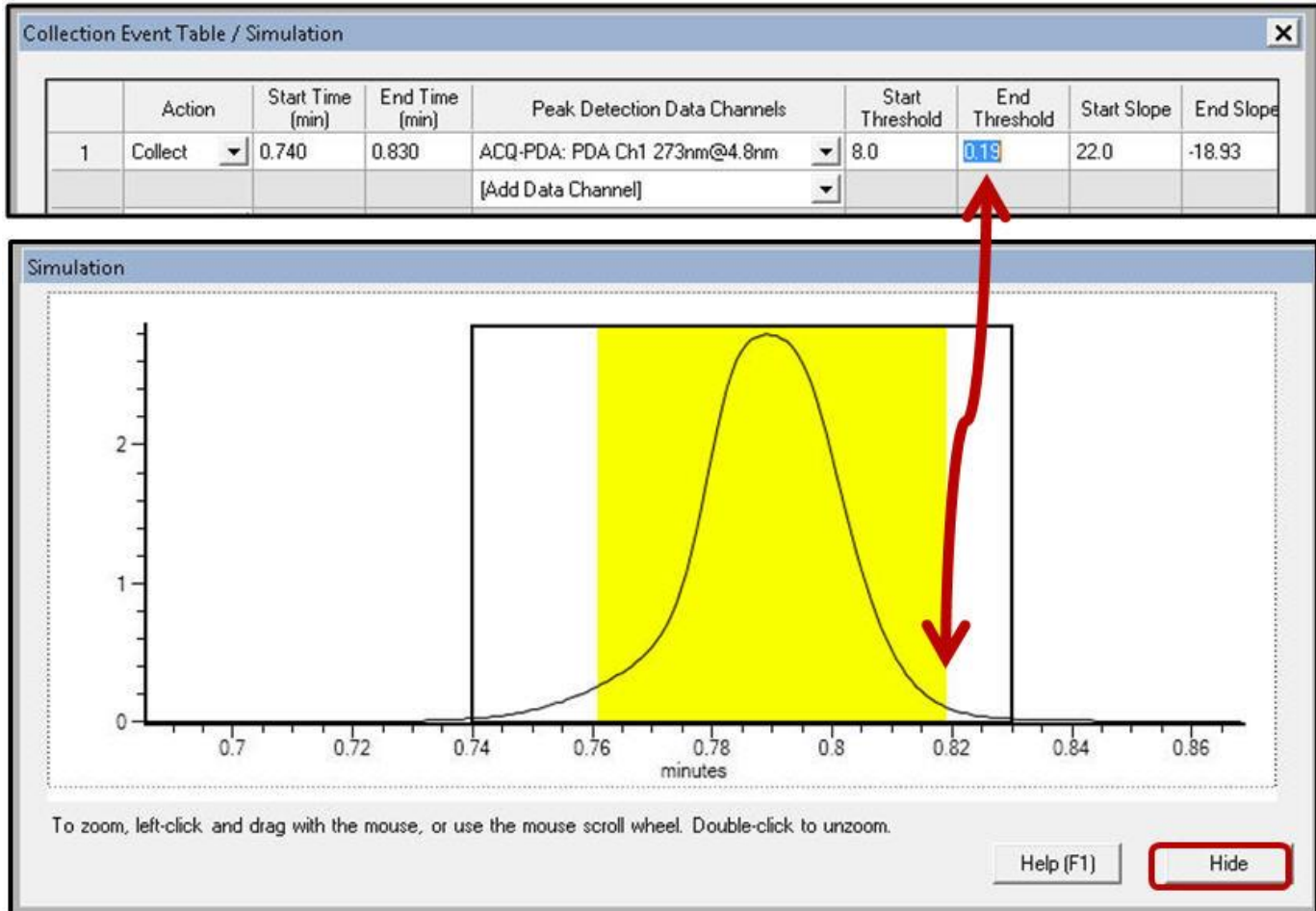


17. Repeat the steps above to copy and paste both Start and End values for Time, Threshold and Slope into the collection channel.

Note: When a "Peak Detection Data Channel" is selected, both Start and End values must be entered into the collection table for threshold and slope.

Collection Event Table / Simulation					
	Action	Start Time (min)	End Time (min)	Peak Detection Data Channels	
1	Collect	0.740	0.830	ACQ-PDA: PDA Ch1 273nm@4.8nm	0.01
				[Add Data Channel]	0.19
					8.32
					-18.93

18. The Simulation window will display the proposed collected peaks region in yellow.
19. The Simulation is interactive with the Collection Event Table. If you manually enter Threshold and Slope values into the table the effects will be displayed in the Simulation window.
20. The Simulation window can be hidden by using the "Hide" button.



21. In the Collection Event Table, select OK to exit out of the Collection\Simulation Function.

Note: If you exit out of the Collection Event window, you will need to re-login to the Empower database and project to get access to the Simulation function.

22. In the Instrument Panel select "File- Save" to save the simulation changes to the Fraction Collection Method.

Notes: If you exit out of the Collection Event table, you will need to re-login to the database and project to access the Simulation function.

You have now completed the basic overview of the "Collection Simulation" function for the Waters Fraction Manager Analytical module.