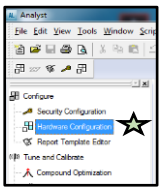
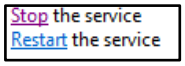
Sciex 6500+ QTrap Operational Steps for Trained Personnel

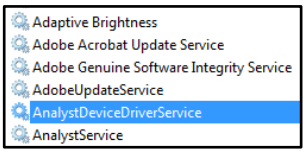
Last Updated 09172017

1. If any of the following instructions does not make sense to you or was not covered during your hand-on training, stop immediately and inform IMSERC staff
2. If Analyst is running, double left click on the Hardware Configuration, deactivate the hardware profile, then exit the program, and click on yes to all save changes requests



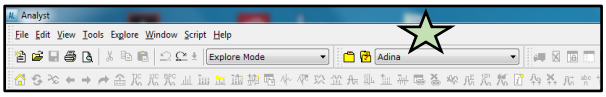
3. Run Services (double left click on the icon on the desktop)
    a. Highlight AnalystDeviceDriveService and then click on Stop the service
    b. Now click on AnalystService and then click on Stop the service



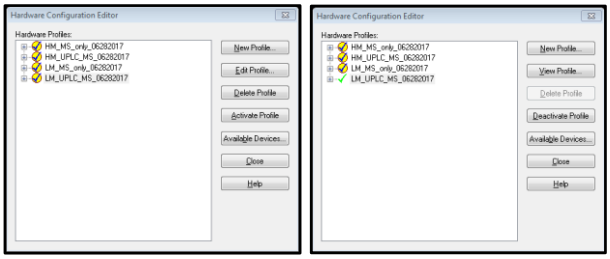


4. Install your column (ensure you have capped both ends of the one you remove)
5. Check mobile phase volumes for all the lines to ensure that they are above 40% fill level and that you have enough for your entire run set
6. You may have to coordinate with IMSERC staff to refill the bottles in the beginning or the middle of your run
7. Double Click on Analyst icon on the desk top

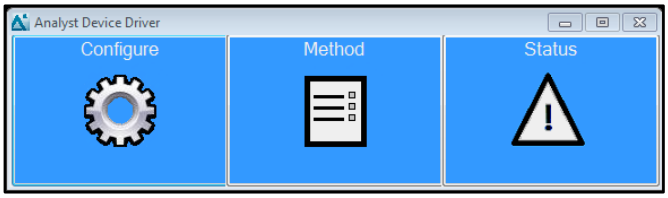
8. Select your project in Analyst
    a. Your project will be set up for you during your hands-on training.



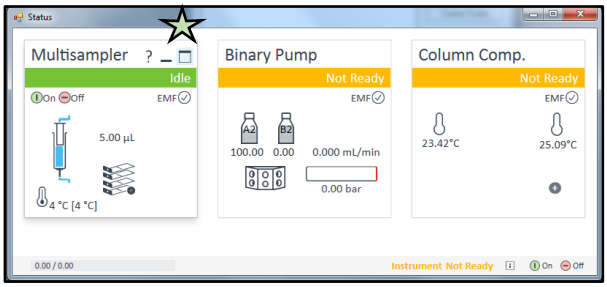
9. Activate the Hardware Profile that corresponds to your method
    a. The green arrow indicates the profile was activated properly
    b. The divert valve on the MS goes to A (source) position, hold down the button under the LED light for a split second to switch it back to B (waste)



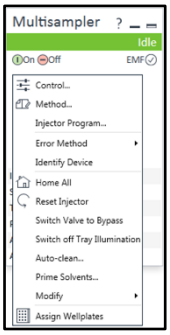
10. Click on AD icon on the bottom menu bar (not the one on the Analyst menu) to bring up ADD window
    a. Click on Status

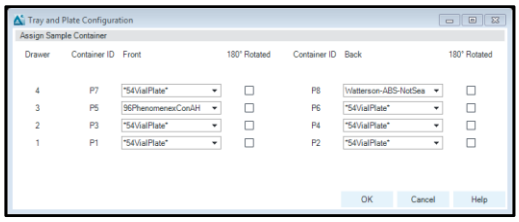


11. You should see Multisampler in green and Binary Pump and Column Compartment in brown color banners
12. Hover the cursor over each box and click on the small resize icon to maximize the modules information panels

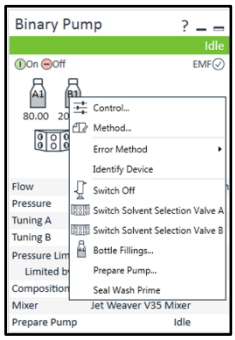


13. Do the following for each module
    a. Multisampler
        i. Auto-Clean; this primes the needle wash port. You should see wash solvent bubble out of the wash port
        ii. Prime Solvents; check to see that solvent drips out of each line, a and b
        iii. Assign Wellplates to all locations that you will be using (only approved plates that have a valid plate map programmed into the software by IMSERC staff can be used; if you cannot pick a map from the menu, then you are not allowed to use the plate)
        iv. Factory default maps have a star at each end of their names
        v. Those programmed by IMSERC staff have no stars
        vi. If you are not completely certain that you are using the right plate name, stop and ask IMSERC staff for help.
        vii. Wrong plate name can lead to damaging the inject needle assembly and you will be responsible for repair cost and downtime.

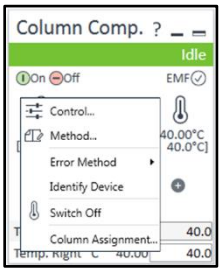




    b. Binary Pump
        i. Check and if needed switch solvent selection valve A and B
        ii. Prepare Pump
        iii. Seal Wash Prime

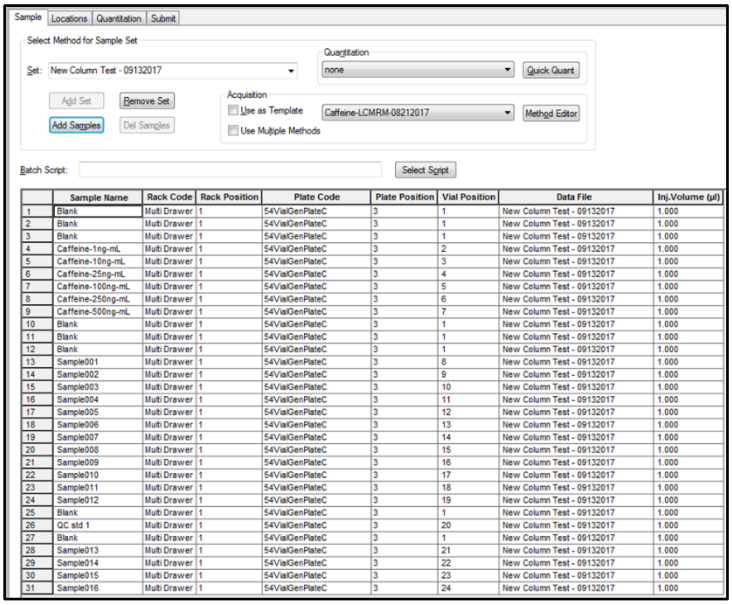


    c. Column Compartment
        i. You can change the temperature set point if it is significantly different that the temperature requested in your method.
        ii. You can change it manually by a right click on the method item and entering the new value



14. If you have not already, ensure that you are in your project folder

15. Create a new or open an existing acquisition batch
    a. Enter a title for the SET.
        i. This will be the name of your *.wiff file that will keep the data generated with every injection. Individual injections do not get their own data file name as is typical of other platforms, but instead, they are kept in a *.wiff file.
        ii. Click on Add Set.
        iii. A batch file can have more than one set. Each set could be a different variable that you are studying. For example, we could have a set called new column and another called old column, in a hypothetical scenario where were comparing two columns with the same set of samples
    b. Select the method you want to use from the drop down menu
        i. During your training, a method will be written for your project
        ii. You will learn how to make minor changes to your method and save it with your name attached
        iii. The steps required for running a method will be part of a more extensive training
        iv. IMSERC staff will work with your on an ongoing basis to deliver the advanced training and it may require more than one session



	Sample Name	Rack Code	Rack Position	Plate Code	Plate Position	Vial Position	Data File	Inj.Volume (µl)
1	Blank	Multi Drawer	1	54VialGenPlateC	3	1	New Column Test - 09132017	1.000
2	Blank	Multi Drawer	1	54VialGenPlateC	3	1	New Column Test - 09132017	1.000
3	Blank	Multi Drawer	1	54VialGenPlateC	3	1	New Column Test - 09132017	1.000
4	Caffeine-1ng-mL	Multi Drawer	1	54VialGenPlateC	3	2	New Column Test - 09132017	1.000
5	Caffeine-10ng-mL	Multi Drawer	1	54VialGenPlateC	3	3	New Column Test - 09132017	1.000
6	Caffeine-25ng-mL	Multi Drawer	1	54VialGenPlateC	3	4	New Column Test - 09132017	1.000
7	Caffeine-100ng-mL	Multi Drawer	1	54VialGenPlateC	3	5	New Column Test - 09132017	1.000
8	Caffeine-250ng-mL	Multi Drawer	1	54VialGenPlateC	3	6	New Column Test - 09132017	1.000
9	Caffeine-500ng-mL	Multi Drawer	1	54VialGenPlateC	3	7	New Column Test - 09132017	1.000
10	Blank	Multi Drawer	1	54VialGenPlateC	3	1	New Column Test - 09132017	1.000
11	Blank	Multi Drawer	1	54VialGenPlateC	3	1	New Column Test - 09132017	1.000
12	Blank	Multi Drawer	1	54VialGenPlateC	3	1	New Column Test - 09132017	1.000
13	Sample001	Multi Drawer	1	54VialGenPlateC	3	8	New Column Test - 09132017	1.000
14	Sample002	Multi Drawer	1	54VialGenPlateC	3	9	New Column Test - 09132017	1.000
15	Sample003	Multi Drawer	1	54VialGenPlateC	3	10	New Column Test - 09132017	1.000
16	Sample004	Multi Drawer	1	54VialGenPlateC	3	11	New Column Test - 09132017	1.000
17	Sample005	Multi Drawer	1	54VialGenPlateC	3	12	New Column Test - 09132017	1.000
18	Sample006	Multi Drawer	1	54VialGenPlateC	3	13	New Column Test - 09132017	1.000
19	Sample007	Multi Drawer	1	54VialGenPlateC	3	14	New Column Test - 09132017	1.000
20	Sample008	Multi Drawer	1	54VialGenPlateC	3	15	New Column Test - 09132017	1.000
21	Sample009	Multi Drawer	1	54VialGenPlateC	3	16	New Column Test - 09132017	1.000
22	Sample010	Multi Drawer	1	54VialGenPlateC	3	17	New Column Test - 09132017	1.000
23	Sample011	Multi Drawer	1	54VialGenPlateC	3	18	New Column Test - 09132017	1.000
24	Sample012	Multi Drawer	1	54VialGenPlateC	3	19	New Column Test - 09132017	1.000
25	Blank	Multi Drawer	1	54VialGenPlateC	3	1	New Column Test - 09132017	1.000
26	QC std 1	Multi Drawer	1	54VialGenPlateC	3	20	New Column Test - 09132017	1.000
27	Blank	Multi Drawer	1	54VialGenPlateC	3	1	New Column Test - 09132017	1.000
28	Sample013	Multi Drawer	1	54VialGenPlateC	3	21	New Column Test - 09132017	1.000
29	Sample014	Multi Drawer	1	54VialGenPlateC	3	22	New Column Test - 09132017	1.000
30	Sample015	Multi Drawer	1	54VialGenPlateC	3	23	New Column Test - 09132017	1.000
31	Sample016	Multi Drawer	1	54VialGenPlateC	3	24	New Column Test - 09132017	1.000

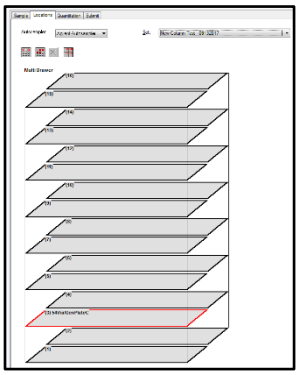
    c. Click on Add Samples and if you don't know how many samples, just enter 12 to get started
        i. Sample Name
            1. Fill the sample name column
            2. You must run three blanks to get the UPLC column conditioned
            3. Fill the rest of the column with the sequence of samples you want to run
            4. A typical run list is blanks, calibration curve, blanks, 12 samples, blank, known standard, blank, 12 samples, etc.
        ii. Rack Code
            1. MultiDrawer
        iii. Rack Position
            1. Always 1
        iv. Plate code
            1. Click in the cell and from the drop down menu, select *54VialGenPlateC for standard 2 mL LC vials
            2. Click in the cell and from the drop down menu, select *96VialGenPlateC for 96 wellplate (shallow or deep)
            3. Note that specific plate maps are assigned when preparing the Multisampler (section 13a.iii)
        v. Plate Position
            1. From the drop down menu in each cell, select number 1 through 8 (since we have installed full height drawers) where the samples have been loaded
        vi. Vial Position

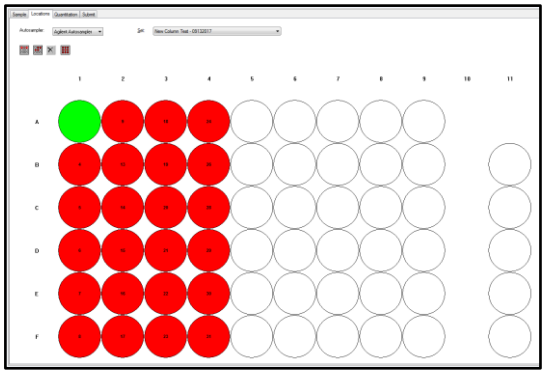
1. Numbers will be 1 through 54 or 1 through 96, depending on the actual plates being used
2. You will work with multiples of 6 for 54 vial plate and multiples of 8 for the 96 well wellplates
3. For 54 vial plates, vials 1 through 6 are located at A1, B1, through F1, and then number seven is A2 location, etc.
4. For 96 well wellplate, positions 1 through 8 are at A1, B1, through H1, and then number 9 is at A2 location
5. After you have loaded the samples, you install the plate by rotating if 90 degrees counter clockwise so that the A1 position is now at front-left

vii. Data File
1. This column should be populated with the Set name
2. You can override it by simply typing a different title in each cell

viii. Inj. Volume (uL)
1. This value is imported automatically from the method you selected.
2. You can override it by typing a different number
3. You must make manual note of the volume change since the data file information, after the data is collected, will only show you the volume that was entered in the selected method and not the override value

d. Click on Locations tab
i. Click on the tray where the samples are loaded, example shown is position 3.
ii. Plate position numbering starts at the bottom front for 1, bottom back for 2, one level higher and front is 3, and so on …

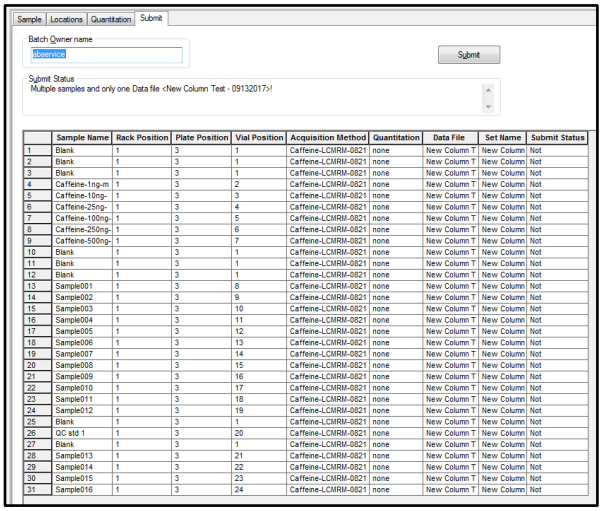




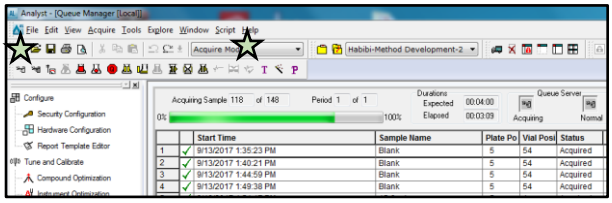
iii. At the top left corner, click on the forth icon, the square filled with red dots
iv. You will now see the location of each vial with a map of the plate
1. Red circles will have the acquisition row number in the middle
2. Green circles means you are injecting from that vial location more than once
3. Hover the curser on one of the circles and vial number will be revealed

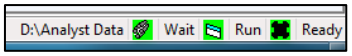
16. Once you finished filling out batch file sample tab, save it by clicking on File on the top menu, and then click on save as.
a. Your naming convention should incluce Your Last Name – PI Last Name – Date
b. You can have this name applied to other files as long as file extensions are different
17. If the compounds that are being analyzed have a quantitation method already (see below), then you can assign a quantitation method and fill out the quantitation tab

18. Click on submit tab
    a. If any errors are found, they will show in the submit status box
    b. For example, if you use multiple Rack Codes, you will have to correct it in the Sample tab before you can submit samples
    c. Once all errors are addressed, you can highlight each line in any order and submit, i.e. to be added to the queue
    d. Analyst allows you to continuously submit samples
    e. If you submit samples that you may not want to run, go to the queue tab and highlight and delete the submission(s)



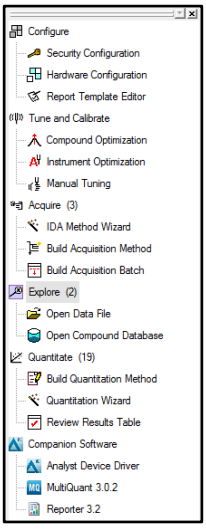
19. Click on queue
    a. View queue icon is on the top left
    b. All of the submitted samples should be in a table with the status Waiting
    c. Ready & Equilibrate
        i. Click on the icon with a red check mark, to the right of the horizontal hourglass, to get the instrument ready; all three hardware modules should be green
        ii. Click on the icon without a check mark, to the left of the horizontal hourglass, to equilibrate the column.
        iii. If you had turned on all of the modules through the ADD, and the mobile phase for the initial condition had been running for more than 5 minutes, you can skip the equilibrate step

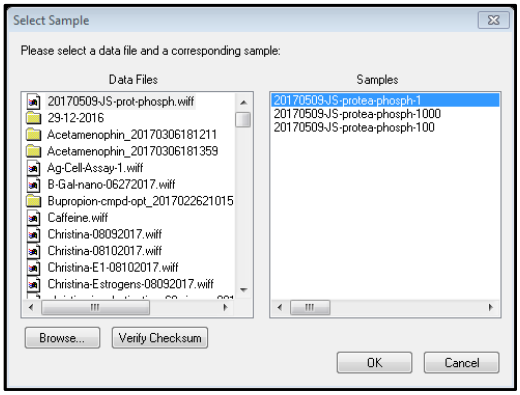


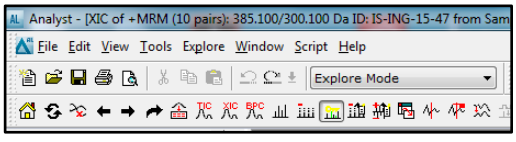


20. Click on Start Sample
    a. Hover the mouse over the first Erlenmeyer flask from the left
    b. When all conditions are satisfied and the system is ready to inject the first sample, the Start Sample Erlenmeyer flask will have a solid black color, otherwise it will be gray

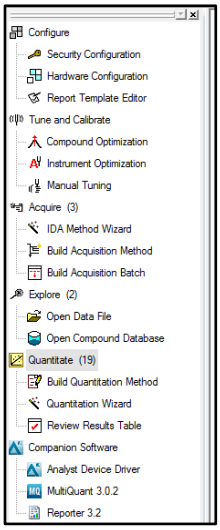
21. Viewing Data
    a. Click on Explore
    b. Double Click on Open Data File
    c. Select Sample window opens
    d. Select the *.wiff file in the Data Files box that corresponds to your data
    e. Click on a sample in the Samples box
    f. The data corresponding to that injection is now displayed
    g. If you want to navigate to other data, use the black arrows
    h. Right clicking on the experimental title will open up the scan types, the assigned names and the transition table, in the case of MRM experiments

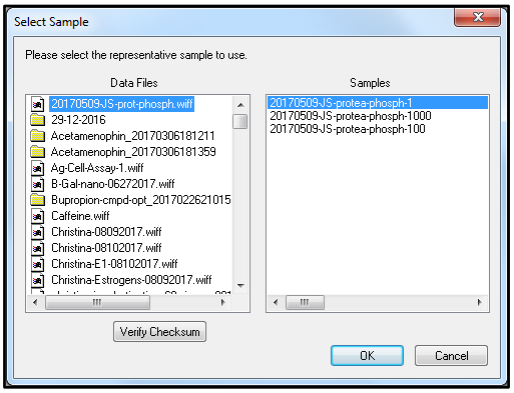


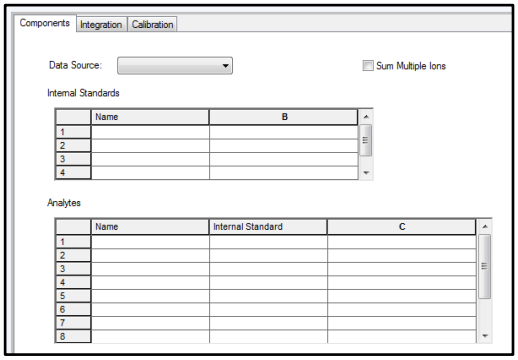


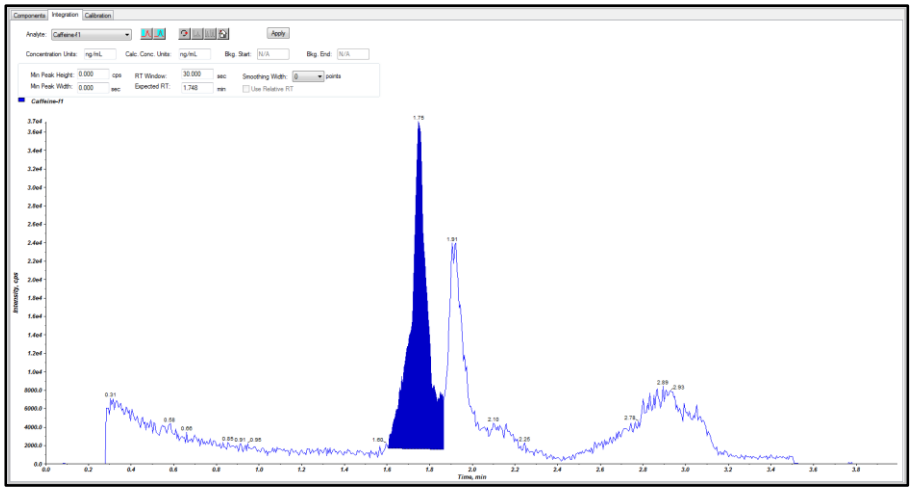


22. Quantitate
    a. Click on the quantitate segment
    b. Build Quantitation Method
        i. Double click on Build Quantitation Method
        ii. In the Select Sample window, select the *.wiff file that contains the representative sample data
        iii. Select a representative sample, one that you are sure the detector is not saturated or the S/N is not acceptable – for example a standard with a concentration in the middle of the linear dynamic range
        iv. Click on Okay
        v. Now in the Components tab, assign the transitions to the Internal Standards and the Analytes tables
        vi. You can only use values available in the drop down menus, as you click on each cell
        vii. Don't forget to remove the internal standard transition from the Analytes table and fill in the Internal Standard column in the Analytes table
        viii. Click on the Integration tab
        ix. Inspect the peak detected
        x. You can change the peak finding criteria (RT window and expected RT, for example) if the peak selected is not the desired peak
        xi. To include peak tailing/fronting, change the peak width value
        xii. You can also change the units of the concentration values
        xiii. Click on Calibration tab







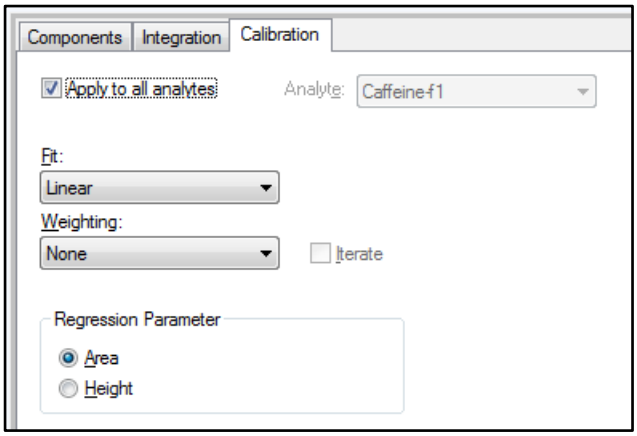


xiv. Typically, the fit is linear and no weighting is use and the conditions applied to all analytes

xv. While inspecting the results table, any changes to these conditions and/or excluding data points from the standard curve can be tested

xvi. Now save the quantitation method by clicking on file and perform a save as operation

xvii. The saved file will have a *.qmf extension



c. Quantitation Wizard

i. Double click on quantitation wizard

ii. Select a *.wiff file and highlight all of the samples that you want to include in the quantitation processing and click on the right arrow to transfer them to the Selected Samples box

iii. Then click on next twice

iv. Select the method you wrote for this set from the drop down menu

v. Click Finish

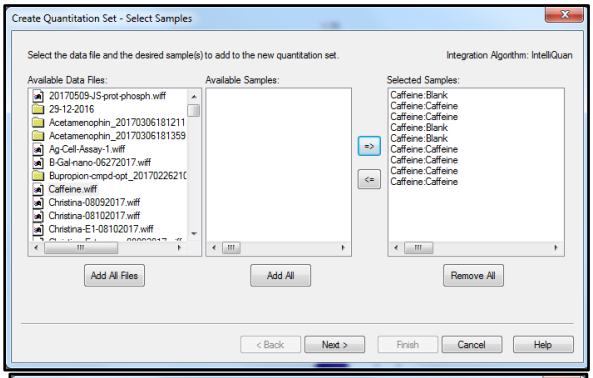
vi. A results table will now be shown

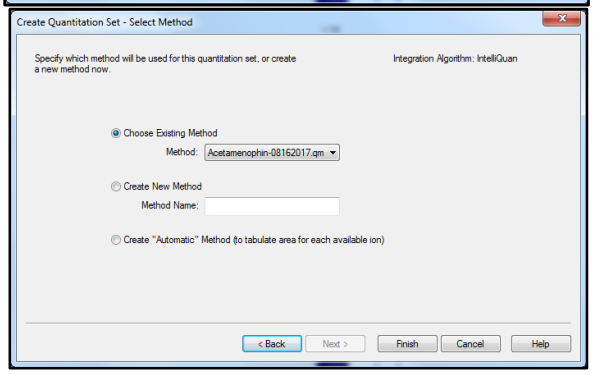
vii. Save this table

viii. Click on the calibration pane to see how good the calibration fit is

ix. During the training you will learn how to exclude points and discuss acceptance criteria

x. You can also open and view other saved results table by clicking on Review Results Table





23. Reporting Data
    a. Click on Companion Software
    b. Double click on the Reporter 3.2
    c. Try different report formats and save the report as a word or pdf file