Waters UPC ² System opera	tion with Empower 3 with a PDA, QDA and ISM makeup Pump
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ACQUITY UltraPerformance Convergence Technology UPC^{2™} Operation with Empower 3

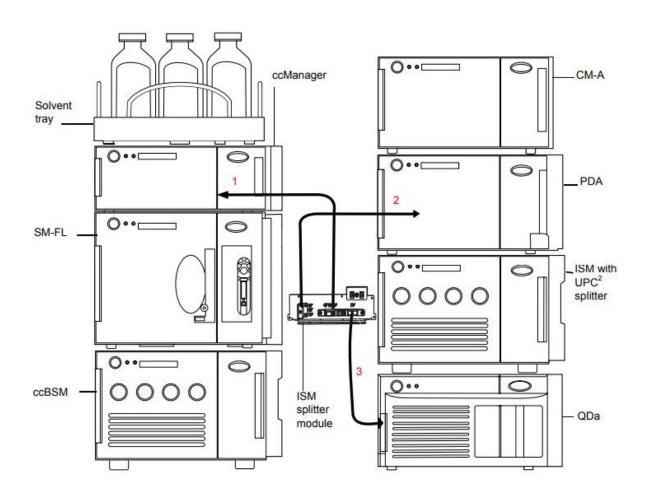


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A: Objective

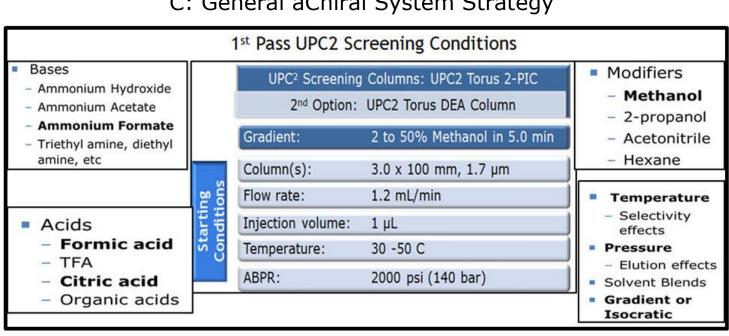
Objective: This is a basic introduction to daily startup, acquisition and system diagnostics for the ACQUITY UPC² PDA system using Empower Software.

B. Supplies and Sample Setup

- 1. UPC² 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. UPC² 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 UPC² Startup project (included):
- 5. Detection: PDA (ELSD or QDA with ISM)
- 6. Co-Solvents:
 - B1: Methanol
 - B2: Acetonitrile
 - > B3: Ethanol or Isopropanol
 - 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^2 Empower project for start conditions.



D: Empower 3 Login



1. Double click on the Empower **icon**. ^{Empower} 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



C: General aChiral System Strategy

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.

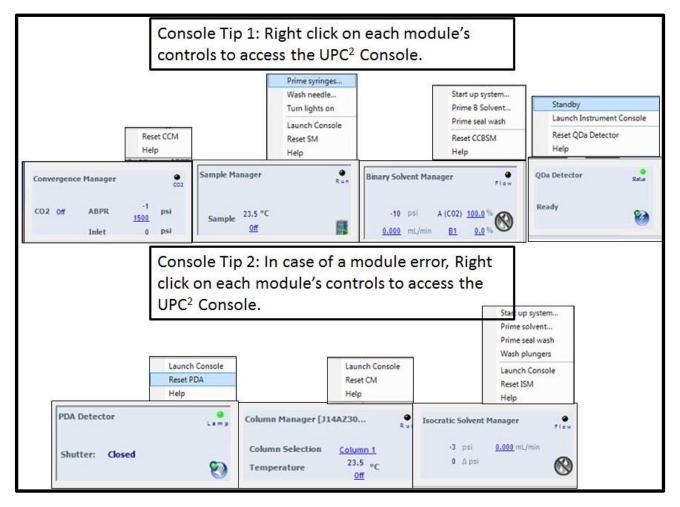
Run Samples			
Project in which to acquire data:	Use 'Run Samples' to run new samples at your Workstation. Select the desired project and system from the displayed lists. When in the Run Samples Window, use the system control panel to equilibrate your system, or use the Sample Set Wizard to lead you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

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

10	UPC2 Sv	stem 4	A in UR	C2 Bas	ic Training as	Bruce/Ad	ministrator - Editing SS Me	thod: UPC2 Bas	ic Training - Run	Samples			
F	ile Edit	View	Inje	ct Act	tions Custor	nize He	lp						
	6/2			0	8	@	1 📓 🗶 📭 I	6	Α.				
1	Run Only			•	Continue on Fa	ut j	Apply Table Preference	nces Sample S	iet Method			J	
L							Sample Set Method: UPC2 Ba	sic Training					PDA Chi 254nm@4.8nm-Compens.
1	Plate/Well	Inj Vol (uL)	# of Injs	Label	SampleName	Level	Function		od Set / t Method	Run Time (Minutes)	Next Inj. Delay (Minutes)	Blank	₹ 0.02398
1	the second secon						Equilibrate	UPC2 Gradient	MS	8.00	0.00		0.02396-
2		2.0		U0200			Inject Immediate Samples	UPC2 Gradient		3.20	0.80	V	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
									B.				₹ 0.15562 0.15562 0.15560 34.00 36.00 38.00 40.00 42.00 Minutes
	I → IA Since	cle λ	Sampl	es (Sa	ample Sets A	Running	C.]	<u>þ</u>					0.13164 0.13162 0.13162 0.13162 0.13162
	Sample M		er 0 °C		Run	Rur	Detector ming itter: Open	. . ,	Instrument Mel	1977		Setu	3
	Sample	10.0	0		0	300	D.	0					
	Converge	nce M	anage	r		Colu	ımn Manager	e Run	Binary Solver	nt Manager	e.	Flow	
	CO2 0ff		ABPR Inlet	2	2 psi 000 45 psi		umn Selection <u>Column</u> mperature <u>55.0</u>		42 P <u>0.000</u> m	si A (C iL/min <u>E</u>	02) <u>98.0</u> 81 2.0	8	
Fo	or Help, pre	ess F1	8				Mor	itoring				UPC	2 Basic Training BPR Press 🕅 # 🖉 🖉 37.73

E: Basic UPC² Daily System Startup

- 1. Before starting the UPC² system, make sure the desired sample loop, CO_2 , co-solvent, and samples are ready and placed on the system. Ensure the CO_2 tank is connected and the value 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.

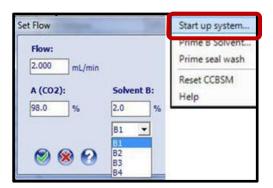


3. For startup, click on the Convergence Manager console's ABPR "Pressure Setting". Enter a pressure of 2000, and then click the check box. The ABPR starts pressurizing once the BSM starts pumping CO₂.

psi



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.

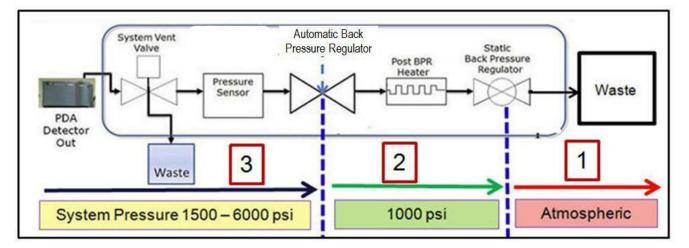
	SM - Sample Manager -	CCBSM – Binary Solvent
	Cycles	Manager Time
Overnight	2 Cycles	1-2 Minutes
Startup	6 Cycles	4 Minutes

nal: Characterize Equilibrate to Method
r trime B2 ime B4

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).

System Startup	Table Terry	- 100 CONT	System Startup	the state of the s
Prime Solvents Option	al: Characterize Equilibrat	e to Method	Prime Solvents Optional: Characterize	e Equilibrate to Method
SM CM CCBSM Other Sample Manager Sample: 10 ° C	SM CM CCBSM Other Column Manager Column: 55 °C	Column Selection: Column 1 Column 1 Column 2 Bypass Waste	 SM CM CCBSM Other Binary Solvent Manager Flow: 2.200 mL/min A (CO2): Solvent B: 	SM CM CCBSM Other
Set Defaults		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:

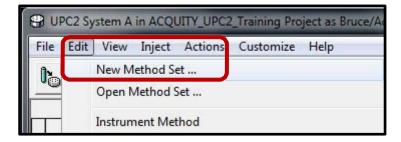


- a. Step 1: Flow rate is turned on and the Static Back Pressure Regulator cartridge builds pressure to \sim 1100 psi.
- b. Step 2: The ABPR (Automatic or Dynamic Back Pressure Regulator) is activated once the system pressure reaches ~1100 psi and holds the system pressure constant between pressures 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 CO₂ from being exhausted as dry-ice.
- 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:
 - a. CO_2 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: 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.



 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 Image: Comparison of the set o		2
□ □ □ Method Set - ☆ Data Channels - ☆ Derived Channels	Instrument Method Default Processing Method Default Report Method	✓ Edit ✓ Edit
	Samples	Report Method
		*

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:	strument Method Please select the instrument method which is relevant to the data you will be using with this method set.	X
	▼ Create New	
	< Back Next > Cancel	Help

6. The UPC² system with PDA, QDA and ISM has 7 different modules.





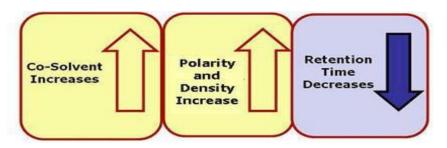
7. Click the ACQ-ccBSM icon to setup the Gradient and Pump conditions. Conditions below are generic for method development using a 3.0 x100cm 1.7um Torus 2-Pic column.

eral [
olvents					re Limits		Seal Wash:
A:	CO2			Low:	0	psi	1.0 min
1 -	Methanol		- 🔟	High:	6000	psi	
							1
adien	t Time (min)	Flow (mL/min)	%A (CO2)	%В	Curve		**
	Time		%A (CO2) 98.0	%B 2.0	Curve		Gradient Start:
٨Ŧ	Time (min)	(mL/min)		80753	0.000000	•	Gradient Start:
⊿ ₽	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:

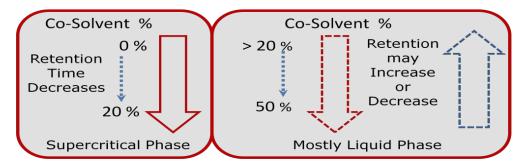
- 8. General ACQ-ccBSM recommendations:
 - a. See the chart below for Torus general flow rate recommendations.
 - b. Use IPA as the pump Seal Wash and frequency of 0.5mls/minute.
 - c. SFC columns should be stored in 100% $CO_2_{\ }$ Use to 100% CO_2 for 3 minutes.

Column	1009	% CO 2	60/40 C	O ₂ /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_2 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. <mark>1</mark>
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 ₄ 0H (Ammonium Hydroxide)	20 mM Am. Ac. (Ammonium Acetate)	0.2% TFA (Trifluoroacetic acid)
Torus 2-PIC (2-Picolylamine)	~	-	~
Torus DIOL (High density Diol)	—	V	~
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. <mark>1</mark>
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.

/ash Solvents	Temperature Control
Weak Wash Name:	Column: Alarm Band:
Methanol	Off
Strong Wash Name:	Sample:
MEOH :IPA 50:50	10.0 ▼ ℃ □ ± 5 °
Weak Wash Volume: 600 µL Strong Wash Volume: 300 µL	Loop Offline: Disable v min Load Ahead Active Preheater:

- 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. 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.



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

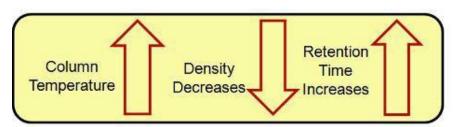
a. Click the "Advanced function to setup columns for 2 separate temperatures.

Column Manager	Column S	election C Advanced
General Data		
Temperature		
Column:	Alarm Band:	
35.0 ▼ °C	± 5.0 °C	Shutdown all columns
Active Preheater:	Use Console Configuration	-
Column Selection		
Valve Position:	Equilibration Time	
Column 1 💌	0.1 min	
External Valve 1:	External Valve 2:	External Valve 3:
No Change 💌	No Change 💌	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.

olumn Manager Seneral Data Events	Iumn Selection Advance	ed
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 °C 4: Off °C 5: Off °C 6: Off °C	
Position 5 Position 6 Position 7 Position 8	Alarm Band: ± 5.0 °C	

c. 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 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 UPC² PDA icon

to setup wavelength range, resolution and data rate.

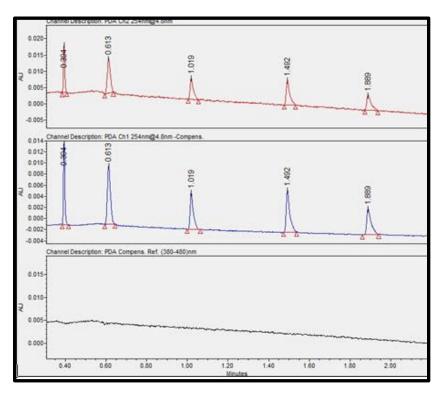
Zamp On		
✓ Enable 3D Data	For the second s	
λ Range:	210 nm to 400 nm	
Resolution:	1.2 v nm	
Sampling Rate:	Filter Time Constant: Expo	osure Time:
20 • points/sec	Normal 💌 0.1000 sec Auto	o 🚽 msec
Interpolate 2nd order	filter Region 🔲 Interpolate 656 nm	Line Region

- 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 CO₂ 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 1	Data mode Absorbance -Compensa -	λ 254	4.8	•	nm resolutio
Channel 2	Absorbance	254	4.8	-	nm resolutio
Channel 3	Absorbance Absorbance -Compensated Absorbance -MBF Max Plot Difference Sum Ratio				
	Max Plot Difference Sum				

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

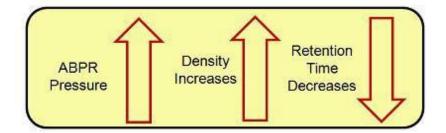




- 14. Click the UPC² Convergence Manager used to keep the CO2 in a liquid/supercritical state during the analysis (1500 psi -6000 psi).
 - a. 2000 psi is a good ABPR pressure start point.

al Dat	a			
	R Pressure (ON		
	e Gradient:			
	Time (min)	Pressure (psi)		
1	Initial	2000		
2	-			
3	-			
4	-		÷	
5			4	
6				
7			-	

- 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.
- 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.



- Blue = ABPR Pressure Red = Retention Time Longer CO2 Retention **No Pressure** In Drop for Time **Gas Phase** Column Shorter Retention Time 1100 1800 2000 3000 5000 6000 ABPR Pressure
- 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. .

elect data channels to		☑ Show diagnosti
Channel ABPR Pressure	Pressure of automated back	preseure regulator (pei)
	Pressure of CO2 Inlet (psi)	pressure regulator (psi)



- 15. Click the ACQUITY QDA detector to setup MS acquisition. If working with unknowns, use the default acquisition settings for both positive and negative switching.
 - a. If you have prior knowledge of you compounds use positive mode or negative mode only for better sensitivity and faster data rates.

Note: For optimal sensitivity keep the ABPR in the lower range (<2200 psi). A smaller split ratio will result in greater MS signal generated per mass of injected analytes.

	_ Mode	
QDa [™] Detector	Mass Detector	C Advanced
General SIR Events		
I Operate	?	
MS Scan		
Mass Range:		
100 Da- 600 Da		
Cone Voltage		
▼ Positive Scan		
▼ Negative Scan		
✓ Negative Scan 15 V		
General		
Sampling Rate		
Target 10 💌 points/sec Actual 7.1	points/sec	
Capillary Voltage		
Positive 1.5 kV		
Negative 0.8 kV		
	100	

b. Click "Advanced" to input SIR data for your specified compounds. Remember to input the mono-isotopic mass of the molecule. Use the default cone voltage to start. Increased voltage may lead to higher fragmentation.

)Da™	1 Detector				-Mode C Mass Detector C Adva	inced				Function Details fo
unctions	s Events			2			Chann		R: ES+, 0	.00 min - 1.00 n
	15				Hide Function Details	?		Name	Mass (Da)	Cone Voltage (V)
		Polarity	Start (min)	Stop (min)	Run Time: 1.00 min		1	Acetamino	151.20	15
1	SIR	Positive	0.00	1.00	SIR of 2 masses		2	Sildenafil	475.20	15
2							3			
3				1			4			
4							5			
5							6			
6					*******		7			
7							8			
8							9			
9				ļ		-	1	- t :		
Samp	ling Rate				Сарі	llarv				
Tar	rget 10	points/s	sec Actua	l <mark>10.0</mark> points/s		kV				
Comment	t:				Probe 600 °C Neg 0.8	kV				



- 16. Click the ACQUITY ISM makeup pump. The makeup pump is used to aide in MS ionization and adding to the flow because of evaporation of the CO2.
 - a. Typically makeup solvents can be acidic (Formic, Acetic) or basic (Ammonium Formate Ammonium Carbonate).
 - b. Additives can be in higher concentrations as it is post column.
 - c. The defaults settings below are a good starting point.

Solvent					Pressu	ire Limits -	-	?
90	:10 MeOH:	water 1% 💌		Change 👻	Low:	0	psi	1.1
90 Ac Ac	:10 Water:A etic Acid etonitrile etonitrile + (vater 1% FA ICN).1% Formic A Combination E	•	15000 sh Period:	psi	min		
ow Grad	dient:							
٨P	Time	Flow (mL/min)	Curve	<u> </u>				
1	Initial	0.450	Initial					**)
2	7.00	0.000	11					
3				-1				

- d. An alternative is to create a flow rate gradient that decreases the amount of makeup flow as the % co-solvent increases. High co-solvents require less makeup flow.
- e. Make sure to prime the ISM before operation.

		CC BS	M Gra	dient	ISN					
Gr	adient				Flow Gradient					
Γ	Z۳	Time (min)	Flow (mL/min)	%A (CO2)	%B	Curve	[] []	Time	Flow (mL/min)	Curv
Г	1	Initial	1.200	97.0	3.0	Initial	1	Initial	0.450	Initia
	2	1.00	1.200	90.0	10.0	6	2	1.00	0.450	6
	3	2.50	1.200	70.0	30.0	6	3	2.50	0.200	6
	4	4.00	1.200	40.0	60.0	6	4	4.00	0.100	6
	5	5.00	1.200	97.0	3.0	6	5	5.00	0.450	11

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

New Method Set : Select In	strument Method	X
	Please select the instrument method which is relevant to the data you will be using with this method set. UPC2 Generic Gradient	
	< Back Next > Cancel H	lelp

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

Note: The UPC^2 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

Operation of the Waters UPC² System with Empower 3 Page # 22 19. Optionally Click "Define PDA Derived Channels" and or "Define MS Derived Channels". This may be bypassed since 2D wavelength or SIR channels were selected in the UPC² instrument method. Click Next.

Define Derived Channels	and the loss of the	X
2 1 1 2 3 3 1	If you wish, you can now define one or more de channel represents a channel that is based (or channel. Derived channels are useful for applications su ratios, derivatives, or blending.	derived) from a direct data
	Define a derived channel by extracting data from a PDA channel Define a derived channel by	Define PDA Derived Channel
	extracting data from an MS 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	t this time, just click on 'Next'.
	< Back Next >	Cancel Help

20. Save the Method Set and click Finish.

	Method Name: UP	C2 Generic Method S	iet	
2	Default Comments:			
	Comments: Bas	sic Method Set		
	Method Comments:			

21. 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.

Fonts Colors Hidden Columns Plate/Well Inj Vol	Column Sizing Automatic Manual
# of Injs W Label SampleName W Level Function Hide All Show All	Fixed 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.

2790 Layout	Create Ne	w Plate Type	Clear Plates	Plate Sequencing Mode
Plate Ty	ype Name	Plate	Layout Position	
ANSI-48Vial2mLHa	lder v			A1 A2 A3 A4 A5 A6 A7 A8 B1 B2 B3 B4 B5 B6 B7 B8 C1 C2 C3 C4 C5 C6 C7 C8 C1 C2 C3 C4 C5 C6 C7 C8 C1 C2 C3 C4 C5 C6 C7 C8 C1 D2 C3 D4 C5 C6 C7 C8 C1 D2 C3 D4 C5 C6 C7 C8 C1 D2 C3 D4 C5 D5 D7 C8 C1 C2 C3 E4 C5 C6 C7 C8 C1 C2 C3 C4 C5 C5 C7 C8 C1 C2 C3 C4 C5 C7 C8 C7 C8 C1 C2 C3 C4 C5 C7 C8 C7 C8 C7 C8 C7

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

					Sample Set Me	thod: Untitled			111		
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			<u>^</u>			
					Inject Immediate St	nject Controls nject RF Internal Standards nject Immediate Standards nject Immediate Samples Clear Calibration Equilibrate Report					

- 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.

Fi		View	a tin i		tions Custo		1 as System/Admini	strator - Editing SS Metho	d: QDa_ISM	_Test - Ru	n Samples
0	6 6	8	0	۲	<u>*</u>	10 H	🔛 🗶 🖻	Run and Proces	18 💌	Continu	e on Fault
								5	ample Set Me	thod: QDa	ISM_Test
	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:A,1	2.0	3	S0201	Test Sample	Inject Standards	QDa_UPC2_POS	Don't Process or Report	8.00	0.00	í i
2	l í				1	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	i I
4						Equilibrate	ISM QDa Test		8.00		No Change 💌
											No Change
0 0 0 0											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 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.
 - 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.

	Component Editor												C	
File	le Edit View He	lp												998.999 - 1997.99
0) <mark>.</mark>	<u>*</u> * B	Sam	npleSet Type:	STANDA	RDS & UNKI	NOWNS	•						
	Current Vial Row: 7 Vial: 1:B,1 Level: Type: Unknown													
C	Components													
	Value													
8		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	Zoloft	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000			1				ug/ml
3	Penegra	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000	2	-			2		ug/ml
4	Methyl Phenidate	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000							ug/ml
5	Theophyline	0.000000	1.600000	4.000000	8.000000	12.000000	16.000000							ug/ml
1.23			2									2	2) 10	C 6
•	Image: Second											Cancel		

- 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 UPC² 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
C Settings for this Sample Set
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 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 \square





- 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	r System UPC2 System A on Node M58WIN7CHAN	GE - [System]			• ×
ACQUITY UPLC System Binary Solvent Manager Interactive Display	Control Configure Maintain Troubleshoot Help			Acc	buity
Performance Sample Manager PDA Detector Convergence Manager Column Manager Picts Maintenance Counters Logs		Binary Solvent Manager 4432 psi <u>2,500</u> mL/min PDA Detector	CO2) 98.0 2.0 <u>2.0</u> <u>51</u> Convergence Manager	Sample Manager Sample 10.1 ° C 10.0 Room 26.7 ° C Column Manager	Flow Stop Flow Lamp
System Status PDA Detector: LC Data Acquisition: Run Time: 0.0 min 0.0 600.0		Shutter Open	СО2 Оп АВРР 2001 ры Зліет 719 ры	Column Selection <u>Column 1</u> Column 555.0 ° C <u>55.0</u> ° C	

- 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 U	PLC Console fo	r System UPC2 PP2 on	Node C20	0X_103523 - [System]
Interact Perform Sample M PDA Dete Converger	vent Manager tive Display nance anager ctor nce Manager tive Display anager	Control Configure	Maintan	Troubleshoot Help Scan instruments Restart Console Service mode Save service profile Connections INSIGHT" Shutter Closed
Name: Telephone: Email: Description: Here is the issue p	Bruce Wilson 508 478 2000 bruce_Wilson@		Con	ettings CUTY system information apputer information hod and directory information OK Cancel

- 5. UPC² 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 Performance Sample Manager PDA Detector		Heads
	heads	Dynamic leak test
		Reset pumped volume
Convergence Manager		Create log entry

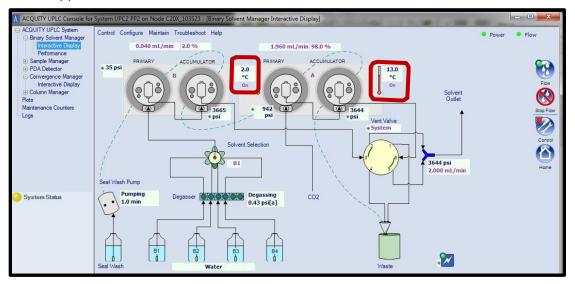
> Select the Pump to test and click Prime. Leave the default test conditions and select "Start".

Pressure Accumulator: Primary: 3000 psi Retry the test if it fails, up to a ma	Pump A (CO2) B C Prime	End Point 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.

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

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:
 - ABPR pressures fluctuations should be <= 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.

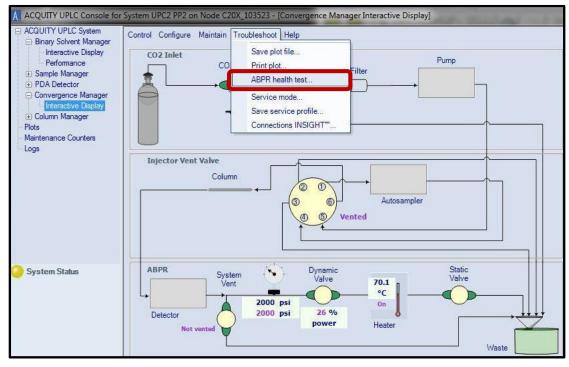


- Check for leaks at the column and all connectors
- Check ambient CO₂ (*3). If this is high, there may be a leak internal to the Convergence Manager. Contact Waters service.

ACQUITY UPLC System Binary Solvert Manager Interactive Display Performance Sample Manager PDA Detector	Control Configure M	Aaintain Troublesh	oot Help				🕈 Pot	er • CO2
 Convergence Manager Interactive Display Column Manager 	CO2	On	CO2 Inlet	943 psi	*2			Stop Flor
Plots Maintenance Counters	ABPR	1998 _{psi}	Heater	70.1 °⊂				Home
Logs	*	1 2000 M	Ambient CO2	542 ppm	*3			8
	ABPR Press			~				Full Ver
System Status	70.00-1							_
System Status	-	02			519.4601 ppm at 5/3	10/2012 9:38 AM		=

• Complete the Convergence Manager's ABPR Health test (step 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% \mbox{CO}_2 (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% \mbox{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 ABPR Health Test	Run time: 0.0 Minutes Stat 0.0 Cose
Stabilizing pressure	Results Minimum Pressure Test: Pass 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.

Contraction of the Associated Association and the second second	System UPC2 PP2 on Node C20X_103523 - [Logs]	ACQUITY UPLC Console for System UPC2 PP2 on Node C20X_103523 - [Plots]
ACQUITY UPLC System Brary Solvent Manager Interactive Display Performance	Control Configure Maintain Troubleshoot Help Dates: Content: System or Module	and during proping
Sample Manager	Al Diagnostic Current System	Performance Valve Position
PDA Detector Convergence Manager	records	Sample Manager Sample Manager PDA Detector
Interactive Display	Date and Time Diagnostic Instrument S/30/2012 9:51 AM ABPP Health Test ACO-CCM#A1255	Convergence Manager
Plots Maintenance Counters	5/30/2012 9:51 AM ABPR Health Test ACQ-CCM#A12SF 5/1/2012 9:36 AM ABPR Health Test ACQ-CCM#A12SF	Columo Manager
Logs	4/13/2012 8:34 AM Dynamic Leak Test ACQ-cc85M#M115	ABPR Pressure
		Logs
		0.0
	• • •	System Pressure
2		
System Status		· · ·
		1000.0-
ų		Flow Rate
1	details of current record	System Status
	ZeroPowerTes MinPressureP PowerTestPas ABPRPressure	
	true 1234.2 true 4513.0	

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