Waters UPC <sup>2</sup> System with F	PDA detection and Empower 3
REVISION NUMBER	1.4
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### ACQUITY UltraPerformance

## Convergence Technology UPC<sup>2™</sup> 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 UPC<sup>2</sup> PDA system using Empower Software. This document covers basic overview and training for methods development of a UPC2 system.

## B. Supplies and Sample Setup

#### 1. UPC<sup>2</sup> aChiral columns:

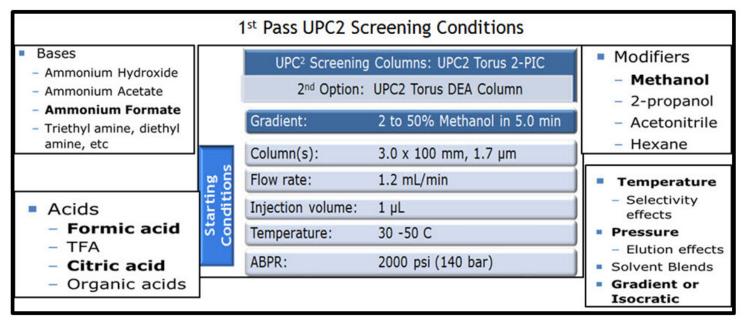
- Torus 2-PIC 1.7um 3.0x100 mm p/n 186007602
- > Torus Method Development Kit 3.0x 100 mm (2-PIC, DEA, Diol, 1-AA) 4 pack p/n 176003580
- Torus Method Screening Kit 2.1x 50 mm (2-PIC, DEA, Diol, 1-AA) 4 pack p/n 176003579
- 2. UPC<sup>2</sup> Chiral columns
  - > Trefoil Chiral Method Development Kit 3.0x150mm 3 Pack (AMY1, CEL1, CEL2) p/n 176003578
  - > Trefoil Chiral Screening Kit 2.1x50mm 3 Pack (AMY1, CEL1, CEL2) p/n 176003577

#### 3. Samples: Quantity 3 for all 3 sample experiments

186007950 UPC2 QC Reference Material
 Test sample for both chiral and achiral modes. Designed for Trefoil, Torus, and Viridis Columns. Four compound mixture in 1 mL diluent of 75:25
 Acetonitrile - Methanol.
 0.50 mg/mL (+/-) trans-Stilbene oxide (chiral separation) test
 0.50 mg/mL Thymine
 0.50 mg/mL Sulfamethoxazole (254 m/z ms performance)
 0.50 mg/mL Sulfamethizole

- 4. Empower 3 UPC<sup>2</sup> Startup project (included):
- 5. Detection: PDA
- 6. Co-Solvents:
  - - B1: MethanolB2: Acetonitrile
    - B2: Acetonithe
      B3: Ethanol or Isopropanol
    - B3: Ethanol of Tsoproparion
       B4: Methanol/Acetonitrile Mix 50:50
- 7. Wash Solvents:
  - Seal Wash: Isopropanol
  - > Weak Needle Wash: Methanol
  - Strong Needle Wash: Methanol: Isopropanol 50:50
- 8. Sample Diluents:
  - > 80:20 Heptane/2-Propanol
  - > 100% Methanol
  - > 75:25 Acetonitrile Methanol
  - > Pipette 1mL of sample into a 5 mL volumetric flask, dilute to volume with above diluents.
- 9. Injection Volume:
  - > 2ul to 8ul (Standard 10ul Sample Loop)
- 10. Instrument method:
  - > Method and Sample Set conditions included in this guide
  - Use the included UPC<sup>2</sup> Empower project for start conditions.

## C: General aChiral System Strategy



### C: Empower 3 Login



1. Double click on the Empower **icon**. The default user name is **System**, and default password is **Manager** (the user name and password may be different if setup by your Empower System Administrator). Click on Run Samples



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

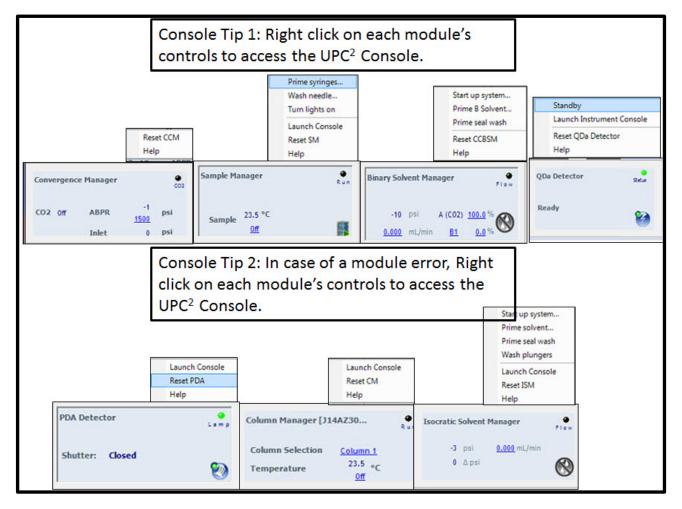
Run Samples			×
Project in which to acquire data:	Use 'Run Samples' to run new samples at your Workstation. Select the desired project and system from the displayed lists. When in the Run Samples Window, use the system control panel to equilibrate your system, or use the Sample Set Wizard to lead you through the process of creating a Sample Set to be run on the system.	Chromatographic ACQUITY UPC2 Alliance 2487	: Systems
		Use QuickStart	Use Open Access Help

- 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. UPC<sup>2</sup> console and direct control functions (details on the next page).
  - E. Real time plot chromatogram and channel display of acquisition.

8	UPC.	2 Svste	em A	in UP	C2 Bas	ic Training as	Bruce/Ad	Iministrator - Editing SS Me	thod: UPC2 Bas	ic Training - Rur	Samples			
F	ile E	dit V	/iew	Inje	ct Act	tions Custor	nize He	lp	Г	2				
1	10	2	8			8	11	0 🔛 🗶 📭 I	6	Α.				
1	Run O	nly			•	Continue on Fa	uit .	Apply Table Preferen	ces Sample S	iet Method			J	
	-						_	Sample Set Method: UPC2 Bas	sic Training					PDA Ch1 254nm@4.8nm -Compens.
1	Plate/		Inj Vol (uL.)	# of Injs	Label	SampleName	Level	Function		od Set / t Method	Run Time (Minutes)	Next Inj. Delay (Minutes)	Blank	₹ 0.02398
1								Equilibrate	UPC2 Gradient	MS	8.00	0.00		0.02396
2	1:A,1 1:A,1		2.0		U0200			Inject immediate Samples	UPC2 Gradient		3.20	0.80	2	34.00 36.00 38.00 40.00 42.00
3	1:A,1		2.0	10	U0201	Gradient		Inject Samples	UPC2 Gradient	Basic Traning	3.20	0.80	Г	Minutes
										В.				Q 0.15560 0.15560 34.00 36.00 40.00 42.00 Minutes
		Single	eλs	ample	es ( S	ample Sets A	Running	<u> </u>	Ŋ					€ 0.13164 0.13163 0.13162 0.13162 0.13162 0.13162 0.13162 0.13162
	0.000	le Mai				e Run	PDA	Detector	L	Instrument Me UPC2 Gradier		00 8P		3
	Sam	ple	10.0 <u>10.0</u>			0	Shu	tter: Open D.	0	Edit	Mon	lor _	Setu	
	Conve	rgend	ce Ma	anage	r	CO2	Colu	imn Manager	Run	Binary Solve	nt Manager	e.	Flow	
	C02	011		nlet	2	2 psi 900 45 psi		lumn Selection <u>Column 1</u> mperature <u>55.0</u>	°C	42 p <u>0.000</u> m		02) <u>98.0</u> 31 2.0	0.00	
Fo	r Help	, press	s F1					Mon	itoring				UPC	2 Basic Training BPR Press 🕅 # 🖉 🖉 37.73

## E: Basic UPC<sup>2</sup> Daily System Startup

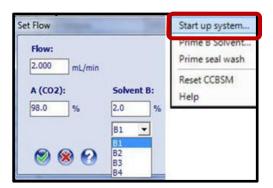
- 1. Before starting the UPC<sup>2</sup> system, make sure the desired sample loop, CO<sub>2</sub>, co-solvent, and samples are ready and placed on the system. Ensure the CO<sub>2</sub> tank is connected and the valve is in the open position.
- 2. Each UPC<sub>2</sub> module has individual controls that 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 CO<sub>2</sub>.



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



5. For standard UPC<sup>2</sup> 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.

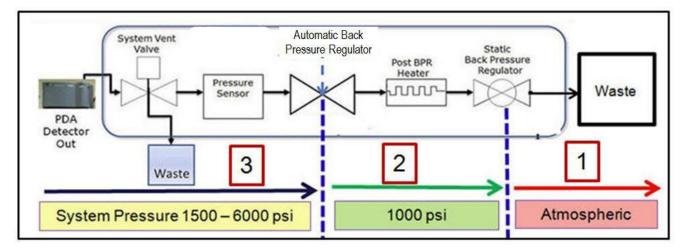
	SM - Sample Manager -	CCBSM – Binary Solvent
	Cycles	Manager Time
Overnight	2 Cycles	1-2 Minutes
Startup	6 Cycles	4 Minutes

System Startup	System Startup
Prime Solvents Optional: Characterize Equilibrate to Method	Prime Solvents Optional: Characterize Equilibrate to Method
SM CCBSM	SM CCBSM
Sample Manager Strong wash Weak wash Sample syringe	Binary Solvent Manager         B Solvent       Seal Wash         Ø Prime B1       Ø Prime B2         Ø Prime B3       Ø Prime B4         Duration of prime:       1.0
Set Defaults Start Clos	se Set Defaults Close

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 UPC<sup>2</sup> column).

System Startup	Name and Address of the Owner, Name	System Startup	And in case of the local division of the
Prime Solvents Option	al: Characterize Equilibrate to Method	Prime Solvents Optional: Characterize	Equilibrate to Method
SM CM CCBSM Other Sample Manager Sample: 10 ° C	SM CM CCBSM Other Column Manager Column: S5 °C Column Selection Column 1 Column 1 Column 2 Bypass Waste		SM CM CCBSM Other
Set Defaults	Start	B2 B3 B4 Close Set Defaults	Start Close

7.  $UPC^2$  operation uses the following 3 step process to keep the  $CO_2$  in a liquid-supercritical state:

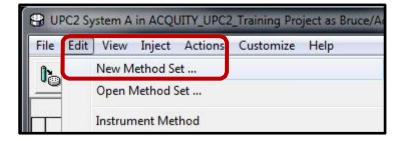


- a. Step 1: Flow rate is turned on and the Static Back Pressure Regulator cartridge builds pressure to ~1100 psi.
- b. Step 2: The ABPR (Automatic or Dynamic Back Pressure Regulator) is activated once the system pressure reaches ~1100 psi and holds the system pressure constant between pressures of 1500 6000 psi (recommended 1700 3000 psi). The ABPR uses a solenoid with a needle and seat.
- c. Step 3: Post ABPR (DBPR) Heat Exchanger heats mobile phase at the outlet of the ABPR to prevent CO<sub>2</sub> from being exhausted as dry-ice.
- Incoming CO<sub>2</sub> 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:
  - a. CO<sub>2</sub> inlet pressures between ~ 650-750 psi are displayed with a "yellow warning icon".
  - b.  $CO_2$  inlet pressures less than ~650 is displayed in red and will not allow system operation.



# F: UPC<sup>2</sup> Instrument Methods and Method Sets

- 1. UPC<sup>2</sup> 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 UPC<sup>2</sup> 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).

Untitled - Method Set Editor		
File Edit View Help		
	Instrument Method	Edit
E D Method Set	Default Processing Method	Edit
	Default Report Method	Edit
Channel Sets Run	Samples Report N	lethod
	Use the wizard to create this new method set?	
	Yes No Cancel	

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".

New Method Set : Select In	nstrument Method Please select the instrument method which is relevant to the data you will be using with this method set.	×
	Create New	
	< Back Next > Cancel	Help

6. The UPC<sup>2</sup> system with PDA has 5 different instrument modules.





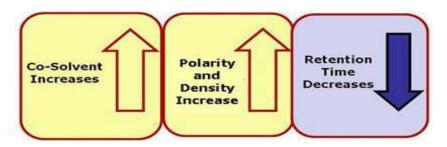
7. Click the ACQ-ccBSM icon to setup the Gradient and Pump conditions. Conditions below are generic for method development using a 3.0 x100cm 1.7um Torus 2-Pic column.

neral [	Data						
Solvents	CO <sub>2</sub>			Pressure Low:		psi	Seal Wash: 1.0 min
31 -	Methanol		- 0	High:	6000	psi	
radien ∕Ω <sup>™</sup>	t: Time (min)	Flow (mL/min)	%A (CO2)	%В	Curve		***
radien	Time		%A (CO2) 98.0	%B 2.0	Curve		Gradient Start:
۸۳	Time (min)	(mL/min)	Constraint and	0.000	0.000		Gradient Start:
<b>⊿</b> ∎ 1	Time (min) Initial	(mL/min) 1.200	98.0	2.0	Initial		Gradient Start: • At injection
2 1	Time (min) Initial 5.00	(mL/min) 1.200 1.200	98.0 50.0	2.0 50.0	Initial		Gradient Start:
1 2 3	Time (min) Initial 5.00 5.50	(mL/min) 1.200 1.200 1.200	98.0 50.0 98.0	2.0 50.0 2.0	Initial 6 1		Gradient Start: • At injection

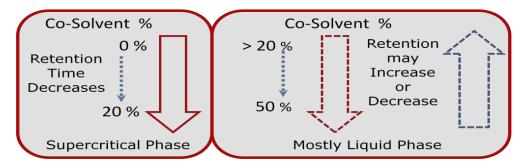
- 8. General ACQ-ccBSM recommendations:
  - a. See the chart below for Torus general flow rate recommendations.
  - b. Use IPA as the pump Seal Wash and frequency of 0.5mls/minute.
  - c. SFC columns should be stored in 100%  $CO_2$ . Use to 100%  $CO_2$  for 3 minutes.

Column	1009	% <b>CO</b> 2	60/40 C	0 <sub>2</sub> /MeOH
Dimensions (mm)	Predicted max flow rate (mL/min)	Predicted max pressure (psi)	Predicted max flow rate (mL/min)	Predicted max pressure (psi)
2.1 x 50 mm	3.20	5943	1.55	5928
2.1 x 75 mm	2.25	5925	1.05	5915
2.1 x 100 mm	1.75	5995	0.80	5949
2.1 x 150 mm	1.20	6030	0.55	6053
3.0 x 50 mm	3.75	4295	2.95	5970
3.0 x 75 mm	3.50	5157	2.10	6026
3.0 x 100 mm	3.25	5938	1.60	5991
3.0 x 150 mm	2.30	5947	1.10	6048

- 9. Gradient recommendations: Start from 2% to 40-60% co-solvent application dependent.
  - > The stronger the polarity of the co-solvent, the shorter the retention time.
  - > Changing the % co-solvent changes:
    - Polarity of the mobile phase (becomes stronger).
    - Density of the mobile phase (becomes greater).
    - Both factors contribute to a decrease in retention time.



- a. Mixtures of CO<sub>2</sub> and co-solvents are supercritical in the column when:
  - > Co-solvent percentages are approximately less or equal to 20%.
  - Co-solvents percentages above 20% may create mixtures that are mostly liquid phase and will affect retention times, but not in a linear fashion.
  - > High % co-solvents affect the flow rate range because of pressure limitations.



b. Example co-solvents are listed below (most common are in Bold):

Solvents	Polarity	Solvents	Polarity
Isobutyl Alcohol	4	1 Propanol	3.9
Tetrahydrofuran	4	Methanol	5.1
Ethyl Acetate	4.4	Ethanol	5.2
Heptane	0.1	Acetonitrile	5.8
n-Hexane	0.1	Isooctane	0.1
Isopropyl Alcohol	3.9	MTBE (methyl tertiary-butyl ether)	1.24
DMSO Dimethyl Sulphoxide	4.4	NMP (N- Methyl Pyrrolidone)	
DMF Dimethyformamide		DMA Dimethy Acetamide	
Heptane/IPA (90:10)		Heptane/IPA (70:30)	
<3% Water		Methanol/IPA (50:50)	

- c. Co-solvent additives (buffer salts, acids and bases) can improve peak shape and change retentivity of polar compounds.
  - > Basic additives improve peak shape and/or elution of basic analytes.
  - > Acids additives improve peak shape and/or elution of acidic analytes.

Recommended additives for Torus Column chemistries	20 mM NH <sub>4</sub> 0H (Ammonium Hydroxide)	20 mM Am. Ac. (Ammonium Acetate)	<b>0.2% TFA</b> (Trifluoroacetic acid)
Torus 2-PIC (2-Picolylamine)	~	—	~
Torus DIOL (High density Diol)	—	~	~
Torus DEA (Diethylamine)	~	_	
Torus 1-AA (1-Aminoanthracene)	~	V	~

10. Select the proper sample diluent your sample.

- a. For aChiral samples use  $\sim 0.2 0.5$  mg/ml concentration.
- b. For Chiral samples use  $\sim 0.4 0.8$  mg/ml concentration.
- c. For polar compounds:
  - Regardless of column type use a non-polar diluent (Heptane)
- d. For non-polar compounds:
  - > If you have a non-polar a-chiral column, use a polar diluent (Acetonitrile)
  - > If you have a polar a-chiral column, use a non-polar diluent (Heptane).
- e. For mixed mode samples that have both polar and non-polar compounds consider:
   > Heptane /IPA (90:10) or Heptane /IPA (90:30) or other mixed mode mixtures.

Solvents	Polarity	Solvents	Polarity
Isobutyl Alcohol	4	1 Propanol	3.9
Tetrahydrofuran	4	Methanol	5.1
Ethyl Acetate	4.4	Ethanol	5.2
Heptane	0.1	Acetonitrile	5.8
n-Hexane	0.1	Isooctane	0.1
Isopropyl Alcohol	3.9	MTBE (methyl tertiary-butyl ether)	1.24
DMSO Dimethyl Sulphoxide	4.4	NMP (N- Methyl Pyrrolidone)	
DMF Dimethyformamide		DMA Dimethy Acetamide	
Heptane/IPA (90:10)		Heptane/IPA (70:30)	
<3% Water		Methanol/IPA (50:50)	



11. Click the UPC2 Sample Manager icon to setup the injections. Conditions below are for generic configuration of UPC2 Injections.

eral Data Events	
Wash Solvents	Temperature Control
Weak Wash Name:	Column: Alarm Band:
Methanol 👻 🔟	Off <u></u> ℃ □ ± 5.0 ℃
Strong Wash Name:	Sample:
MEOH :IPA 50:50 🗨	10.0 🔽 ℃ 🖂 ± 5 ℃
Weak Wash Volume:	
600 μL	Loop Offline:
Strong Wash Volume:	Disable 💌 min
300 μL	Load Ahead
	Active Preheater:
Max Sample Volume: 0.00 µL	Use Console Configuration 🔻

- a. Sample Temperature settings:
  - Sample temperature settings greater than the freezing point of the sample diluent (DMSO diluents temperatures ~20C).
- b. General Wash Solvents:
  - Generic Wash: Use the same solvent for the weak and strong washes as the cosolvent used in the method.
  - > Alternative Wash (sample dependent): 60 MEOH/40 IPA
- c. UPC<sup>2</sup> 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.



12. Click the ACQ-CM icon to setup valve positions and temperatures.

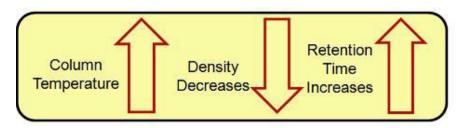
a. Click the "Advanced function to setup columns for 2 separate temperatures.

Column Manager	Column Selection C Advanced
General Data Temperature Column: 35.0 • °C Active Preheater:	✓ Alarm Band: ± 5.0 °C □ Shutdown all columns Jse Console Configuration ▼
Column Selection Valve Position: Column 1	Equilibration Time
External Valve 1: No Change	External Valve 2: External Valve 3: No Change 💌

- b. In the Advanced Column Manager tab. The advanced tab is used to configure 2 different column temperatures (Up to 6 if you have the CM-Aux configurations).
  - Select "Valve Position" of both valves to "No Change". This allows column switching to be selected in the "Sample Set Method".
  - If you select specific valve positons, you will need to make specific instrument methods for each column position.

General   Data   Events	Iumn Selection  Advanced
Valve Position: Left Valve: No Change	- Temperature Control: 1: 35.0 ▼ °C 2: 45.0 ▼ °C
Right Valve: No Change Position 1 Position 2 Position 3 Position 4	3: Off
Position 5 Position 6 Position 7 Position 8	✓ Alam Band: ± 5.0 °C ✓ Shutdown all temperatures

c. General UPC<sup>2</sup> 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 25-65C.
- A column temperature below 35C takes the CO2 out of the supercritical phase, though as long as the pressures are above 1500 psi, the separation should work.



13. Click the UPC<sup>2</sup> PDA icon

to setup wavelength range, resolution and data rate.

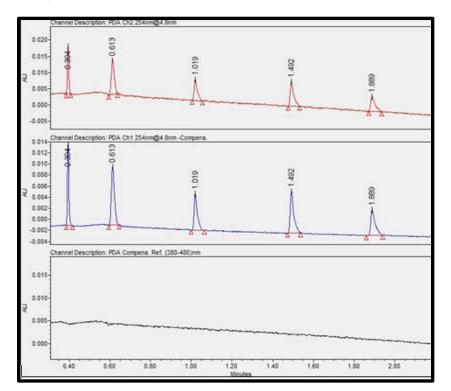
eneral 2D Channels Ar	alog Out   Events	
Lamp On		
🔽 Enable 3D Data		
$\lambda$ Range:	210 nm to 400 nm	
Resolution:	1.2 • nm	
Sampling Rate:	Filter Time Constant: Ex	cposure Time:
20 v points/sec	Normal 💌 0.1000 sec A	uto 💌 msec
Interpolate 2nd order	ilter Region 🔲 Interpolate 656 r	nm Line Region
Use UV blocking filter	(below 210nm) Negative Absorbanc	e Margin: -0.07

- 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 co-solvent.
  - > 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 peaks a minimum or 15 points across a peak).

- > Leave the Filter time at the default settings (automatically adjusts).
- The negative absorbance margin is needed when the absorbance baseline saturates or exhibits a flat, noiseless trace within the chromatogram.
- b. The 2D Channels tab can extract up to 8 individual wavelengths.
- c. The refractive effects of CO<sub>2</sub> can produce baseline disturbances. "Absorbance-Compensated" 2D collection is used to account for refractive index disturbances. Use Compensated Reference of "380-480" to subtract this from the chromatogram.

Channel 2 Absorbance     ✓ 254     4.8     mm resi			054		_	
Absorbance	_	Absorbance -Compensa 💌	254	4.8	-	nm resolution
	hannel 2		254	4.8	•	nm resolution
Absorbance -MBF Max Plot Difference Sum Ratio	hannel 3	Absorbance -Compensated Absorbance -MBF Max Plot Difference Sum				

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

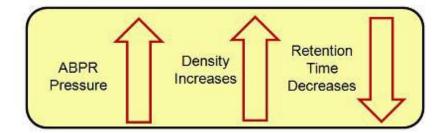




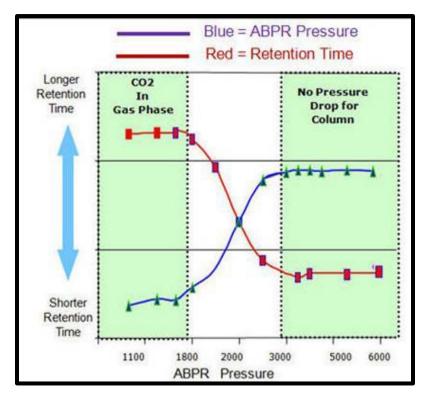
- 14. Click the UPC<sup>2</sup> Convergence Manager used to keep the CO2 in a liquid/supercritical state during the analysis (1500 psi -6000 psi).
  - a. 2000 psi is a good ABPR pressure start point.

Acqu	<u>iit</u> y†u	JPC <sup>2</sup>	Conve	ergence Manager
Gener	al Data	a		
		R Pressure (	- N	?
		e Gradient:	אוכ	
		Time (min)	Pressure (psi)	
	1	Initial	2000	
	2			
	3			
	4			
	5			
	6			
	7			▼
	Commer	it:		

- 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 1600 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 1600-3500. Gradients may be used, though not required.



- g. Select the Data tab of the Convergence Manager.
  - > Select ABPR pressure and CO2 inlet pressure data channels.
  - > These can be used for diagnosing instrument acquisition issues. .

neral Data   elect data channels to a	acquire:	☑ Show diagnostics
Channel	Description	re priow diagnostics
	Pressure of automated backpressure regulator (psi)	
	Pressure of CO2 Inlet (psi)	

15. Select the desired Instrument Name in the list, and then select Next.

New Method Set : Select In	strument Method	×
2 2 2 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2 3 2	Please select the instrument method which is relevant to the data you will be using with this method set.	
	<pre>Cleate New </pre> Cancel He	-lp

16. Select the desired Processing Method and Report Method, and select Next.

Note: The UPC<sup>2</sup> training project has a basic default processing method and report method. For the training select these two methods.

Select Default Methods		×
	Choose methods for processing, reporting, and exporting channels. Processing Method: UPC2 Generic Processing Method	
	Report Method: UPC2 Basic report Export Method: ((No Exporting)	Edit
	< Back Next > Cancel	Help

17. Optionally Click "Define PDA Derived Channels". This may be bypassed since 2D wavelength was selected in the UPC<sup>2</sup> instrument method. Click Next.

Define Derived Channels		X
2	If you wish, you can now define one or more d channel represents a channel that is based (or channel.	
3	Derived channels are useful for applications su ratios, derivatives, or blending.	uch as baseline subtraction,
	Define a derived channel by extracting data from a PDA channel	Define PDA Derived Channel
3 3 3	Define a derived channel by extracting data from an MS channel	Define MS Derived Channel
	Define a channel set by combining data from concentration and viscosity channels	Define GPCV Channel Set
	If you don't want to create a derived channel a	at this time, just click on 'Next'.
	< Back Next >	Cancel Help
	< Back Next >	Cancel Help

18. Save the Method Set and click Finish.

	Method Name:	UPC2 Gener	ic Method Set		
2	Default Comments:	C		-	
3 3 2 2 2 3 3 1	Comments:	Basic Metho	d Set		
2 3	Method Comments:				

19. 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 **Samples** 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.

Hidden Columns	- Column Sizing
Plate/Well	Automatic     Manual
☐ # of Injs ☑ Label ☐ SampleName	Fixed Column
	•
Hide All Show All	

3. Select the Plates icon to select the vial format

	1m
.+	1
11	1 million

4. Select the style of plate and how many samples/standards then click "Insert". ACQUITY UPC<sup>2</sup> sample plates must use ANSI format. Then select OK.

2790 Layout	Create New	v Plate Type	Clear Plates	Plate Sequencing Mode
Plate Ty	/pe Name	Plate	Layout Position	
ANSI-48Vial2mLHc	older 💌	1		A1 A2 A3 (A4) (A5) (A5) (A7) (A8)
		- 22		61         82         83         84         85         86         87         88
		8		0000000
				(F1) (F2) (F3) (F4) (F5) (F5) (F7) (F3)
		23 76		
		- 8		

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

					Sample Set Me	thod: 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	Г
3	1:A,3	3.0	1	UPC2 Training 3	Inject Standards	UPC2 Generic Gradient	4.00	1.00	Г
4	1:A,4	3.0	1	UPC2 Training 4	Inject Standards -	UPC2 Generic Gradient	4.00	1.00	Г
					Inject Standards Inject Samples Inject Controls Inject RF Internal St Inject Immediate Sta Inject Immediate Sa Clear Calibration Equilibrate Report	andards			
	6				Quantitate			*	-

- 6. If the Instrument Method has the column manager configured as "No Change", you can switch columns using the Sample Set Method.
  - a. Column Switching can only occur when using the "Equilibrate" function.
  - b. During column switching, the flow will reduce to no flow, switch the column, and then come back up to initial conditions.
  - c. If the column needs flushed, it is recommend to add a "condition column" row. This runs the gradient without injecting or collecting data.

9	ACQUITY	UPC	2_QDa	_ISM_D	ual in ACQUI	TY_UPC2_QDa_Dual	1 as System/Adminis	strator - Editing SS Method	: QDa_ISM	Test - Ru	n Samples	
F	ile Edit	View	Inje	ct Act	tions Custo	mize Help						
-	<b>b</b> 3	-	0	۲	<u>*</u>	I I I     I I     I	🔛 🗶 🖻	Run and Process	•	Continu	e on Fault	•
								Sa	mple Set Me	thod: QDa_	ISM_Test	
13	Plate/Well	lnj Vol (uL)	# of Injs	Label	SampleName	Function	Method Set / Report or Export Method	Processing	Run Time (Minutes)	Next Inj. Delay (Minutes)	Column Position	
1	1:A,1	2.0	3	S0201	Test Sample	Inject Standards	QDa_UPC2_POS	Don't Process or Report	8.00	0.00		
2				j –		Equilibrate	QDa_UPC2_NEG		8.00		Position 1	
3	1:A,4	2.0	3	S0203	Test Sample	Inject Standards	ISM QDa Test	Don't Process or Report	8.00	0.00		
4	1 11 11	1.0		<u>[</u> ]		Equilibrate	ISM QDa Test		8.00		No Change	-
Г											No Change	
											Position 1 Position 2 Position 3 Position 4 Position 5 Position 6 Position 7	

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 <sup>2</sup> gradient without collecting data
Purge Injector	Purges the UPC <sup>2</sup> Sample Manager
Wet Prime	Primes the UPC <sup>2</sup> BSM with flow using the specified method set.
Sys Prep	Primes the ACQUITY UPC <sup>2</sup> BSM, primes and washes the UPC <sup>2</sup> 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.
  - a. 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.

e	🔤 🔤 🕰	x 🖻	Sam	npleSet Type	STANDA	RDS & UNKI	NOWNS	•						
	Current Vial Row : 7 Vial :		/el:			Type: Uni	known		]					
-	omponents													
3	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 (Vi
	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	Zoloft	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000		2	2			2	ug/ml
3	Penegra	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000							ug/ml
1	Methyl Phenidate	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000		8				8	ug/ml
5	Theophyline	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000		0				0	ug/ml

- b. 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 UPC<sup>2</sup> date with Empower.

Run Sample Set
You have selected lines in this sample set method
Do you wish to :
Inject all rows
C Inject only selected lines
Name for this sample set : UPC Training
Settings for this Sample Set
T Wait For User
Run Mode : Run Only 💌
Suitability Mode : Continue on Fault
Printer : Select Printer
Shutdown Method : UPC2 Generic Gradient
Default Comments:
Comments:
Run Cancel Help

# H: ACQUITY UPC<sup>2</sup> Console and Diagnostics

- 1. The ACQUITY UPC<sup>2</sup> console can be used for diagnostics and system checks of the UPC<sup>2</sup> System.
- 2. Access the UPC<sup>2</sup> console by clicking on the Sample Manager's  $\square$

in the Run Samples window.



- 3. The main console screen has a general status screen for each of the  $UPC^2$  modules.
  - Each module can be selected for more detailed information as well as specific diagnostic checks.

A ACQUITY UPLC Console for	System UPC2 System A on Node M58WIN7CHAN	GE - [System]			• ×
ACQUITY UPLC System     Binary Solvent Manager     Interactive Display	Control Configure Maintain Troubleshoot Help			Acc	Duity
Performance     Sample Manager     PDA Detector     Convergence Manager     Coursegence Manager     Colum Manager     Pots     Maintenance Courters     Logs		4432 psi <u>2.500</u> mL/min PDA Detector	CO2) 98.0 2.0 E1 Convergence Manager	Sample Manager Sample 10.1 ° C 10.0 ° C Room 26.7 ° C Column Manager	Flow Flow Stop Flow Lamp
System Status PDA Detectors LC Data Acquisition: Run Time: 0.0 min 0.0 600.0		Shutter Open	CO2 On ABPR 2001 psi 2000 Inlet 719 psi	Column Selection <u>Column 1</u> Column 555.0 * C <u>55.0</u>	

- 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.
  - Service and method details are sent in a zipped file with no chromatographic data.

ACQUITY UF	LC Console fo	r System UPC2 PP2 o	on Node C20	X_103523 - [System]
Interact Perform Sample Ma PDA Deter Convergen	vent Manager tive Display hance anager ctor tice Manager tive Display anager	Control Configure	Maintan T	Troubleshoot Help Scan instruments Restart Console Service mode Save service profile Connections INSIGHT <sup>m</sup>
Name: Telephone: Email: Description: Here is the issue p	Bruce Wilson 508 478 2000 bruce_Wilson@ Rease review the det		Com	ettings UITY system information puter information and directory information OK Cancel

- 5. UPC<sup>2</sup> BSM Main display screen diagnostic and performance checks:
  - Pressure Ripple (Isocratic conditions) Delta should be <= 1-2% of system pressure. If greater than this, re-prime co-solvent pump and perform the Dynamic Leak Test (step b).</p>



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

ACQUITY UPLC System	Control Configure	Maintain Troubleshoot Help	
Binary Solvent Manager Interactive Display	Control Configure	Heads	
Performance	heads	Dynamic leak test	
Sample Manager     BDA Detector	neaus	Reset pumped volume	
PDA Detector     Convergence Manager		Create log entry	

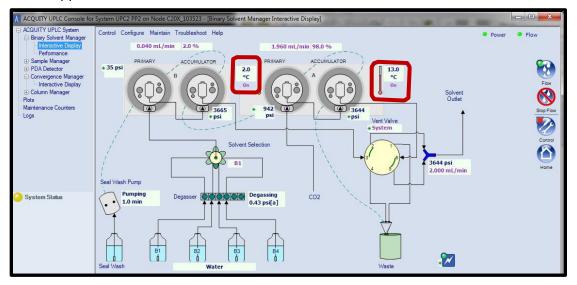
> Select the Pump to test and click Prime. Leave the default test conditions and select "Start".

Accumulator: 5000	psi	Pump	End Point	
Primary: 3000	psi	A (CO2)  B C	Vent Valve   Column	

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.

arameters Status Results					
l l	Accumulator		Primary		
Result:	Passed		Passed		
Leak Rate:	91	nL/min	91	nL/min	
Maximum Pressure:	5052	psi	3065	psi	
Percent of Final Stroke:	27	%	14	0/0	
Compressed Volume:	39	uL	20	uL	
Compression Strokes:	1		1		
Test Attempts:	1			Print	
				1 100 %	
				Start Close	

Use the UPC<sup>2</sup> BSM –Interactive Display to confirm the BSM CO<sub>2</sub> 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. UPC<sup>2</sup> Convergence Manager Main display screen diagnostic and performance checks:
  - ABPR pressures fluctuations should be <= 1% of ABPR pressure settings (\*1). If fluctuations occur above 1% check the following:</p>
    - Check the inlet pump pressure
    - Make sure CO<sub>2</sub> inlet pressure is within acceptable ranges (\*2). Out of range pressures will be colored coded.

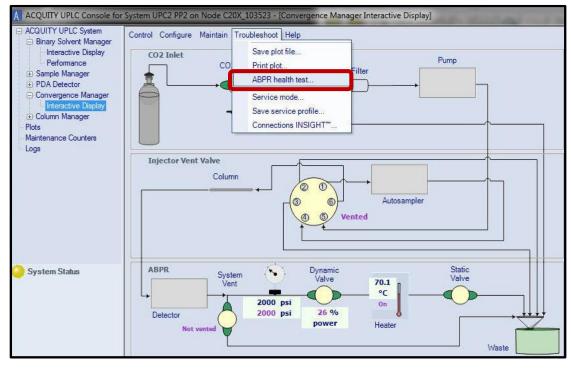


- Check for leaks at the column and all connectors
- Check ambient CO<sub>2</sub> (\*3). If this is high, there may be a leak internal to the Convergence Manager. Contact Waters service.

ACQUITY UPLC System Comparison of the system of the syste	Control Configure Maintain Troc conditions	ubleshoot Help 🔹 Power	• coz
	co2 On	CO2 Inlet 943 psi *2	Stop Flow
	ABPR 1998	DSI CONTRACTOR DE LA CONTRACTOR	Home
	*1 2000	Ambient CO2 542 ppm *3	8
	ABPR Pressure		- Full Ver
	0.0 - ABPR Heater		Unzoom
System Status	ABPR Heater		Unzoon
	0.0- ABPR Heater	[519.4601 ppm at 5/30/2012 9:38 AM]	

• Complete the Convergence Manager's ABPR Health test (step 7)

 UPC<sup>2</sup> 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% CO<sub>2</sub> (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%  $CO_2$  (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.

ABPR Health Test	ABPR Health Test
Setting Column Manager to bypass Run time: 0.0 Minutes 0.0 Results >> Cose	Run time: 0.0 Minutes 0.0 Cose
ABPR Health Test	Results
Stabilizing pressure Run time:	Minimum Pressure Test: Pass Print Pressure at 0% ABPR Power: 1234 psi
0.0 Stop	ABPR Power Test: Pass Pressure at 45% ABPR Power: 4513 psi

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

ACQUITY UPLC System	Control Configure Mai	ntain Troubleshoot Help		ACQUITY UPLC System	Control Configure Maintain Troubleshoot Help	
Binary Solvent Manager     Interactive Display     Performance     Sample Manager     PDA Detector     Convergence Manager	Dates: Content:		System or Module:	<ul> <li>Binary Solvent Manager</li> <li>Interactive Display</li> </ul>		
	Al	Diagnostic •	Current System	Performance	Valve Position	
	records			PDA Detector	Logistical Contraction of the second contrac	
Enteractive Deploy Column Manager Pots Mextension Counters Logs	Date and Time \$/30/2012 9:51 AM \$/1/2012 9:36 AM 4/13/2012 8:34 AM	ABPR Health Test	Instrument ACQ-CCM#A125F ACQ-CCM#A125F ACQ-cc85M#M115	Convergence Manager Extensitive Display Column Manager Notification Marketance Counters Logs	ABPR Pressure	
System Status		nPressureP PowerTestPas	ABPRPressure 4513.0	System Status	5000 0	

10. For other ACQUITY UPC<sup>2</sup> issues, please contact Waters Expert center or your local Waters Field Service Engineer.