

Waters UPC ² System operation with Empower 3.1	
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**ACQUITY UltraPerformance
Convergence Technology
UPC²™ Operation with Empower 3**



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A: Objective

Objective: This is a basic introduction to daily startup, acquisition and system diagnostics for the ACQUITY UPC² PDA data using Empower 3.0 with Feature Release 1 software.

B. Supplies and Sample Setup

The following will be needed for the training. This assumes the UPC² System has been started up by your Waters Field Service Engineer and is in good operating condition.

1. UPC² BEH Column 3x100mm p/n186006564
2. Empower 3 UPC² Startup project
4. PDA or ELSD Detector
5. Methanol – Co-Solvent B1 location
6. Weak/Strong/Needle Wash – Methanol
7. 2ml Vials for ANSI 48 vial rack
8. Heptane and Isopropanol -for Sample Dilution
9. UPC² UV Gradient Test Mix p/n 186006551 - Sample Setup:
 - a. Stock: 1mg/mL Coumarin, 1 mg/mL Flavone, 2 mg/mL Caffeine, 1 mg/mL Thymine and 2 mg/mL Prednisone in 88%/12% 2-Propanol /Water.
 - b. Pipette 1mL of Stock solution into a 10 mL volumetric flask, dilute to volume with 80%/20% Heptane/2-Propanol and mix thoroughly.
 - c. 2uL Injection Volume
 - d. Instrument method and sample conditions will be noted in this guide as well as included in an "Instrument Method" from the training project.

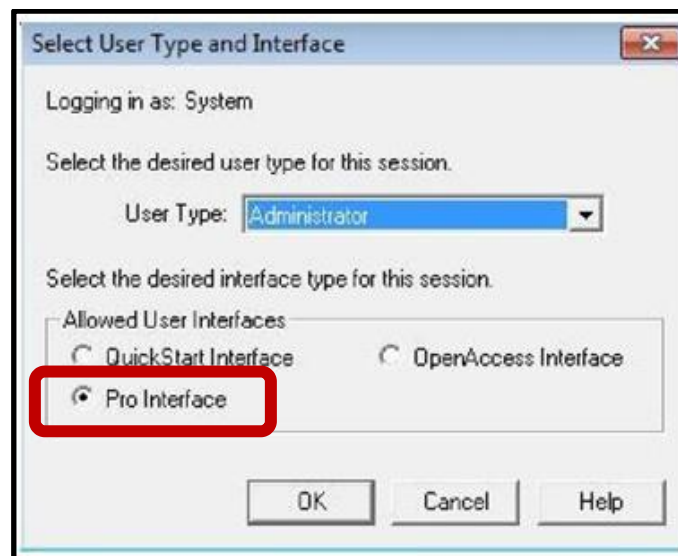
C: Empower 3 Login



1. Double click on the Empower **icon**. Enter in the user name as **System**, and password as **Manager** (the user name and password may be different if setup by your Empower System Administrator). Click on the **Advanced** tab.



2. In the User Type and Interface, select the User Type as Administrator (or other type), and the desired Interface to the **Pro** Interface.

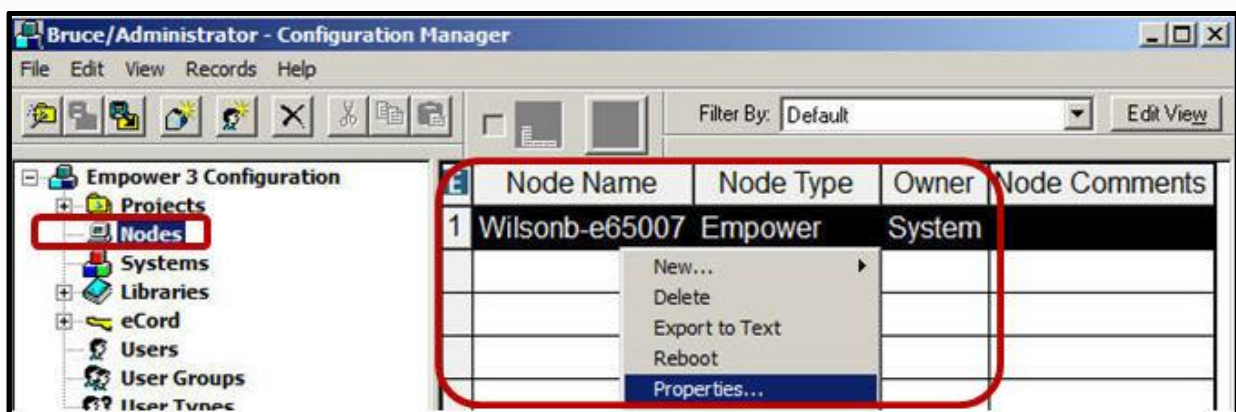


3. The Empower 3 Menu Icons are displayed below. Basic icon descriptions:

- Run Samples – for acquiring UPC² Data and viewing UPC² system diagnostics using the ACQUITY console.
- Browse Project - for viewing acquired data, processing Data, Reviewing data and Reporting data (all functions within a Project).
- Configure System –for the Empower management and configuration. Used to create users, systems and backup/restore projects.
- Process Data, Review Data and Print Data icons are for specific actions within a selected project.

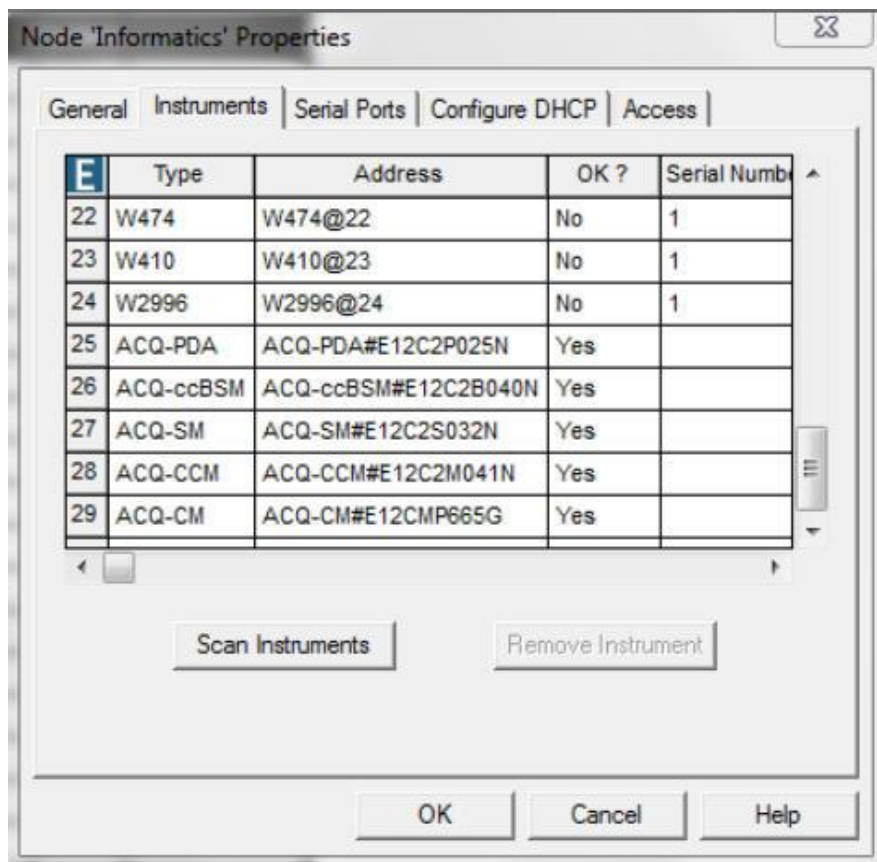


- For configuration and setup of the UPC² system, select the "Configure System" icon. This needs to be configured once for system configuration, although this will be used periodically for Empower management configuration and project management.
- From the Configuration Manager menu select the Node, and then right mouse click on the Node name to select "Properties".

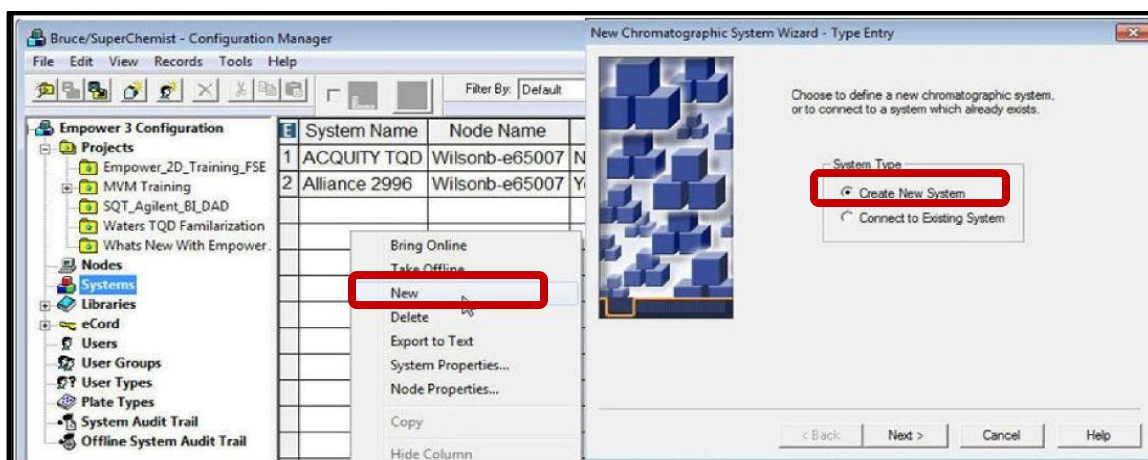


- Click the Instruments tab of the "Node Properties". Confirm that each of the modules listed in the OK column say yes.

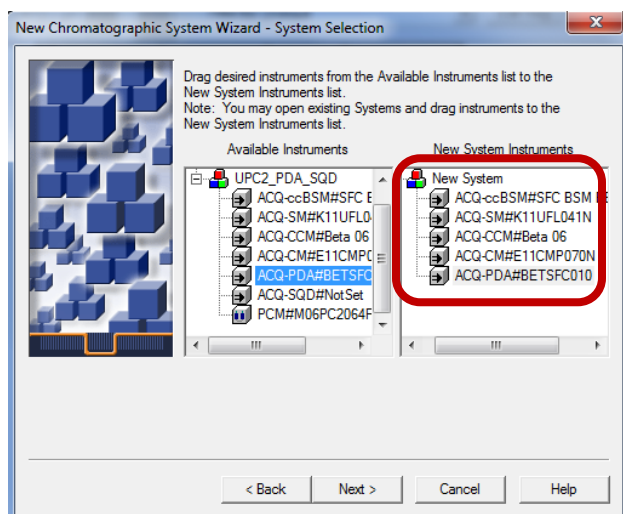
Note: If the modules say “No”, check that each module is powered on and all Ethernet cables are connected, and then click “Scan Instruments”. If still a problem, see the “Ethernet Getting Started Guide” or contact Waters technical support.



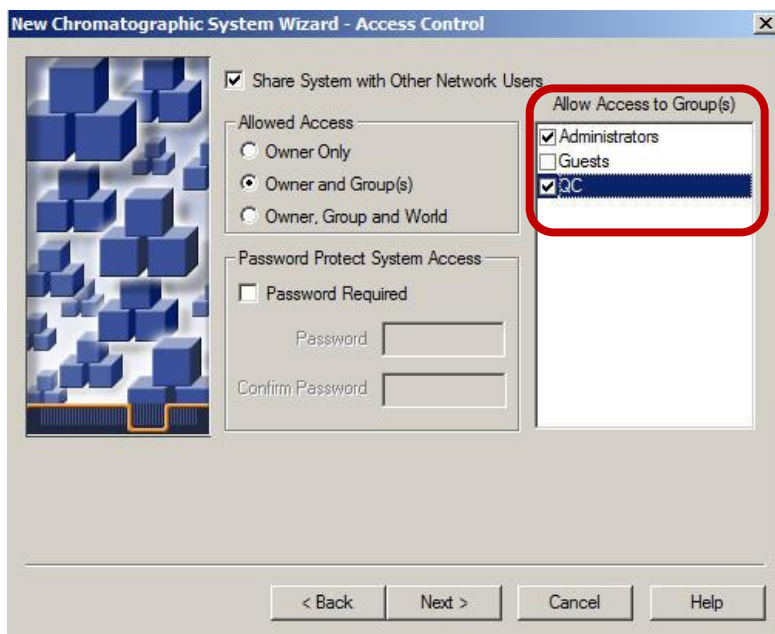
7. In the Configuration manager, select the “Systems” icon. Right mouse click and select “New”, then select “Create New System”.



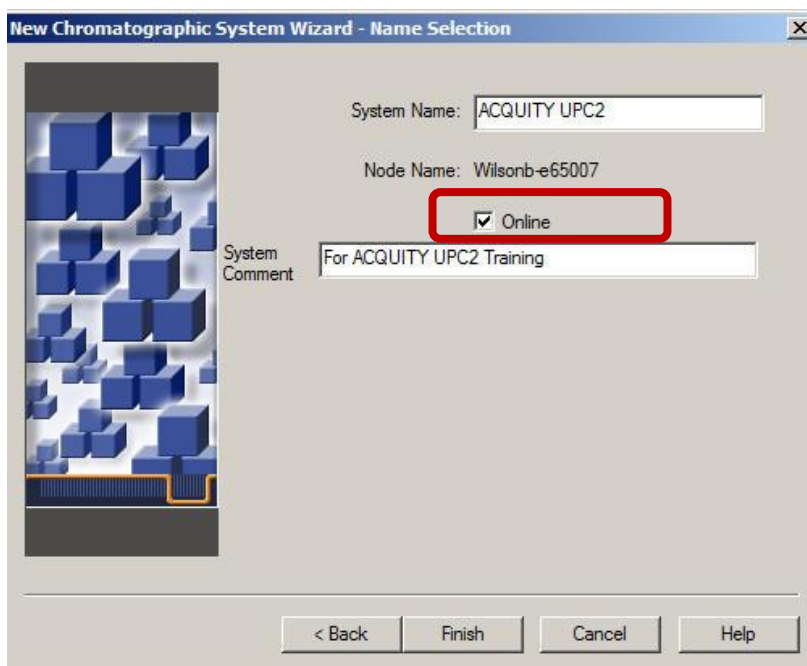
8. Select the UPC2 modules from the list on the right hand side and drag it over to the right to create the System. Click Next.



9. Select "Share System with Other Network Users" and select Allowed Access level. Consult your "Empower System Administrator" for specific Groups who are allowed access. Click Next.



10. Give the System a Name and select that "Online" is checked, and then click "Finish."

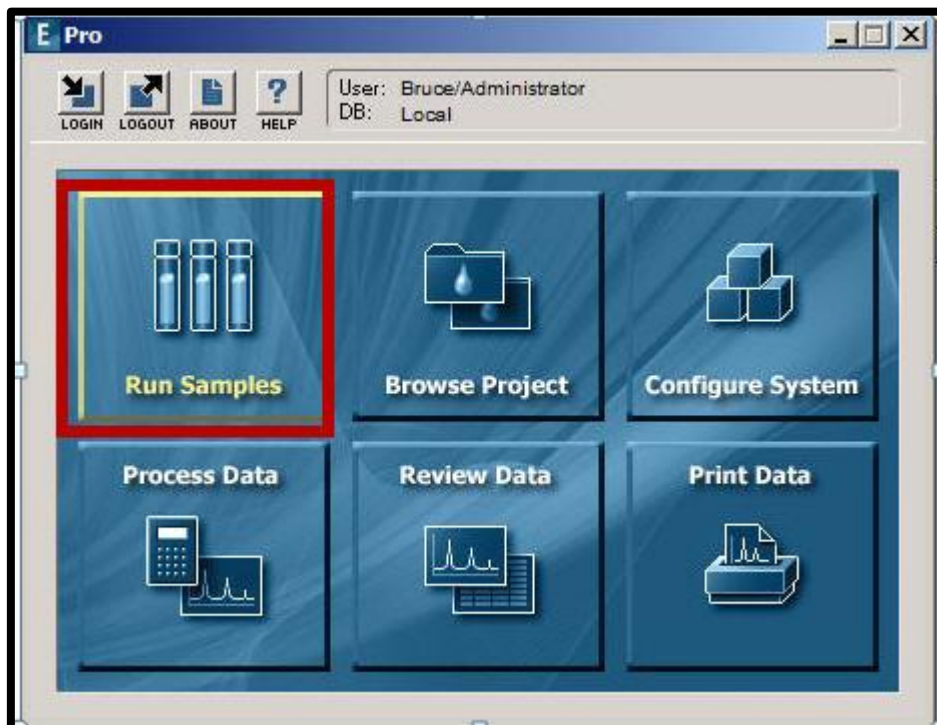


11. Select File – Exit to leave the "Configure Systems" window.

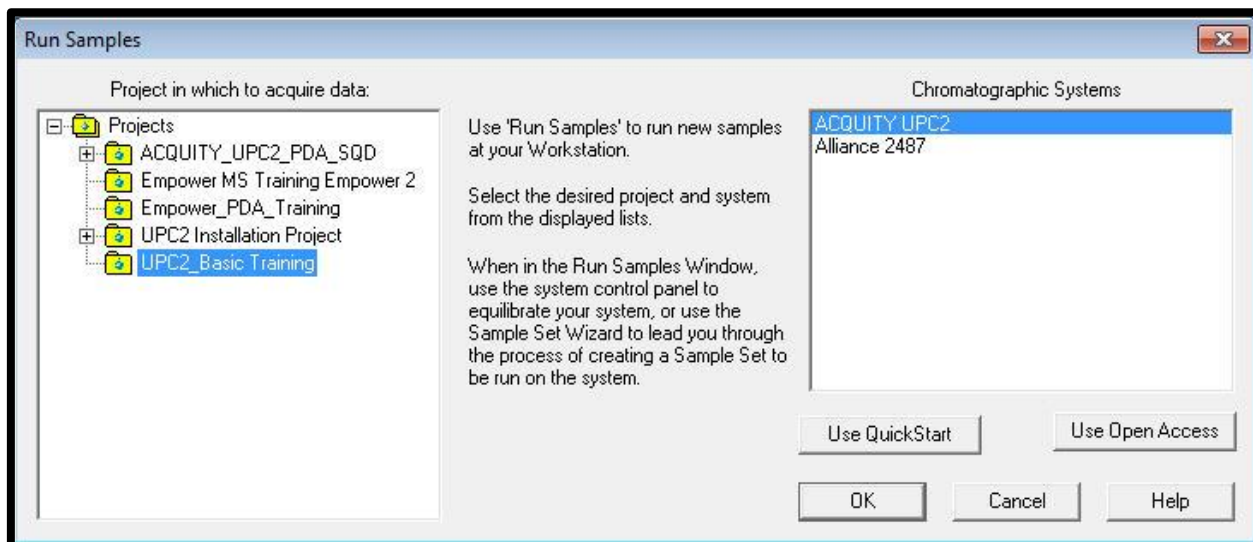
Note: Empower System Management includes global Empower settings, Project creation, backup and restore, and user rights. Consult the help and user manuals for more details.

D: Run Samples with UPC² Basic Overview

1. From the Empower 3 login screen click on "Run Samples".

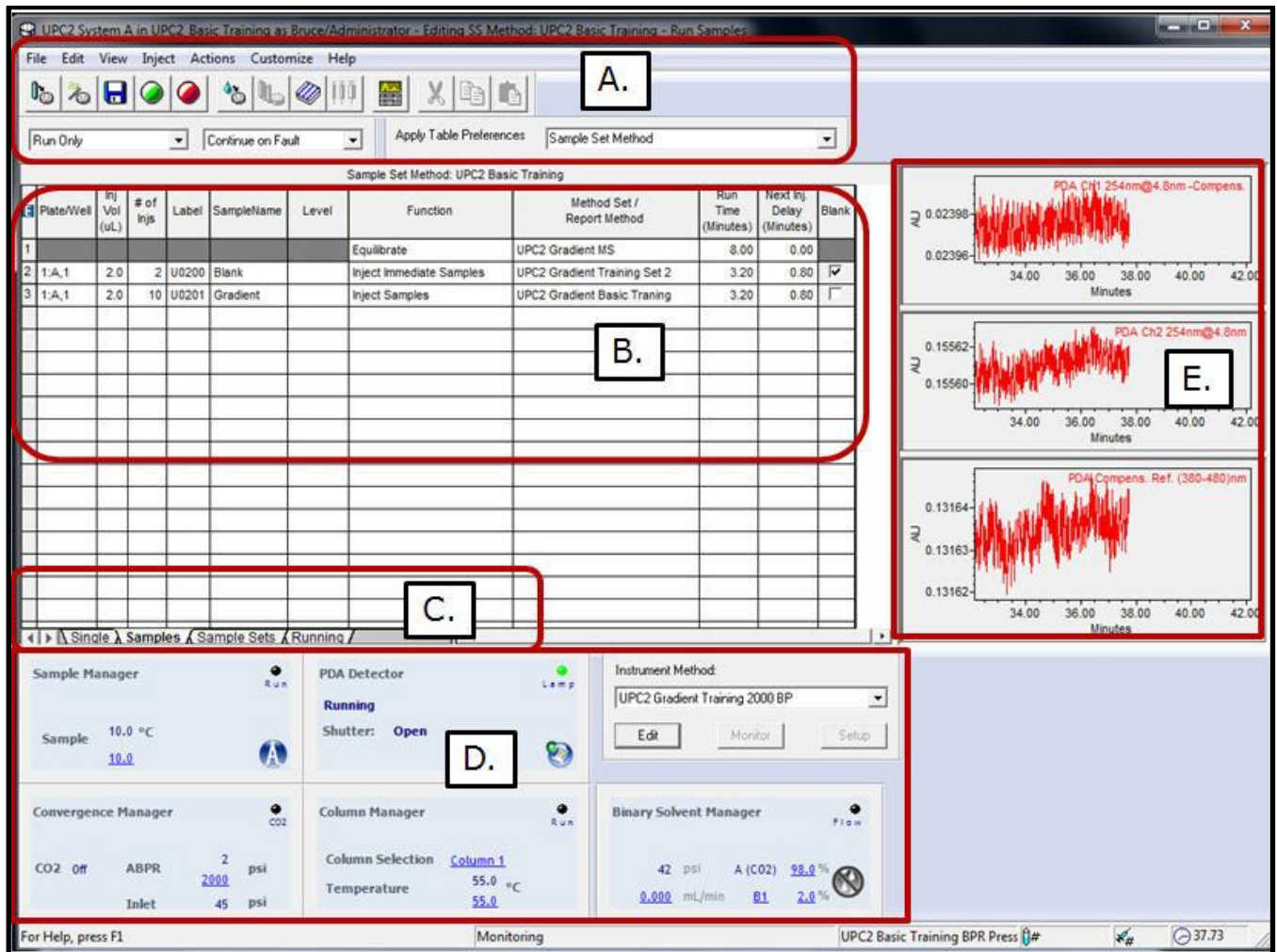


2. From Run Samples menu select the "UPC² Basic Training" Project or other UPC² defined project. Also select the Chromatographic System that was just created, "ACQUITY UPC²" System.



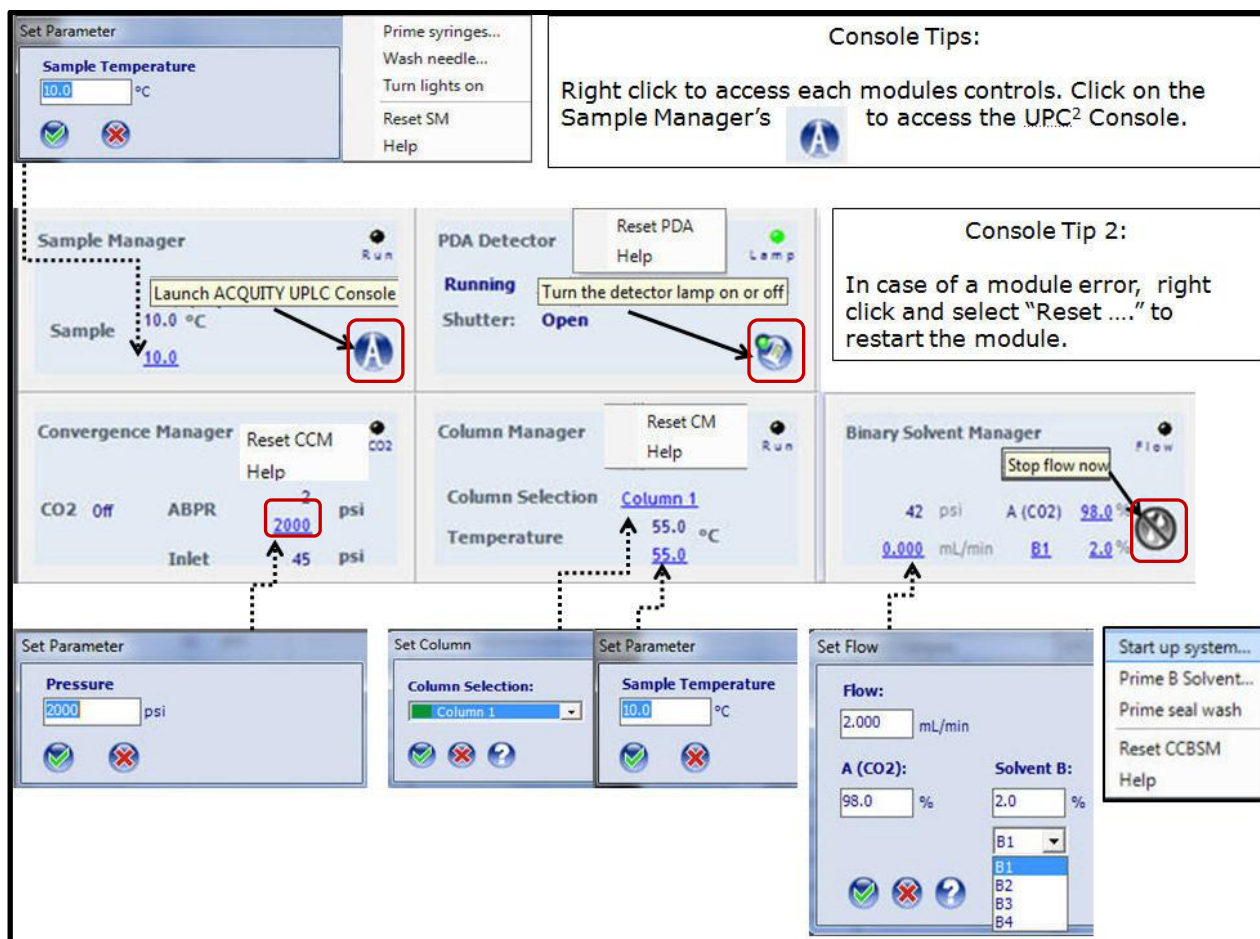
3. The Run Samples window has 5 general sections:

- A. Menu bar, Run Type and Table Preferences
- B. Sample Set table display
- C. Sample table selection tab (Single, Samples, Sample Sets, Running).
- D. UPC² console and direct control functions (details on the next page).
- E. Real time plot chromatogram and channel display of acquisition.

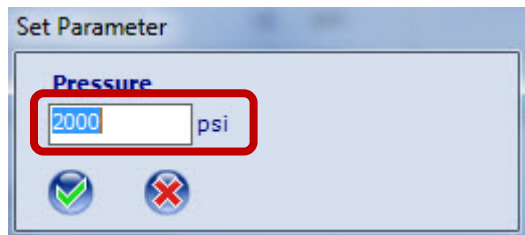


E: Basic UPC² Daily System Startup

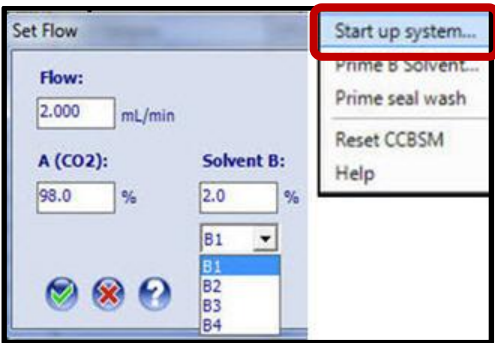
1. The next steps cover the basics of daily UPC² system startup, instrument method and sample acquisition. Before starting make sure the desired sample loop, CO₂, co-solvent, and samples are ready and placed on the system. Ensure the CO₂ tank is connected and the valve is in the open position.
2. Each UPC₂ module has individual controls that can be accessed with right mouse clicks. These include resetting a module in case of a failure, start and stop pump flow, and access of the full ACQUITY console for configuration and diagnostics. Each of the console controls are listed below.



3. For startup, click on the Convergence Manager console's ABPR "Pressure Setting". Enter a pressure of 2000, and then click the check box. The ABPR starts pressurizing once the BSM starts pumping CO₂.

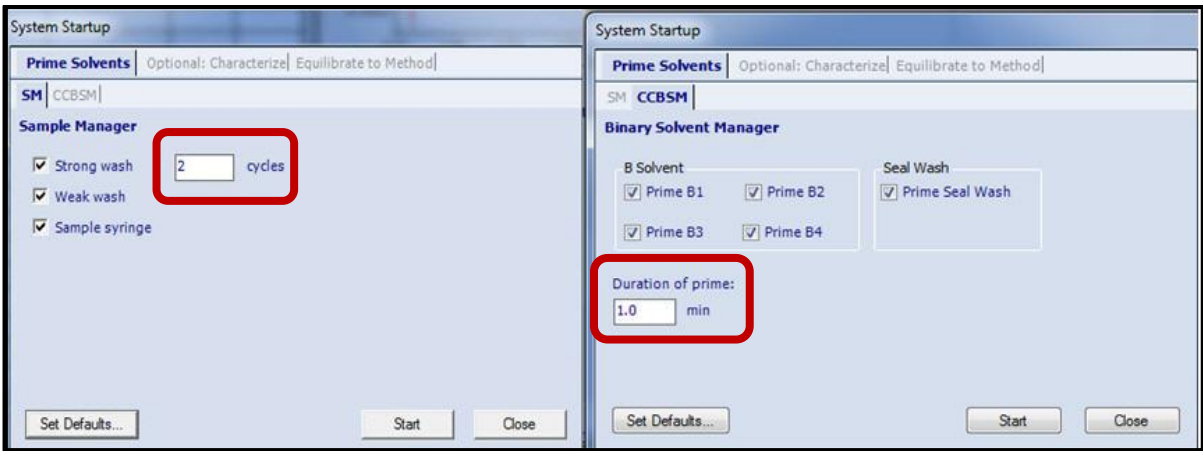


4. Right mouse click on the Binary Solvent Manager and select "Start up system..."

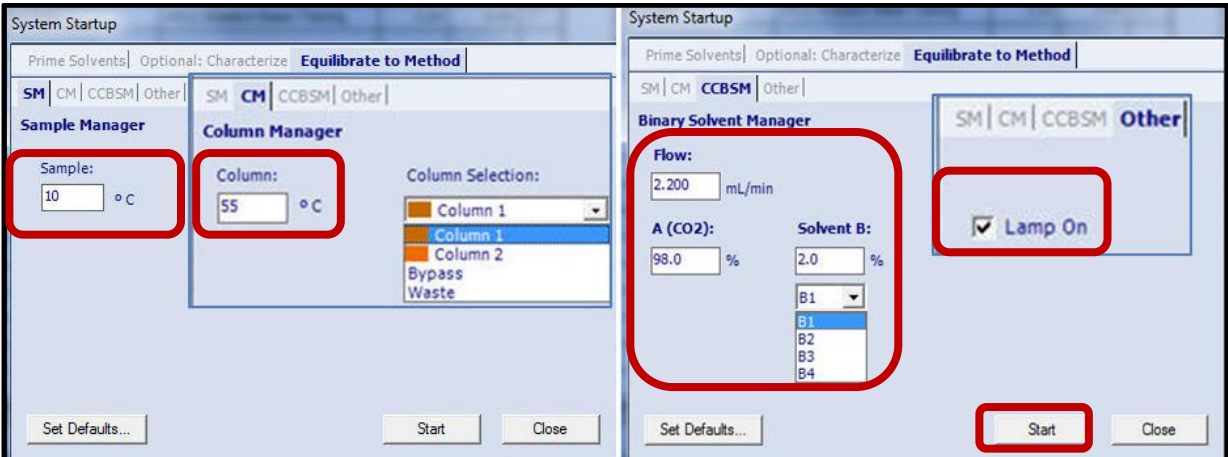


5. For standard UPC² System Startup, use "Prime Solvents" tab and "Equilibrate to Method" tab (the "Optional: Characterize" tab is used when changing sample loops"). For the "Prime Solvent" tab use the following general recommendations.

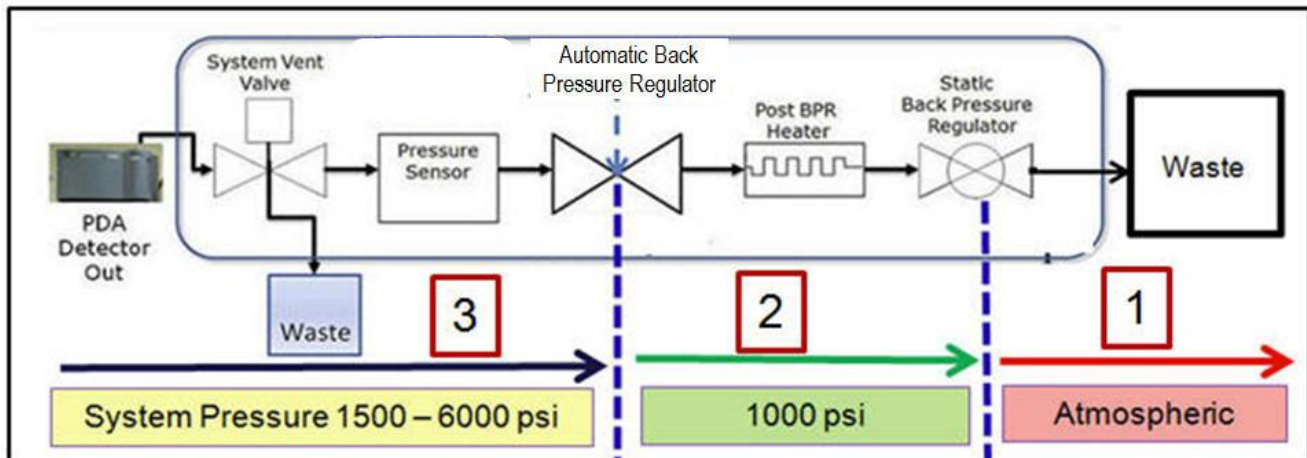
	SM - Sample Manager - Cycles	CCBSM – Binary Solvent Manager Time
Overnight	2 Cycles	1-2 Minutes
Startup	6 Cycles	4 Minutes



6. For the Equilibrate to Method tab, select as per the developed method and click "Start" (below are some general startup conditions for a 3.0mm IDx100mm UPC² column).



7. UPC² operation uses the following 3 step process to keep the CO₂ in a liquid-supercritical state:

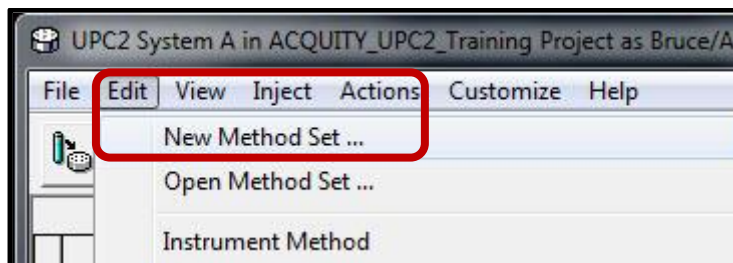


- Step 1: Flow rate is turned on and the Static Back Pressure Regulator cartridge builds pressure to ~1100 psi.
 - Step 2: The ABPR (Automatic or Dynamic Back Pressure Regulator) is activated once the system pressure reaches ~1100 psi and holds the system pressure constant between pressures of 1500 - 6000 psi (recommended 1700 - 3000 psi). The ABPR uses a solenoid with a needle and seat.
 - Step 3: Post ABPR (DBPR) Heat Exchanger – heats mobile phase at the outlet of the ABPR to prevent CO₂ from being exhausted as dry-ice.
8. Incoming CO₂ pressure is required to be above ~750 psi (room temperature dependent) and will be displayed in the Convergence Manager Console display (after the pump is started). The following warning signs will be displayed:
- CO₂ inlet pressures between ~ 650-750 psi are displayed with a "yellow warning icon".
 - CO₂ inlet pressures less than ~650 is displayed in red and will not allow system operation.

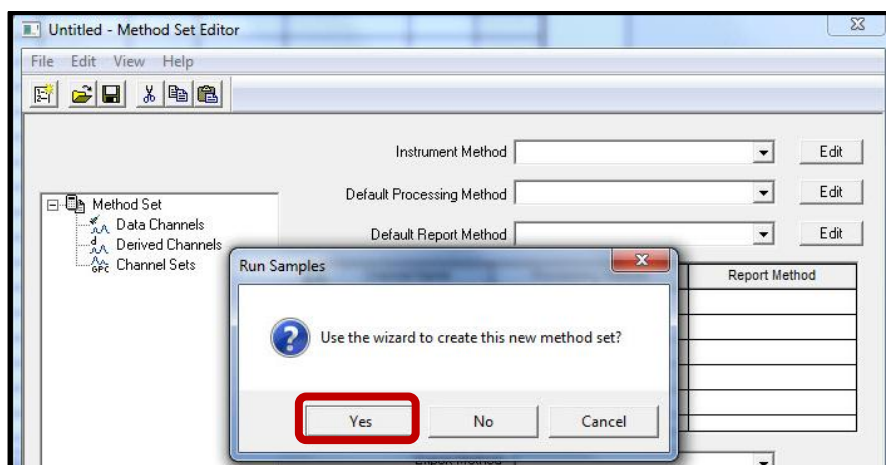


F: UPC² Instrument Methods and Method Sets

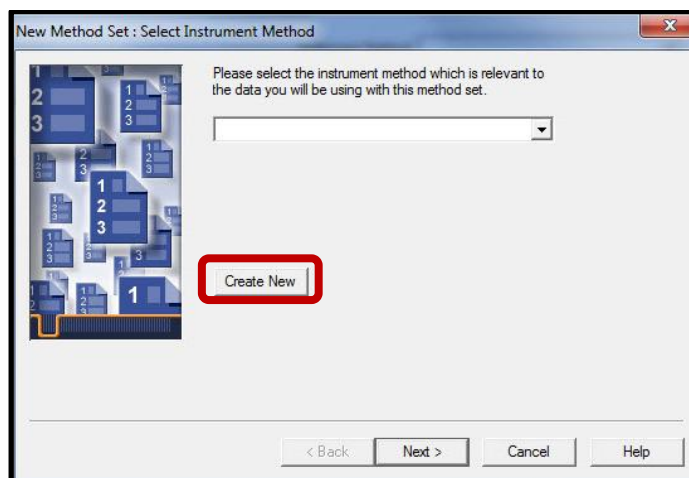
1. UPC² Instrument Methods and Method Sets are already developed for the training sample analysis. The following is an overview for each of the Instrument modules that are part of the UPC² system.
2. Empower has multiple ways to create methods and acquire data. The procedure below is one way to create an Instrument Method and Method Set.
3. From the menu bar select “Edit - New Method Set”. This is used to create the Instrument Method and Method Set.



4. The Method Set Editor will be displayed; select “Yes” to use the wizard to create the Method Set (the Method Set includes the Instrument Method and optionally the Processing Method and Report Methods).



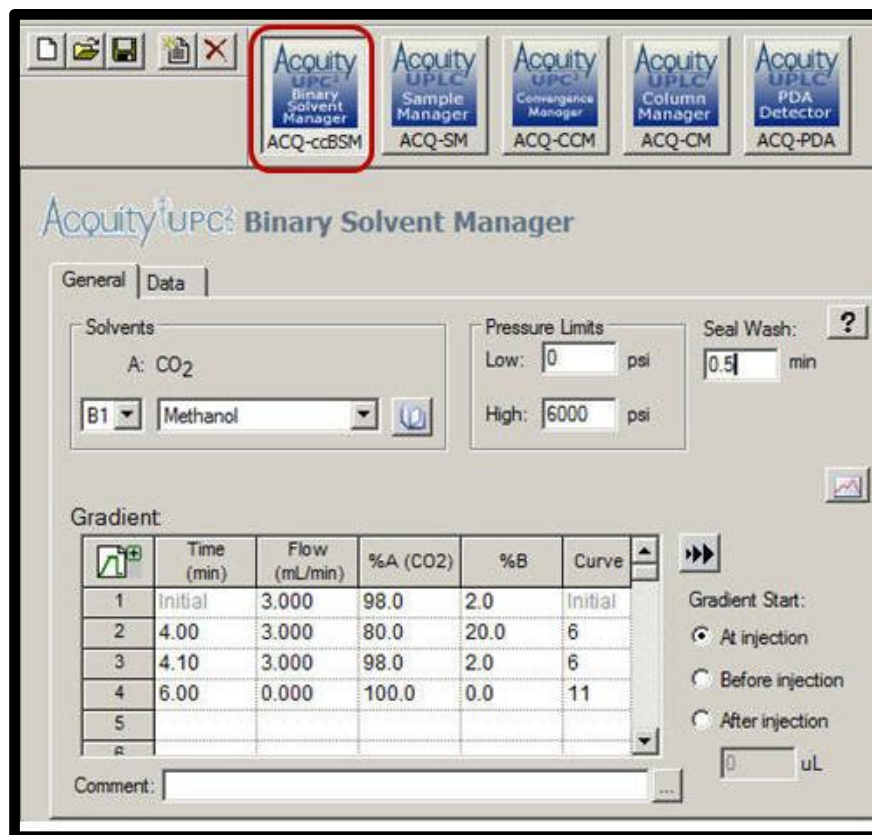
5. If an existing Instrument Method has been created, then select the desired method from the drop down select box. For new methods select “Create New”.



6. The UPC² has 5 to 6 different modules depending upon configuration (an ELSD and MS detector will include a splitter and MS detectors include a makeup pump).



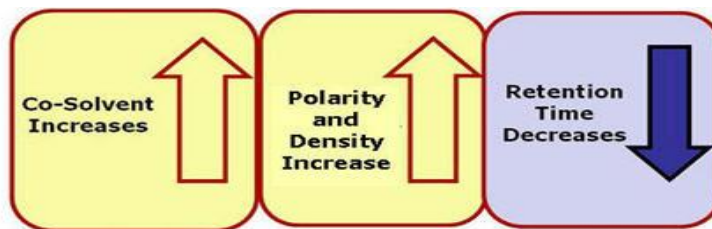
7. Click the ACQ-ccBSM icon to setup the Gradient and Pump conditions. Conditions below are generic for gradients using a 3.0 x100cm 1.7um ACQUITY UPC2 column.



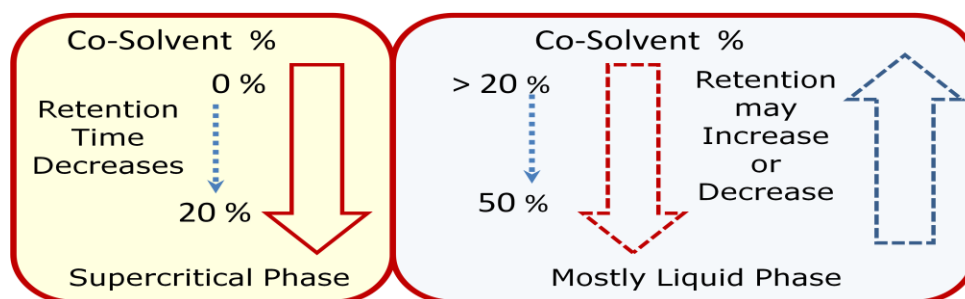
8. General ACQ-ccBSM recommendations:

- For 3.0mm ID UPC² 1.7um columns, use a flow rate between 2.0-3.5 mls/minute.
- For 2.1mm ID UPC² columns, use a flow rate between 0.8-1.8 mls/minute because of pressure restrictions (in general it is recommended to use columns >=3.0 ID).
- For legacy 3.0mm and 4.6mm ID columns, it is recommended to use a flow rate between 2.0-4.0 mls/minute (See each columns care and use manual for details).
- Set the Seal Wash at 0.5mls/minute. Use Methanol as the Seal Wash.
- System shutdown should be set to 100% CO₂ for 3 minutes.
- General recommendation of gradient %:
 - Start from 2% co-solvent to 20% co-solvent – application dependent.
 - The stronger the polarity of the co-solvent the shorter the retention time (see the details on the next page).

Co-Solvent affects on Retention Times are: Co-Solvents added to CO₂ generally decrease a component's retention time. As you change the amount of co-solvent, the polarity of the mobile phase is changed. In addition the density of the mobile phase becomes greater. Both factors contribute to a decrease in retention time.



Mixtures of CO₂ and co-solvents are supercritical in the column when co-solvent percentages are approximately less or equal to 20%. Co-solvents percentages above 20% will create mixtures that are mostly liquid phase and will affect retention times, but not in a linear fashion.



- g. Some co-solvents are listed below based upon polarity (most common are in Bold). Most common co-solvent is **Methanol**.

<u>Co-Solvent</u>	<u>Polarity</u>	<u>Co-Solvent</u>	<u>Polarity</u>
Heptane	0.1	Isobutyl Alcohol	4
Hexane	0.1	Tetrahydrofuran	4
Iso-Octane	0.1	Ethyl Acetate	4.4
<i>n</i> -Butyl Chloride	1	Methanol	5.1
Dichloromethane	3.1	Ethanol	5.2
<i>n</i> -Butyl Alcohol	3.9	Acetonitrile	5.8
Isopropyl Alcohol	3.9		

- h. The ACQ-ccBSM can start a pump gradient using the following conditions:
- At Injection: Gradient starts as soon as the injection occurs. The UPC² system has a delay (dwell volume) of up to ~400ul. A sample injected will be at isocratic conditions in the column for the dwell volume before the Gradient starts.
 - Before Injection: Gradient starts before injection occurs, which can be used to account for the Dwell volume. It is recommended to keep the volume at <=400 ul.
 - After Injection: Used to match a previous normal phase or SFC system's dwell volume (gradient hold or delay). Enter the previous systems dwell time for use of this function.

The screenshot shows a 'Gradient Start:' menu with three radio button options: 'At injection' (which is selected), 'Before injection', and 'After injection'. Below these options is a text input field containing the number '10' followed by the unit 'uL'.



9. Click the UPC² Sample Manager icon to setup the injection technique. Conditions below are for generic configuration of Injections.

- a. Sample Temperature settings:
- Sample temperature settings should not be lower than the freezing point of the sample diluent (if DMSO is used it is advised to set the Sample Temperature to ~20C).
- b. General Wash Solvents:
- Generic Wash: Use the same solvent for the weak and strong washes as the co-solvent used in the method.
 - Alternative Wash (sample dependent): 60 MEOH/40 IPA
- c. UPC² Sample Manager uses only PLUNO (Partial Loop Needle Overfill). PLUNO is detailed below:
- Automatic settings require 15 ul of additional sample
 - Uses 14ul of Needle Overfill pre-sample draw (this may adjusted in the "Advanced" icon button of the Sample Manager).
 - Uses 1ul of Needle Overfill post-sample draw (cannot be adjusted or changed).
 - No Weak Wash is injected in to column – only mobile phase and sample injected.
 - Air gaps are not injected into column
 - Sample does not come into contact with weak wash.
 - Recommended range for injection size is 25-75% of the loop volume.
 - Other Injection modes are not supported with UPC²

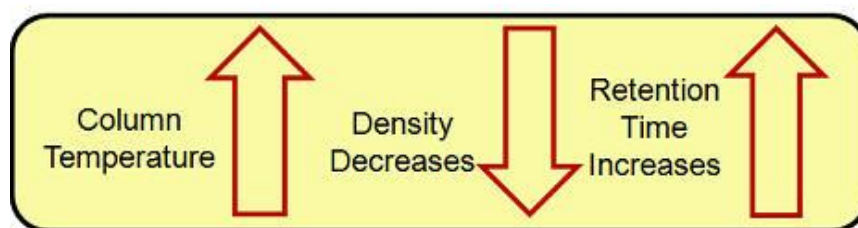
- d. Load Ahead: minimizes the injection cycle time of samples through the system.
- The sample manager overlaps the sample preparation portion of the injection cycle for the next sample while injecting the current sample for analysis.
 - For UPC² Load Ahead, do not use the "Loop Offline" parameter.
 - Note: The first injection of a sample set, and injection sets with different methods, cannot utilize the load-ahead mode.
- e. Recommended Sample Diluents are listed below (Sample Dependent).
- Methanol
 - 80%/20% Heptane/2-Propanol
 - Others: THF, Hexane, Chlorobutane, Dichloromethane, Chloroform



10. Click the UPC² Column Manager icon to setup column configuration and temperatures.

The screenshot shows the 'Acquity Column Manager' window. It has a 'General' tab selected. In the 'Temperature' section, 'Column' is set to '55.0 °C', 'Alarm Band' is checked and set to '± 5.0 °C', and 'Shutdown all columns' is unchecked. In the 'Column Selection' section, 'Valve Position' is 'Column 1' and 'Equilibration Time' is '0.1 min'. There are three 'External Valve' settings (1, 2, and 3), all set to 'No Change'. A 'Comment' field is at the bottom.

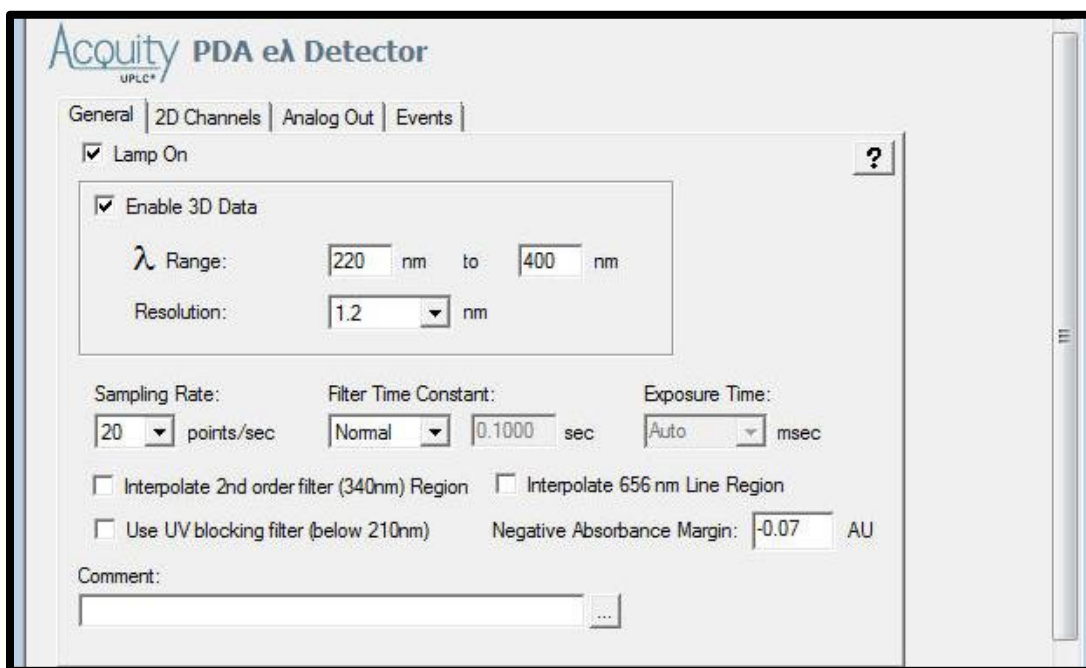
- a. General UPC² Column Temperatures details: SFC Separations are affected by the density in the column. Higher Column temperatures decrease density and increase retention time. Lower column temperatures increase density and decrease retention time.



- Recommended column temperatures ranges for UPC² are from 35-65C.
- A column temperature below 35C takes the CO₂ out of the supercritical phase, and may produce retention time and selectivity reproducibility issues.



11. Click the UPC² PDA icon to setup wavelength range, resolution and data rate.

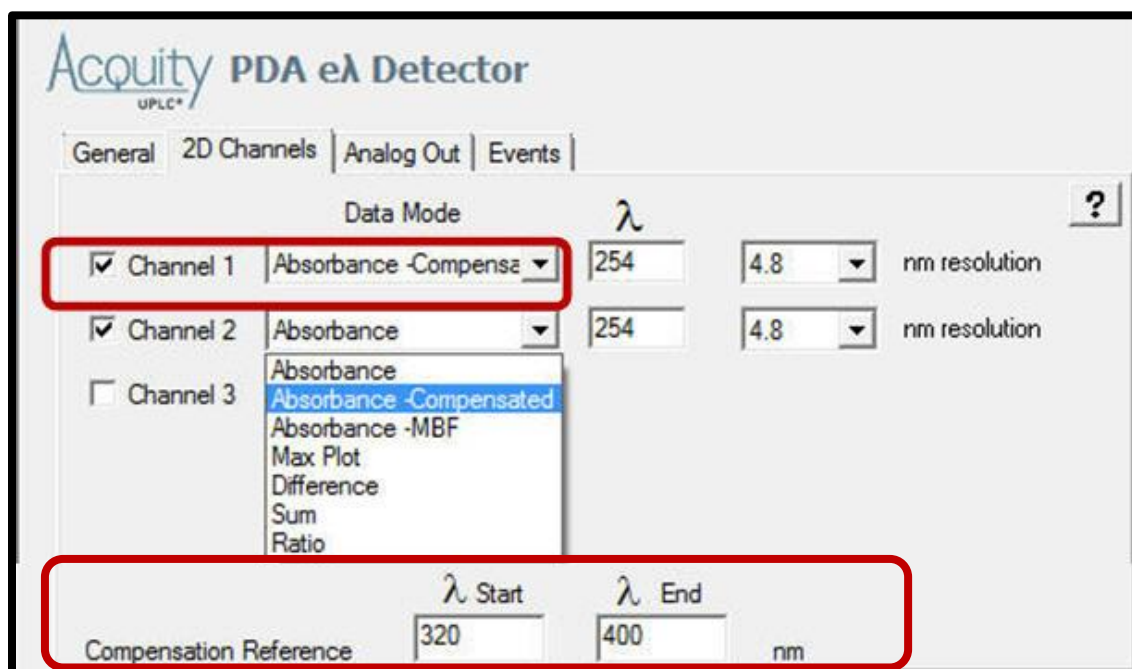


a. For 3D data and spectral analysis:

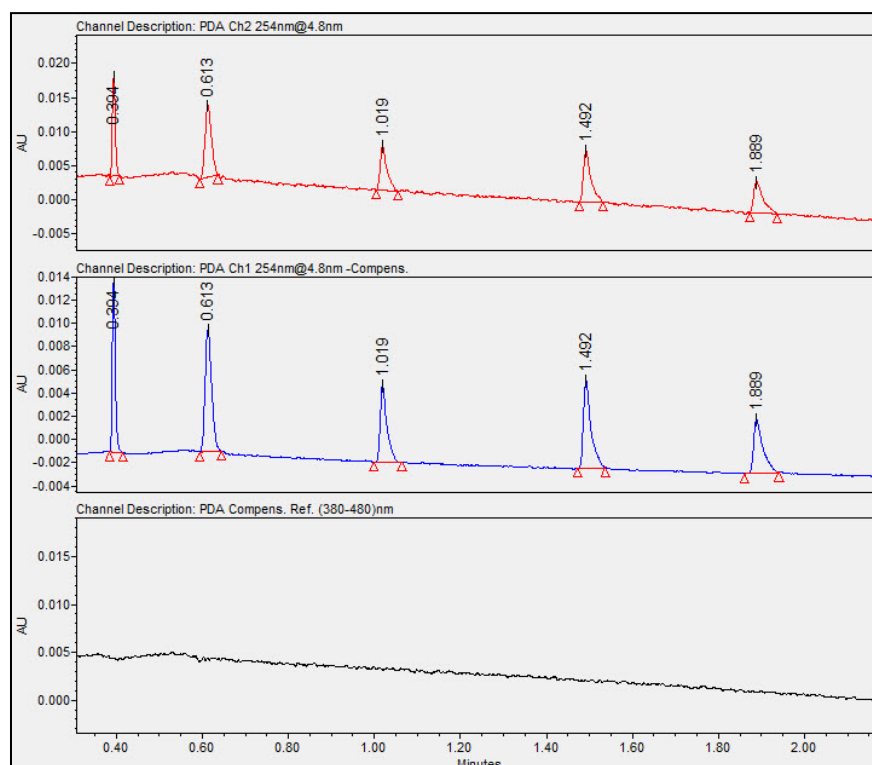
- Collect a wavelength range wide enough to include all spectral information for each component. Usually start at 210nm or greater – dependent upon the co-solvent selected.
- Use a resolution of 1.2nm for Peak Purity and Spectral Library investigation.
- Sample rates should be ~10 -20 points a second for most analysis conditions (peak integration/quantitation requires that a peak have a minimum of 15 points across a peak). Too many data points will increase noise.
- Usually recommended to leave the Filter time constant at the default settings (this will automatically adjust based upon the data rate).
- The negative absorbance margin is used to accommodate increases in optical transmission during a gradient run. This is typically needed when the absorbance baseline saturates or exhibits a flat, noiseless trace within the chromatogram.

b. Click the 2D Channels tab to collect individual wavelengths (in addition to 3D Data). You may extract up to 8 individual wavelengths.

- c. The refractive effects of CO₂ can produce baseline disturbances. It is generally recommended to use "Absorbance-Compensated" 2D collection to account these refractive index disturbances. Use a Compensated Reference of 320-400 to subtract this for the chromatogram (the Compensated Reference range must be within the 3D data collected).



- d. The Chromatograms below show how "Compensation Reference" works.





12. Click the UPC² Convergence Manager to setup the ABPR system backpressure. This is used to keep the CO₂ in a liquid/supercritical state during the analysis (1500 psi -6000 psi).

Acquity⁺UPC² Convergence Manager

General | Data

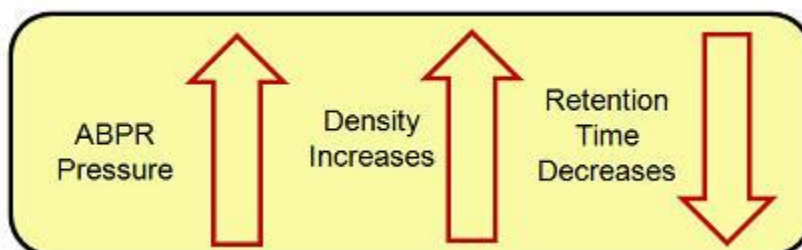
☒ ABPR Pressure ON

Pressure Gradient:

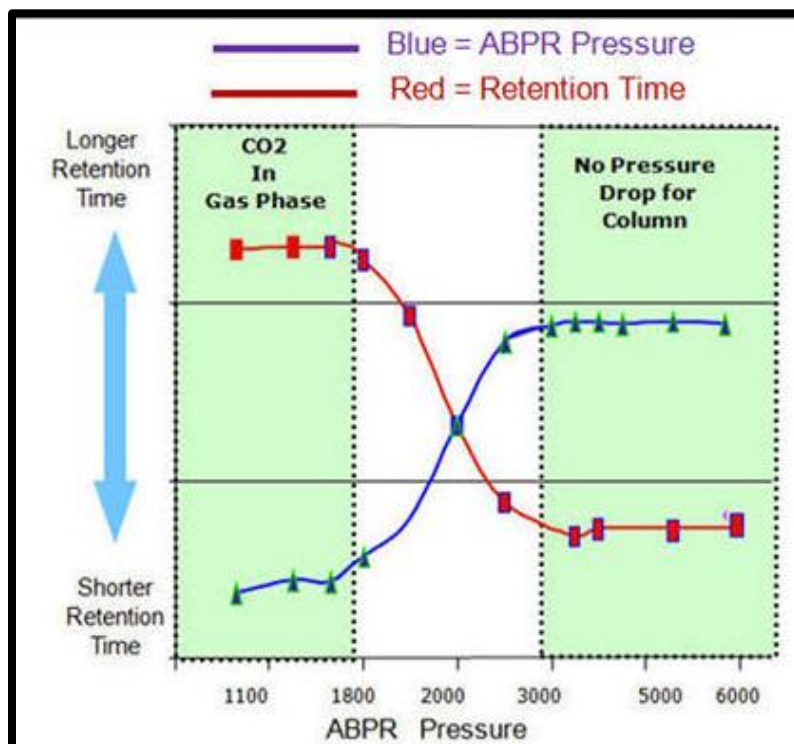
	Time (min)	Pressure (psi)
1	Initial	2000
2		
3		
4		
5		
6		
7		

Comment:
General Starting Conditions

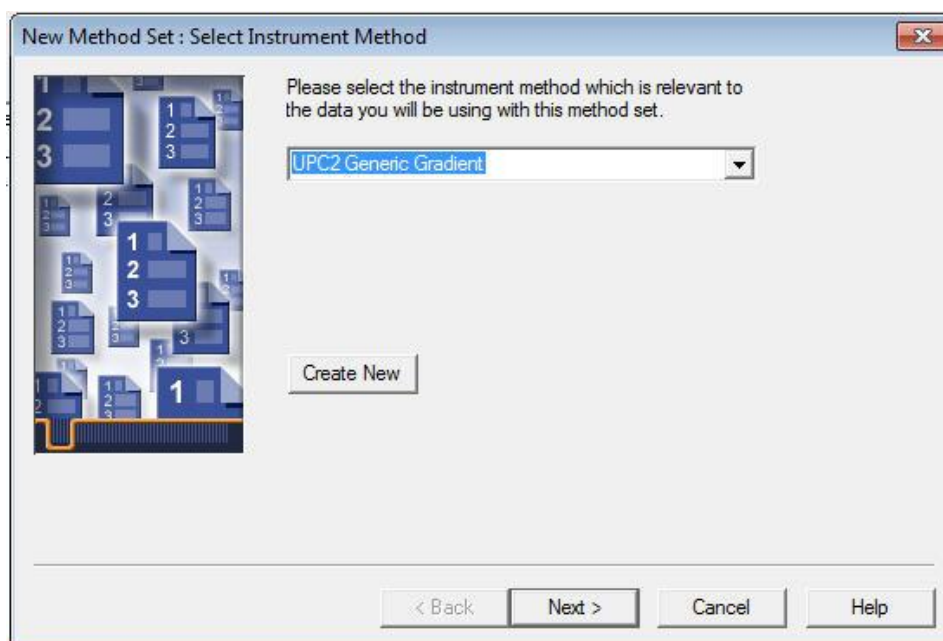
13. General information about the Convergence manager and ABPR (Automatic Backpressure Regulator) settings:
- b. SFC Separations are affected by density. The ABPR back pressure settings affect retention time by changing the density before the release of pressure.
 - c. As ABPR pressure increases, the density increases and retention time will decrease.
 - d. Lower Pressures than 1800 may affect the separation in a non-reproducible way as the condition changes from a supercritical phase to a more gaseous phase – which then affects the polarity and density.
 - e. Higher pressures than 3500 may prohibit the pressure drop for the column. ABPR pressure is another dimension that can be used in developing methods and separations.



- f. General settings for the ABPR are between 1700-3500. Gradients may be used, though not required.

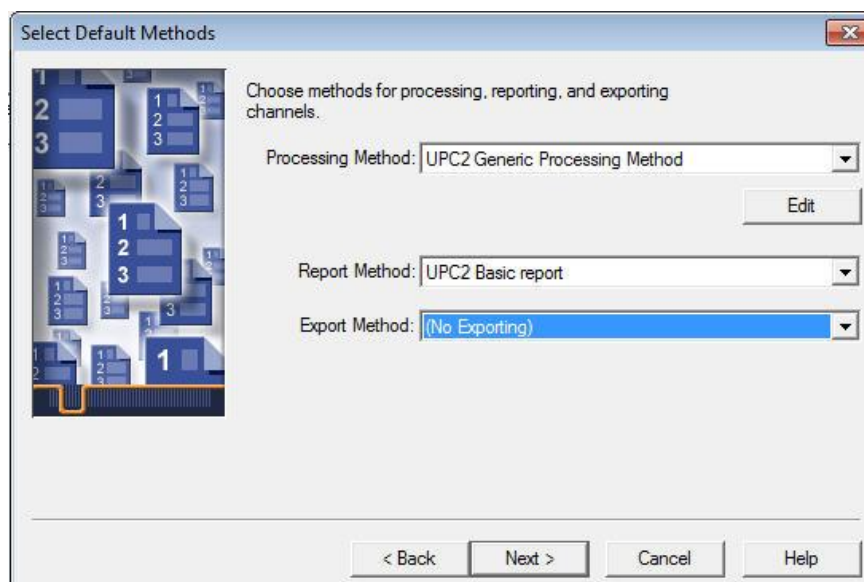


- g. After creating all the Instrument Method conditions, select File-Save Instrument Method As, and enter the instrument method name as xxxxxxxx, and then select File – Exit. The Method Set wizard is re-displayed.
- h. Select the desired Instrument Name in the list, and then select **Next**.

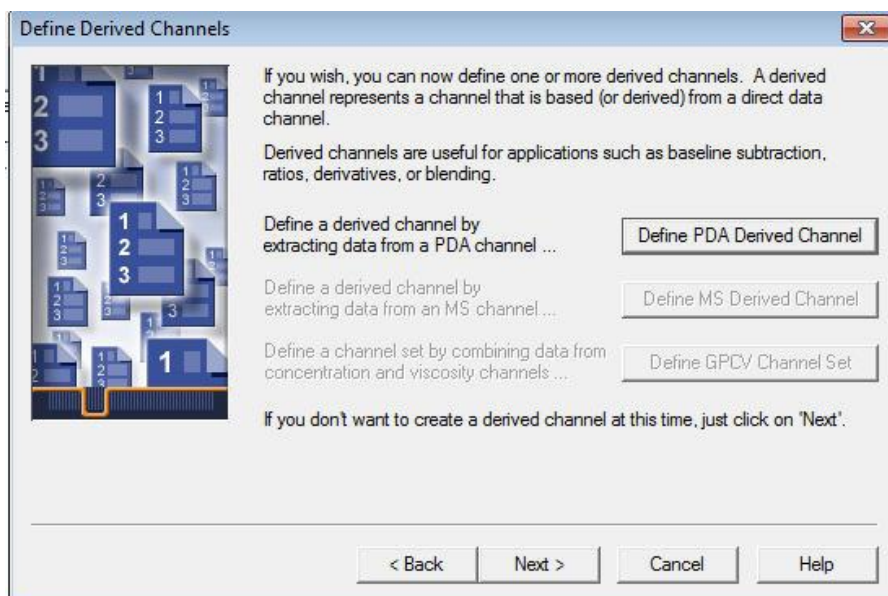


14. Select the desired Processing Method and Report Method, and select **Next**.

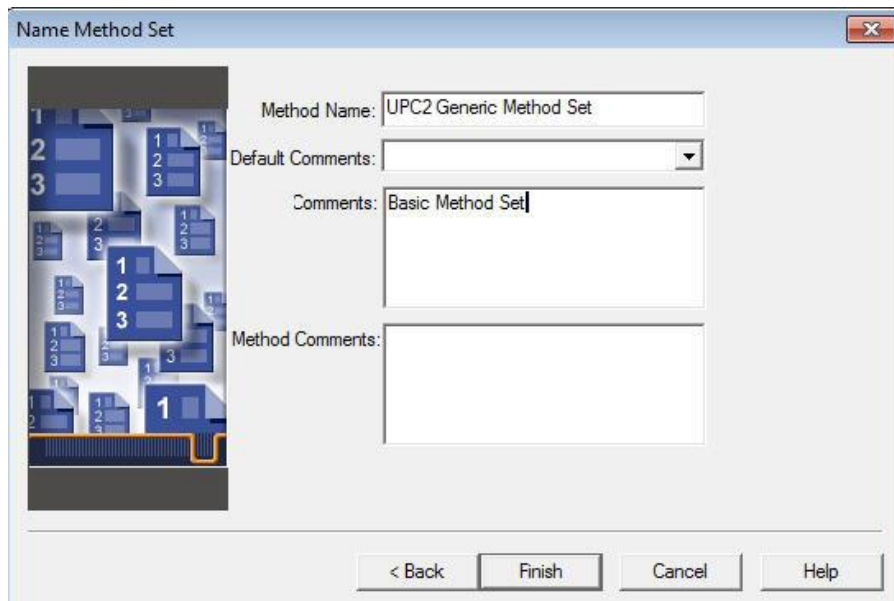
Note: The UPC² training project has a basic default processing method and report method. For the training select these two methods.



15. Optionally Click "Define PDA Derived Channels". This may be bypassed since 2D wavelength channels were selected in the UPC² instrument method. Click Next.



16. Save the Method Set and click Finish.



Method Name: UPC2 Generic Method Set

Default Comments: [dropdown arrow]

Comments: Basic Method Set

Method Comments:

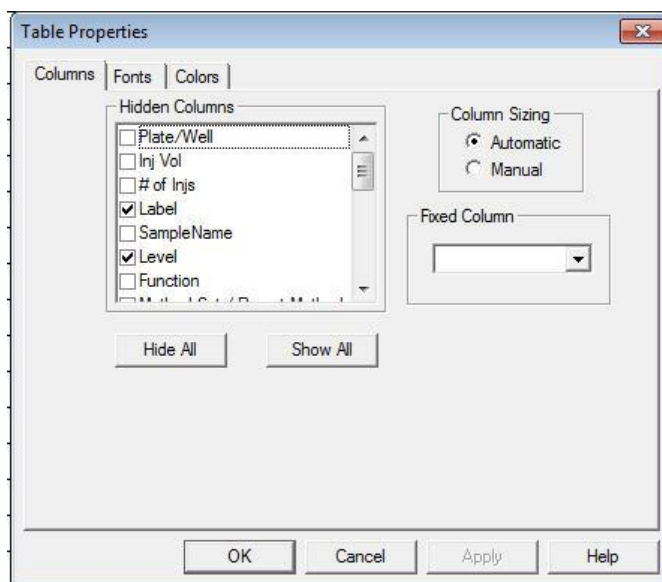
< Back Finish Cancel Help

17. You have now completed the basics of the Instrument method and Method Set.

G: Making a Sample Set and Acquiring Data

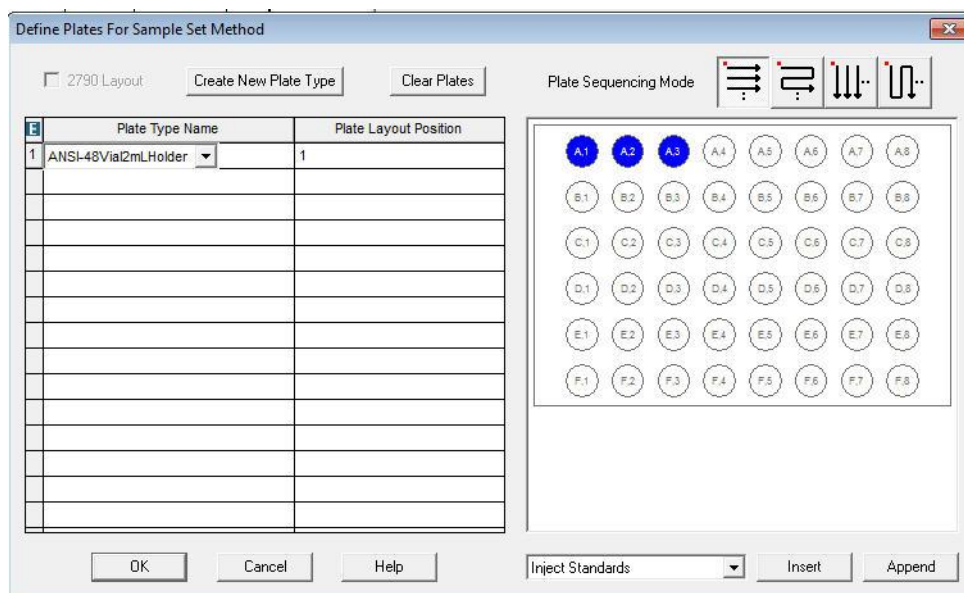
1. Manually create a Sample Set by clicking on the  tab.

2. Right click on the  column to access the Samples Table properties to customize the displayed channels. Items checked are hidden from display. To calculate signal to noise from a Blank uncheck the "Blank" column.



3. Select the Plates icon to select the vial format .

4. Select the style of plate and how many samples/standards then click "Insert". ACQUITY UPC² sample plates must use ANSI format. Then select OK.





5. You can manually enter the number of samples or use the wizard

Sample Set Method: Untitled									
E	Plate/Well	Inj Vol (uL)	# of Injs	SampleName	Function	Method Set / Report Method	Run Time (Minutes)	Next Inj. Delay (Minutes)	Blank
1	1:A,1	0.0	1	UPC2 Training 1	Inject Standards	UPC2 Generic Gradient	4.00	1.00	☒
2	1:A,2	3.0	1	UPC2 Training 2	Inject Standards	UPC2 Generic Gradient	4.00	1.00	☐
3	1:A,3	3.0	1	UPC2 Training 3	Inject Standards	UPC2 Generic Gradient	4.00	1.00	☐
4	1:A,4	3.0	1	UPC2 Training 4	Inject Standards ▾	UPC2 Generic Gradient	4.00	1.00	☐
					Inject Standards				
					Inject Samples				
					Inject Controls				
					Inject RF Internal Standards				
					Inject Immediate Standards				
					Inject Immediate Samples				
					Clear Calibration				
					Equilibrate				
					Report				
					Quantitate				

6. Plate/Well 1:A,1 is a Blank with mobile phase as the sample. It is also checked in the Blank column so it can be used to calculate signal to noise from a Blank injection.
7. The Function column can be used to detail specific actions (major Functions are listed below).

Function	Description
Inject Standards	Identifies the Sample for Calibration
Inject Samples	Identifies the Sample for Quantitation
Inject Controls	Identifies Standards not used for Calibration
Inject Immediate Standards	Collects Standard data bypassing the injector
Inject Immediate Samples	Collects Sample data bypassing the injector
Clear Calibration	Clears the previous Calibration for new analysis
Equilibrate	Runs at initial conditions without collecting data
Condition Column	Runs the UPC ² gradient without collecting data
Purge Injector	Purges the UPC ² Sample Manager
Wet Prime	Primes the UPC ² BSM with flow using the specified method set.
Sys Prep	Primes the ACQUITY UPC ² BSM, primes and washes the UPC ² Sample Manager and equilibrates in one step (does not start the ABPR on the Convergence Manager).



8. Click the Amounts icon to enter concentration of Standards then select OK.
- If using Internal Standards, select the Sample Set Type to "Standards & Unknowns". Internal Standards usually have the same concentration for Standard and Unknown samples.

Note: The Internal Standard in this example is Diflucan.

Component	Value (Standard)	Value (Standard)	Value (Standard)	Value (Standard)	Value (Standard)	Value (Standard)	Value (Unknown)	Value (Unknown)	Value (Unknown)	Value (Unknown)	Value (Unknown)	Value (Unknown)	Units (Vial)
1 Diflucan	0.000000	25.000000	25.000000	25.000000	25.000000	25.000000	25.000000	25.000000	25.000000	25.000000	25.000000	25.000000	ug/ml
2 Zolof	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000							ug/ml
3 Penegra	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000							ug/ml
4 Methyl Phenidate	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000							ug/ml
5 Theophylline	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000							ug/ml

- Select "File – Exit" to exit the Component Editor. Select "File – Save" to save the changes to Process Only Sample Set, and then File – Exit to the Project View.



9. Select File-Save Sample Set then select the button to start the Sample Set.
10. Enter the Sample Set information as desired and select "Run" to start the Sample Set. You have now completed the basics steps to acquire UPC² data with Empower.

Run Sample Set

You have selected lines in this sample set method

Do you wish to :

☒ Inject all rows

☐ Inject only selected lines

Name for this sample set :

Settings for this Sample Set:

☐ Wait For User

Run Mode :

Suitability Mode :

Printer :

Shutdown Method :

Default Comments :

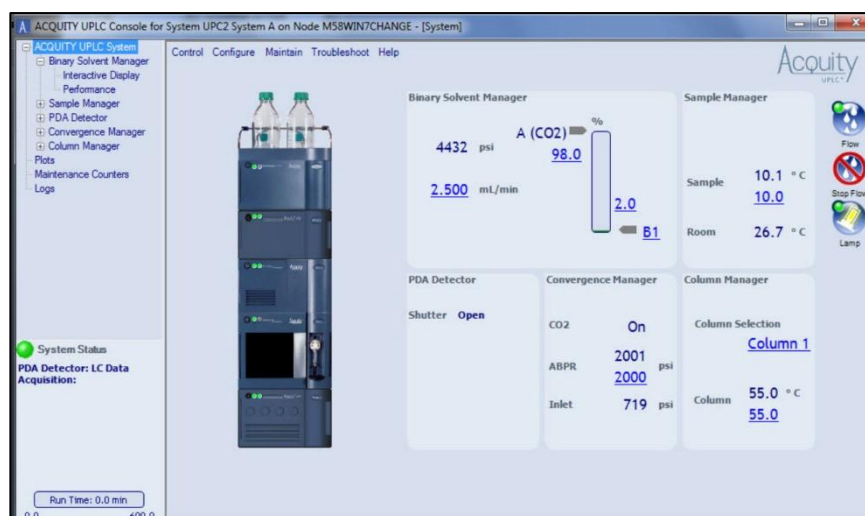
Comments :

H: ACQUITY UPC² Console and Diagnostics

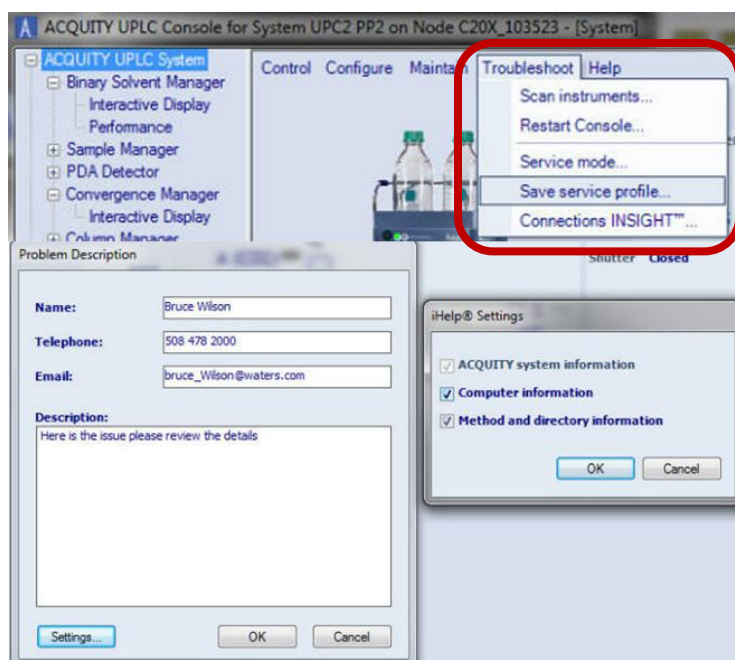
1. The ACQUITY UPC² console can be used for diagnostics and system checks of the UPC² System.
2. Access the UPC² console by clicking on the Sample Manager's  in the Run Samples window.



3. The main console screen has a general status screen for each of the UPC² modules. Each module can be selected for more detailed information as well as specific diagnostic checks.

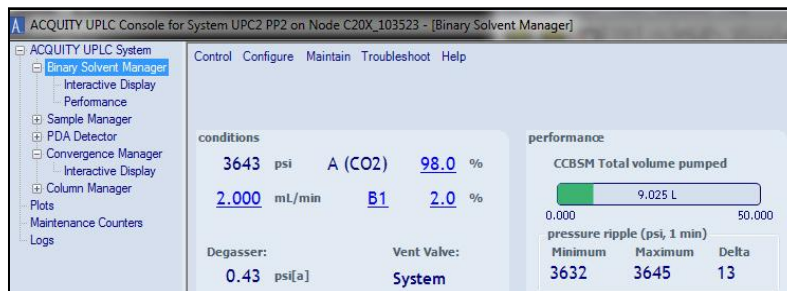


4. For issues that need support and assistance, use Troubleshoot – “Connections Insight” or “Save Service Profile”. Save Service Profile can be emailed to Waters support for troubleshooting issues. You can save the service and method details to send in a zip file (no chromatographic data).

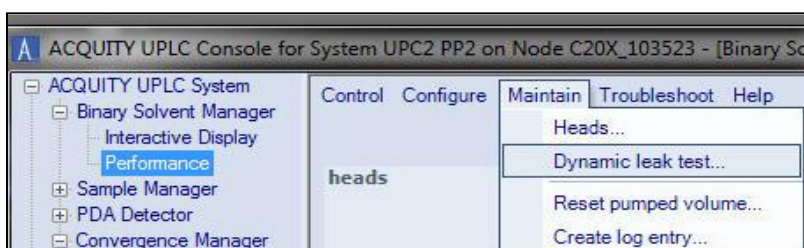


5. UPC² BSM - Main display screen diagnostic and performance checks:

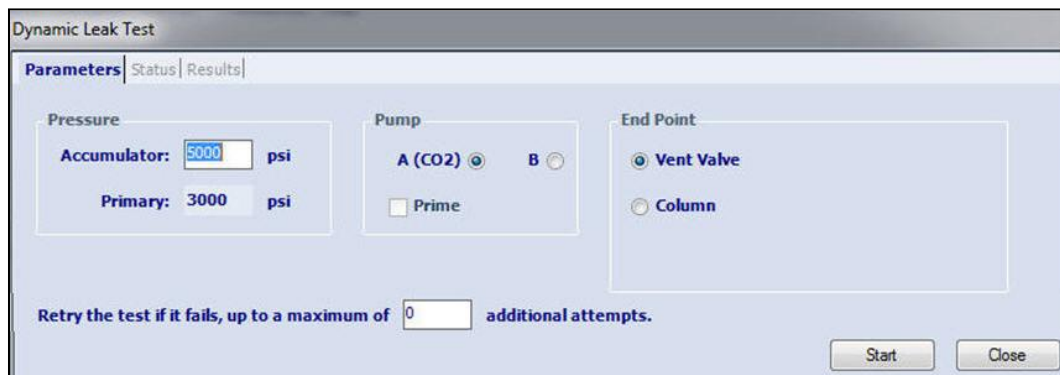
- a. Pressure Ripple (Isocratic conditions) Delta should be $\leq 1\text{-}2\%$ of system pressure. If greater than this, re-prime co-solvent pump and perform the Dynamic Leak Test (step b).



- b. Select the Binary Solvent Manager – Maintain – Dynamic Leak Test.



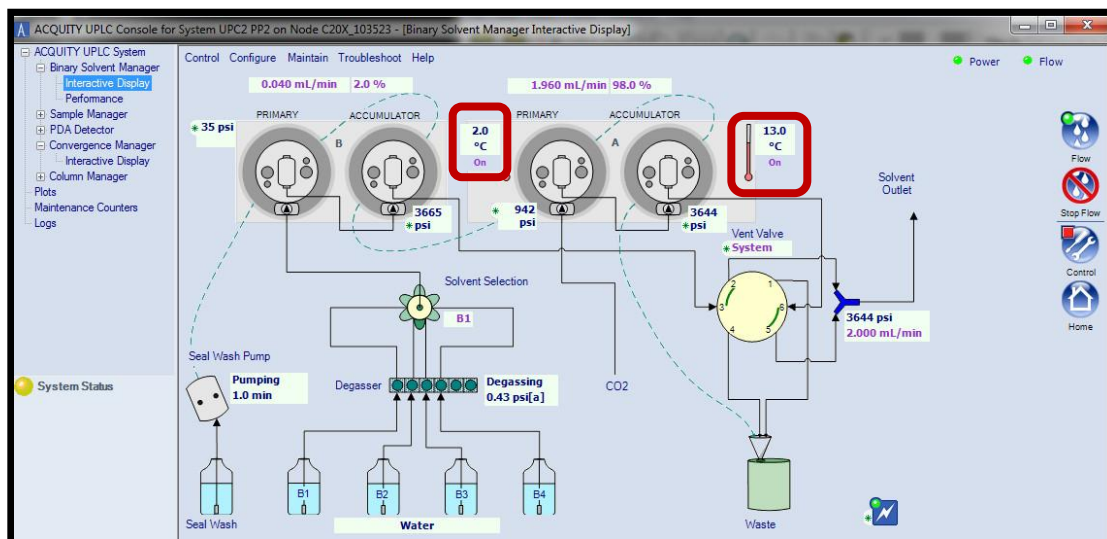
- c. Select the Pump to test and click Prime. Leave the default test conditions and select "Start".



- d. The results for the Accumulator and Primary will be displayed as below. If the results fail – re-prime and try again. If the leak fails again, contact your Waters Service engineer.



- e. Use the UPC² BSM –Interactive Display to confirm the BSM CO₂ cooling temperatures for the Primary pump is at 2.0 C (-0.5 +2.0) and the Actuator pump is at 13.0 C (+/- 2.0). If the temperatures are beyond the range, contact Waters technical services for support.

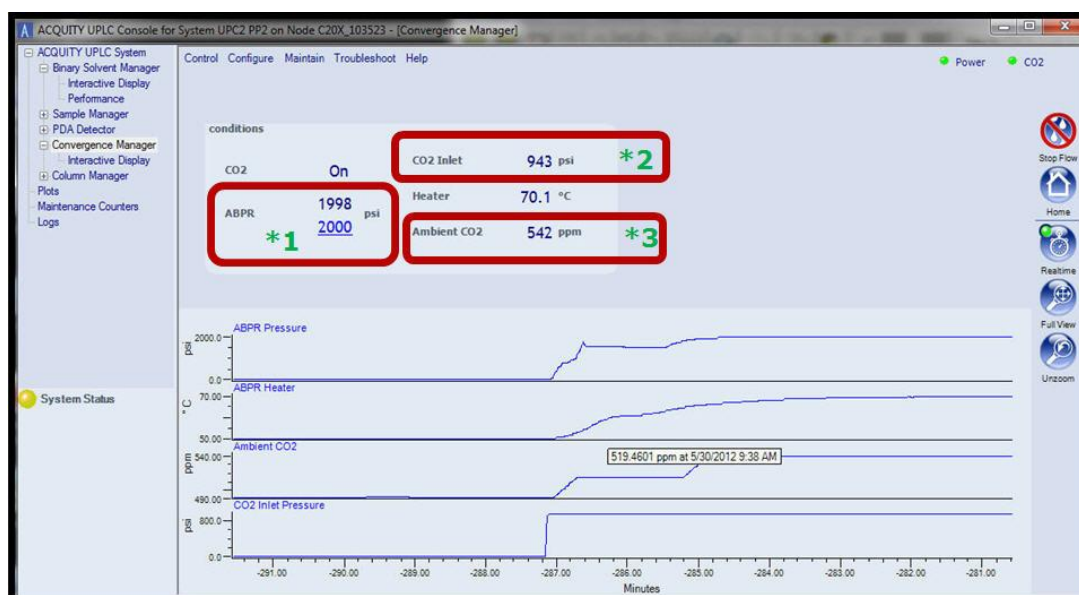


6. UPC² Convergence Manager - Main display screen diagnostic and performance checks:

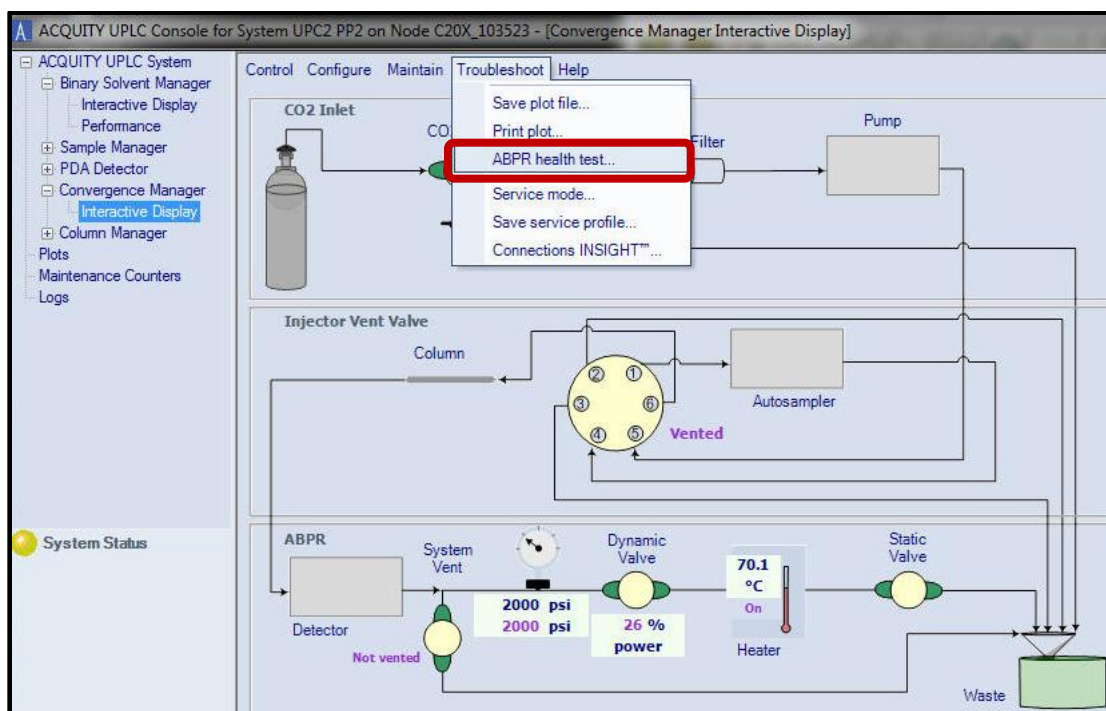
- a. ABPR pressures fluctuations should be $\leq 1\%$ of ABPR pressure settings (*1). If fluctuations occur above 1% check the following:
- Check the inlet pump pressure
 - Make sure CO₂ inlet pressure is within acceptable ranges (*2). Out of range pressures will be colored coded.

CO2 Inlet 943 psi

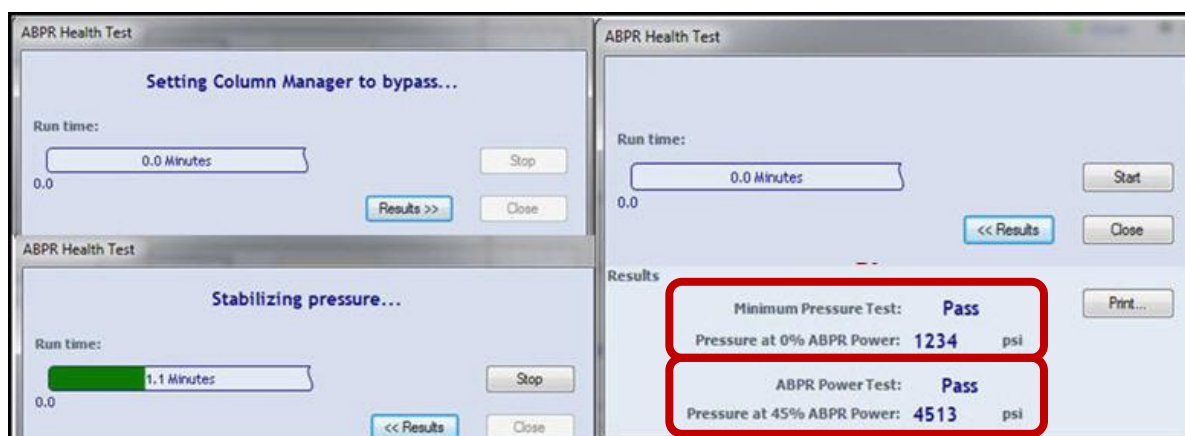
- Check for leaks at the column and all connectors
- Check ambient CO₂ (*3). If this is high, there may be a leak internal to the Convergence Manager. Contact Waters service.
- Complete the Convergence Manager's ABPR Health test (step 7)



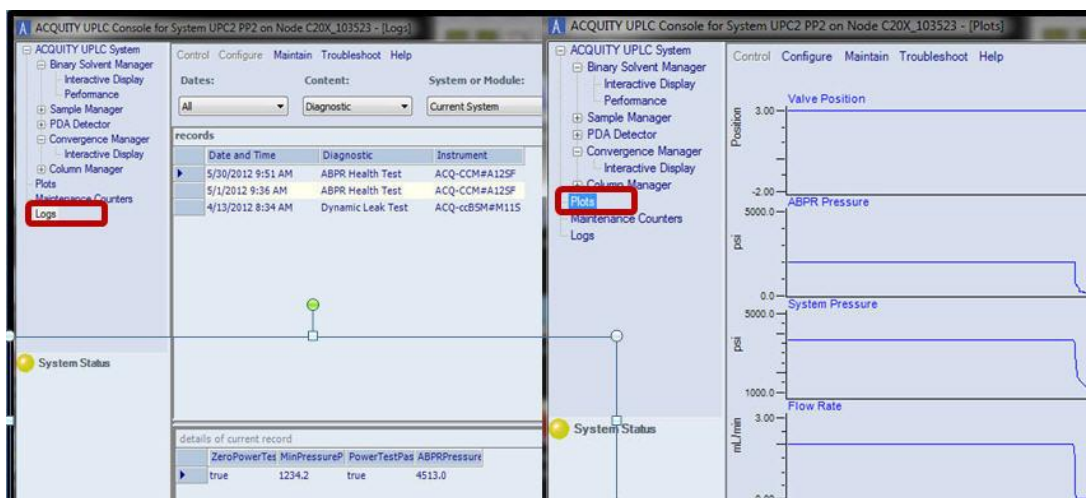
7. UPC² Convergence Manager ABPR Health Test. This should be used if the ABPR pressure settings cannot be reached and maintained. Select from the Convergence Manager, Troubleshoot-ABPR health test.



8. The ABPR Health Test executes the following consecutively (takes about 6 minutes):
- Part 1 - Tests the Static Back Pressure Regulator
 - Sets the Column Manager to the Bypass position and turns off the ABPR.
 - Sets the BSM flow rate to 100% CO₂ (pump A) at 3 ml/min and 1ml/minute for 2 minutes each.
 - Check and ensures the values read are greater than 1000 psi to 1400 psi. If the Static test fails, the Static cartridge will need to be changed.
 - Part 2 - Tests the Dynamic Back Pressure Regulator
 - Keeps the Flow at 1.0 ml/min 100% CO₂ (pump A) and uses the Column Manager Bypass position.
 - Sets APBR power to 45%
 - Waits 2 minutes and confirms the APBR pressure is between 3000 & 5000 psi. If the Dynamic test fails, contact Water's Service.



9. Additional ACQUITY UPLC console items to check:
 - a. View the Logs and select to see "All" content for errors or issues, or choose another content category.
 - b. View the Plots and view the various plots.



10. For other ACQUITY UPC² issues, please contact Waters Expert center or your local Waters Field Service Engineer.