

Column Switching on Hybrid SFC Systems

Technical Note

This technical note describes the methods required to perform automated column switching in SFC/LC and SFC/LC/MS hybrid systems.

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Introduction

Abstract

Agilent recommends that columns be dedicated for either SFC or HPLC use in the hybrid system. Following this recommendation provides the simplest, fastest, and most robust means for mode and column switching. However, there may be cases where the shared use of a single column for both SFC and HPLC operation is required. This shared operation can be accomplished by creating and using intermediate (switch) methods that provide the proper conditioning of the column when switching modes. The methods required to perform the automated switching are described herein.

Background

In SFC, the mobile phase is a combination of dense, high pressure carbon dioxide (CO₂) and an organic modifier. The CO₂ in the mobile phase is kept dense by a backpressure regulator located after the detector. As the CO₂ decompresses from its dense state to a lower pressure gaseous state, it experiences significant decompressive cooling. In normal SFC operation, this cooling is contained and mitigated in the back pressure regulator. However, in a hybrid system, mode switching an active column from SFC mode to HPLC mode will bypass the back pressure regulator and allow the decompressive cooling to occur within the column bed. Should this cooling of the column bed occur in the presence of a liquid with a melting temperature near ambient (such as water or acetic acid), the cooling could result in the liquid freezing and forming a solid blockage within the column. Flushing or filling the column with a significant concentration of methanol before the mode switching is performed greatly reduces the probability of developing a blockage.

The preparation of a shared use column for switching can be automated and easily performed within a sequence. This is accomplished by the creation of two methods, one for switching to SFC, and one for switching to HPLC. The purpose of these methods is to provide a mechanism for altering the mobile phase composition in either mode to be methanol rich to allow the presence of water or modifiers containing additives to be removed from the column before the SFC/HPLC mode valve changes position. When switching to SFC from HPLC, flowing (for a period of five column volumes of) 50 % methanol has been shown to be effective prior to changing modes. When switching from HPLC to SFC, five column volumes of neat methanol is recommended prior to the mode switch.

SFC Mode

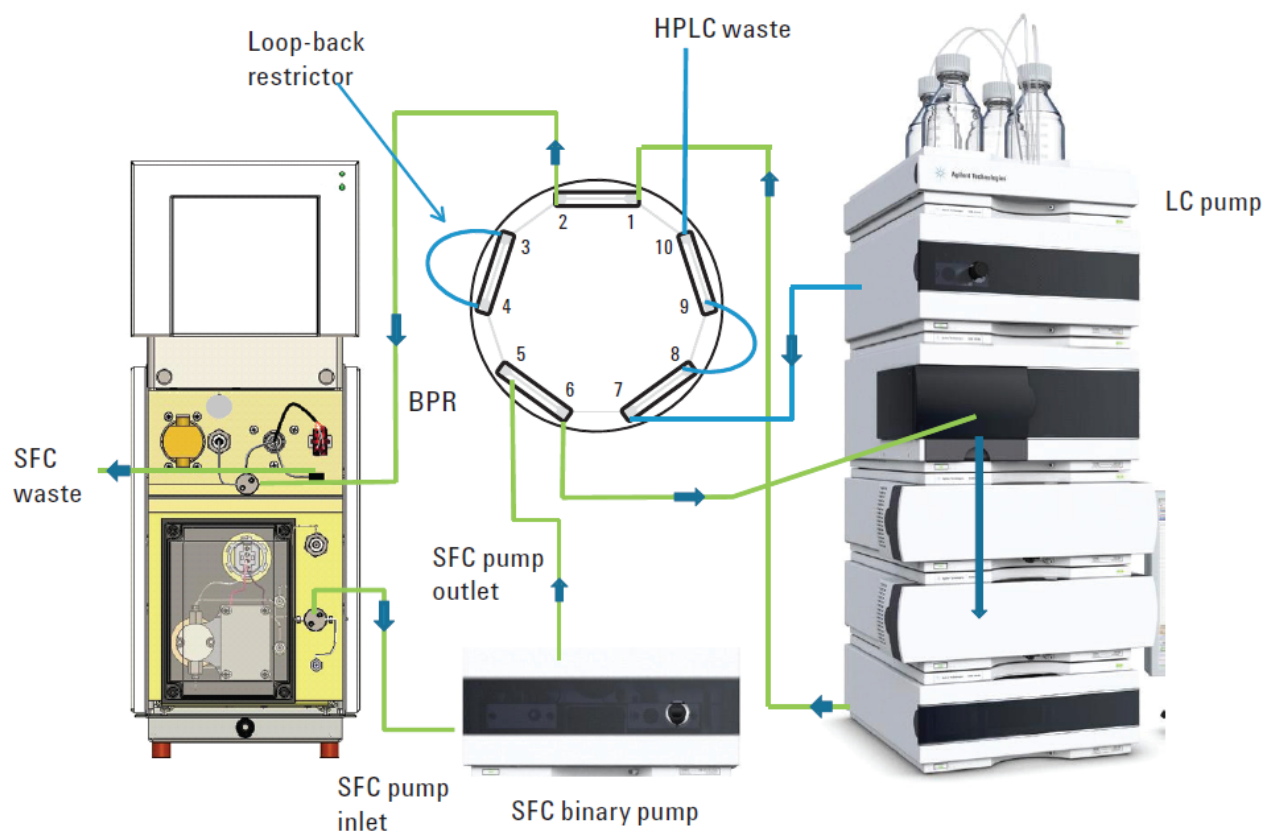


Figure 1 Agilent 1260 Infinity SFC/UHPLC in SFC mode

The method settings for the mode valve provide selections for switch operation that allow this to be easily automated. In the method, the position of the valve can be specified to change when the method loads, when the method ends, or at various times within the method (timetable). We will use these selections to place the mode valve in the correct position, with columns correctly conditioned, at the end of a run utilizing the switch method. During this run, the HPLC pumps, and SFC pumps will operate at reduced flow rates with appropriate solvent composition specified.

While not specifically shown in each example, these strategies apply equally well for the SFC/LC/MS Hybrid. In the examples where operation of the mode switch valve is shown, duplicate the basic method for the MS selection (2pos 6 port) valve. Both valves should follow the same positioning guidelines and same timetable switch settings.

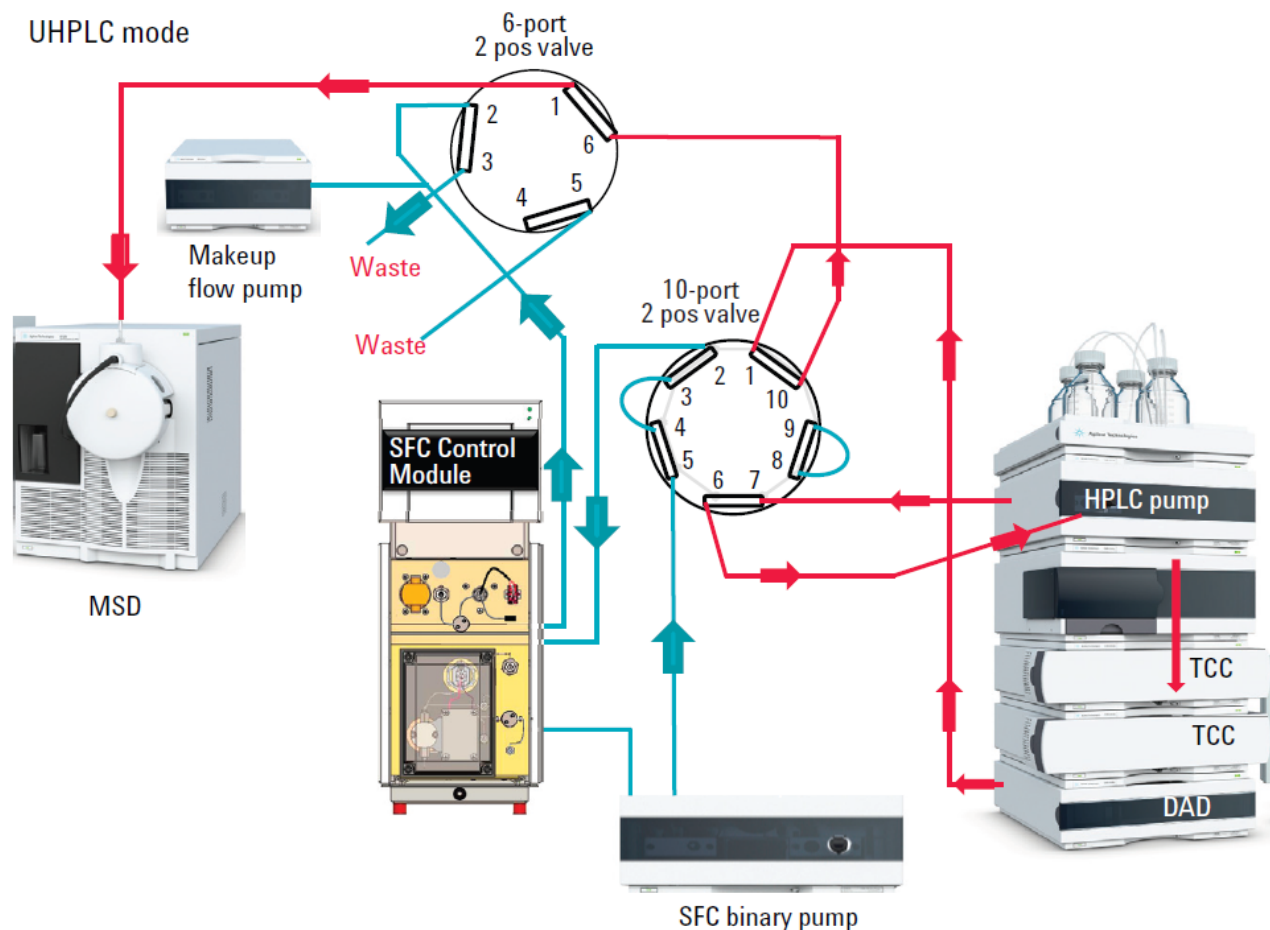
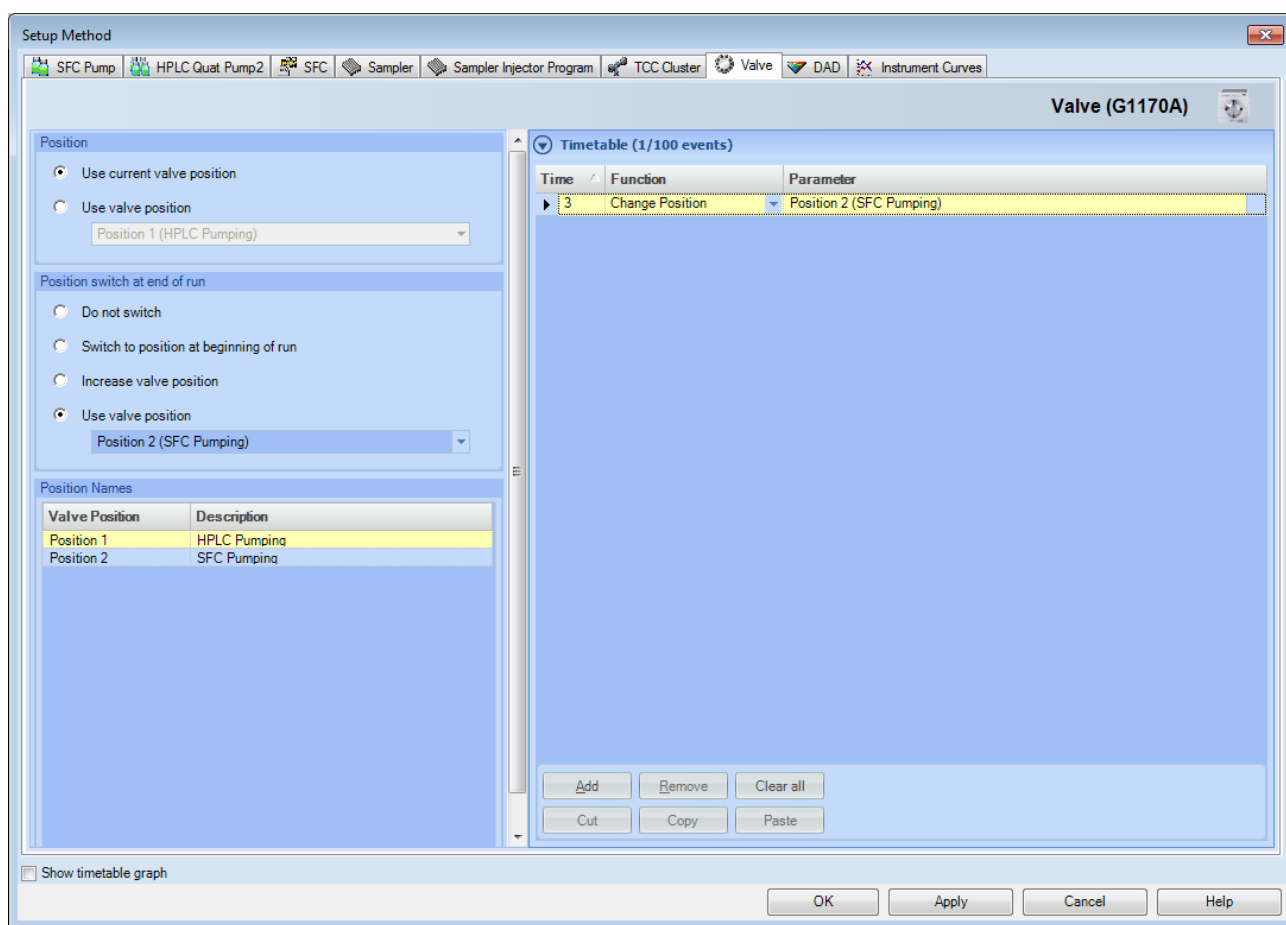


Figure 2 Schematic of the hybrid system in UHPLC/MS mode

Switching From HPLC to SFC

When operating in HPLC mode, the column may contain significant amounts of water. This water could freeze in either the column or near the backpressure regulator upon mode switching. Our strategy is to have the HPLC-2-SFC.M method flow the HPLC pump at 0.5 mL/min with 100 % methanol before switching. During this time, the SFC pumping system will begin to flow at 1.0 mL/min with 50 % methanol. After a period of time, the mode valve will switch signaling the entry into SFC operation. In the example shown below, we have chosen this period of time to be 3.0 min. With longer, large diameter columns, this time should be increased to ensure that at least five column volumes of methanol traverse the column before the mode switch.



The switch method is predicated on the mode valve remaining unchanged upon loading of this method and subsequently changing to SFC mode after a run time of 3.0 min. At the end of the method, the valve is left in the SFC mode. By having the valve position remain initially unchanged and positively switching to SFC mode, there is no harm in running this method from either SFC or HPLC modes – the initial valve position will not matter.

Switching From HPLC to SFC Background

Setup Method

HPLC Quat Pump (G1311A)

Flow: 1.000 mL/min

Solvents:

- A: 0.0 % water
- B: 100.0 % methanol
- C: 0.0 %
- D: 0.0 %

Pressure Limits: Min: 0.00 bar, Max: 400.00 bar

Stoptime: As Injector/No Limit (1.00 min), 1.00 min

Posttime: Off (1.00 min), 1.00 min

Timetable (empty)

Time [min]	A [%]	B [%]	C [%]	D [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	0.0	100.0	0.0	0.0	1.000	400.00

Buttons: Add, Remove, Clear All, Clear Empty, Cut, Copy, Paste, Shift Times, 0.00 min

Show timetable graph

OK, Apply, Cancel, Help

Initially upon method load, and throughout the duration of this method, we will have the HPLC pump flowing neat methanol. This methanol will displace any water or mobile phase additives in the column before the mode switch. After the mode switch, this flow will be directed through the HPLC bypass restrictor to waste.

The screenshot displays the 'Setup Method' window for an SFC Pump (G4302A). The interface includes several control panels:

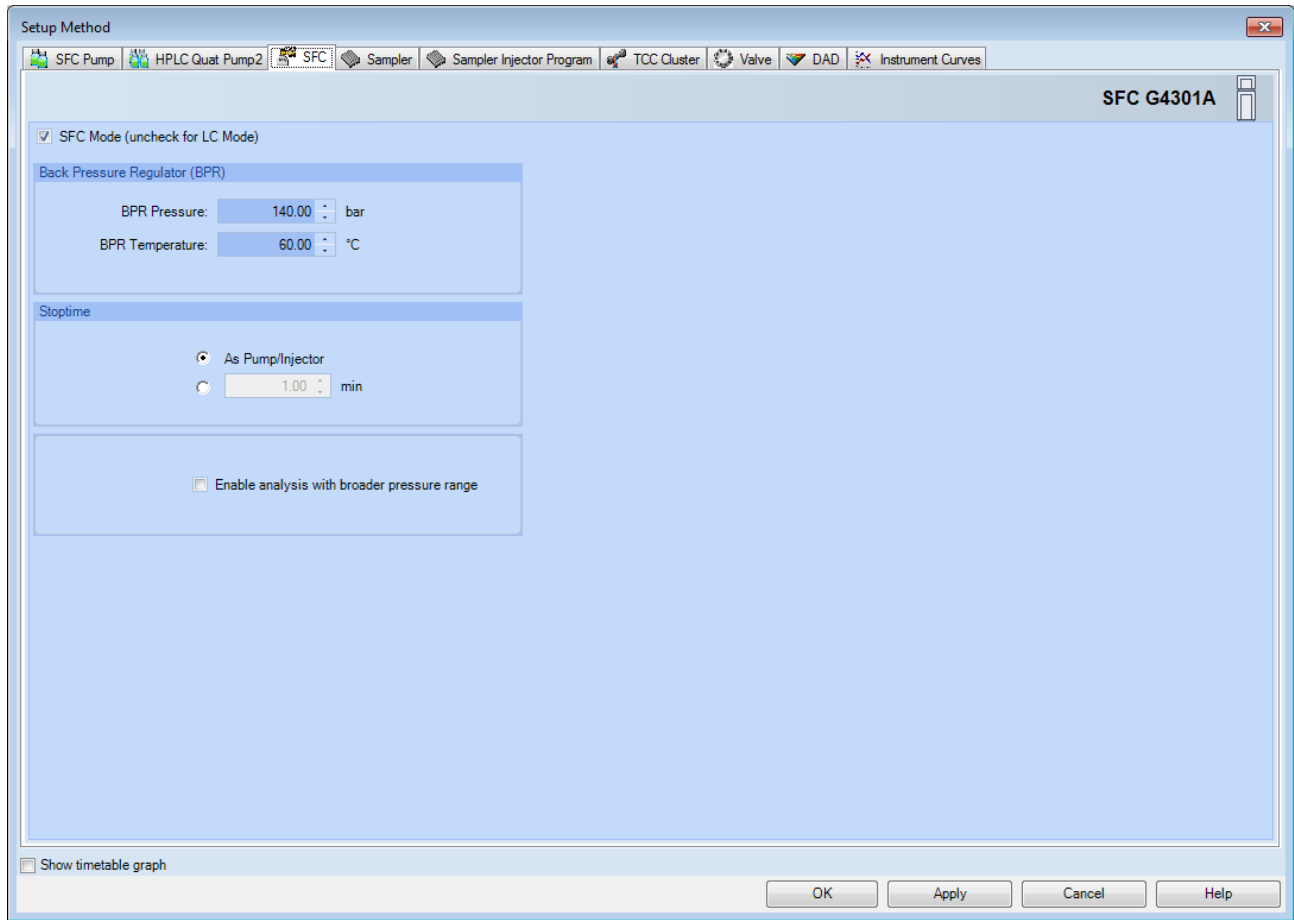
- Flow Rate:** Set to 1.000 mL/min.
- Solvents:**
 - A: 50.0 % CO2 (pre-compress) CO2
 - B: 50.0 % MeOH methanol
- Pressure Limits:** Min: 0.00 bar, Max: 400.00 bar.
- Top/Posttime:**
 - Top: As Injector/No Limit, 1.00 min
 - Post: Off, 1.00 min
- Timetable (4/184 events):**

Time [min]	A [%]	B [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	50.0	50.0	1.000	400.00
3.00	80.0	20.0	1.000	---
4.00	80.0	20.0	1.000	---

At the bottom of the window, there are buttons for 'Add', 'Remove', 'Clear All', 'Clear Empty', 'Cut', 'Copy', 'Paste', 'Shift Times', and a '0.00 min' input field. The 'Show timetable graph' checkbox is also present.

The SFC binary pump is set up to initially pump a flow of 50 % methanol at a flow rate of 1.0 mL/min. Initially, this flow will be directed through the SFC bypass restrictor allowing the SFC pumping system to pressurize and control. Upon the mode valve's switch at 3.0 min, our SFC flow which will be directed through the column is reduced to 20 % methanol.

Switching From HPLC to SFC Background

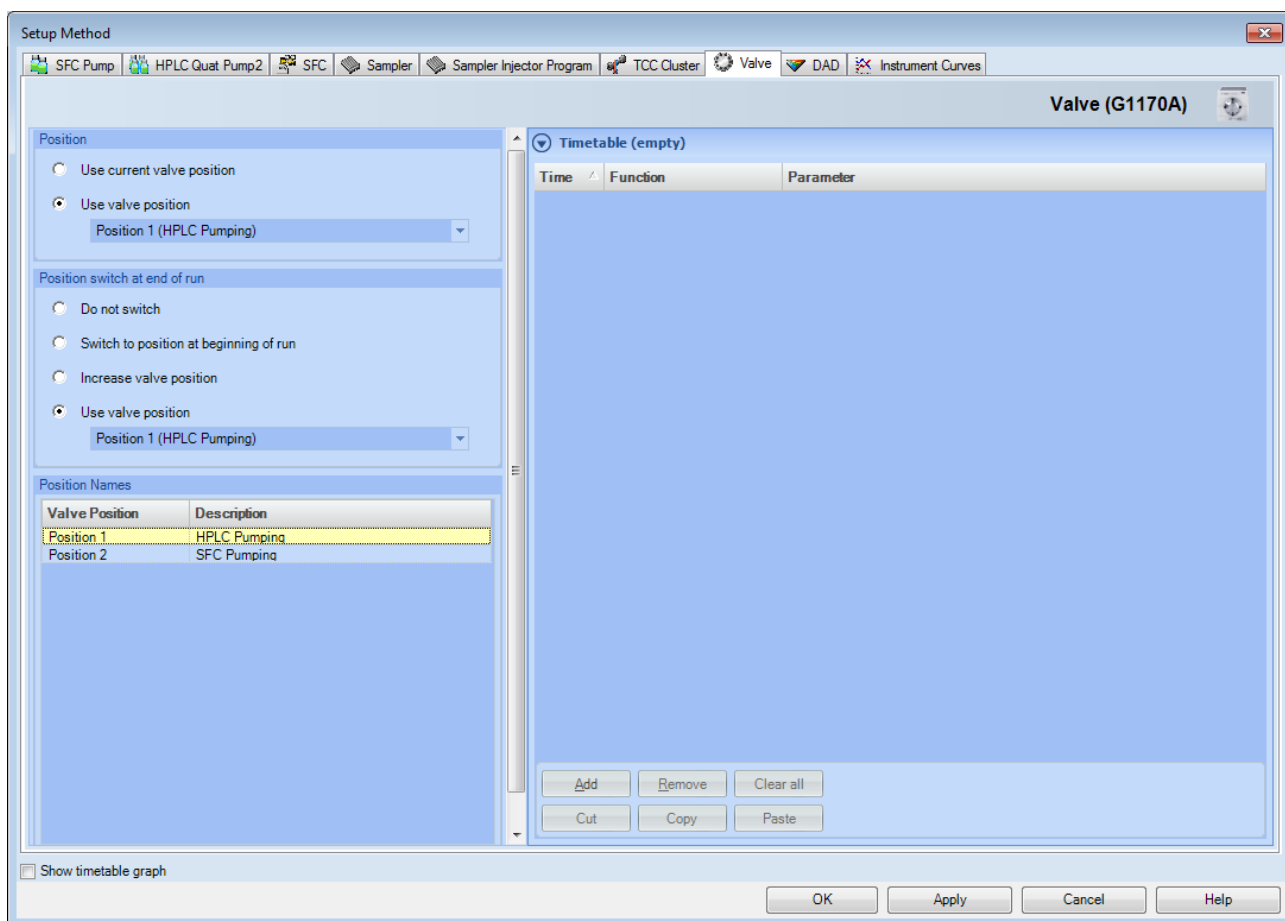


The settings for the SFC control module dictate that this method is operated in **SFC Mode**. This selection shall allow the SFC control module to automatically turn on (if previously in standby) when this method is loaded. As such, the SFC system will begin to pressurize and flow appropriately. While this method may be automatically associated with a sequence run, the run will not begin until after the SFC control module becomes ready. This is beneficial as it allows additional time for the HPLC pump to flow methanol (flushing the column) before the start of the run.

Subsequent to running this switch method, the SFC system will be fully operational and pumping 20 % methanol through the column. The Mode valve will be in the SFC position, and any HPLC flow will be directed down a bypass path. Any subsequent SFC methods may load with their respective setpoints for this column without further intervention. Further, any subsequent SFC methods may specify their HPLC flow rates to be set to 0.0 mL/min.

Switching from SFC to HPLC

In general, switching from SFC operation to HPLC operation is less demanding. Any mobile phase from the HPLC pump will easily displace the rapidly exiting SFC mobile phase as the mode valve is switched. Provided sufficient methanol is present in the HPLC mobile phase, blockages are unlikely.



In switching to HPLC operation, we will not require timetable programming of the injection valve. In this switch, we will simply place the valve into HPLC mode upon method load and have it remain in HPLC mode subsequent to the run.

Switching from SFC to HPLC Background

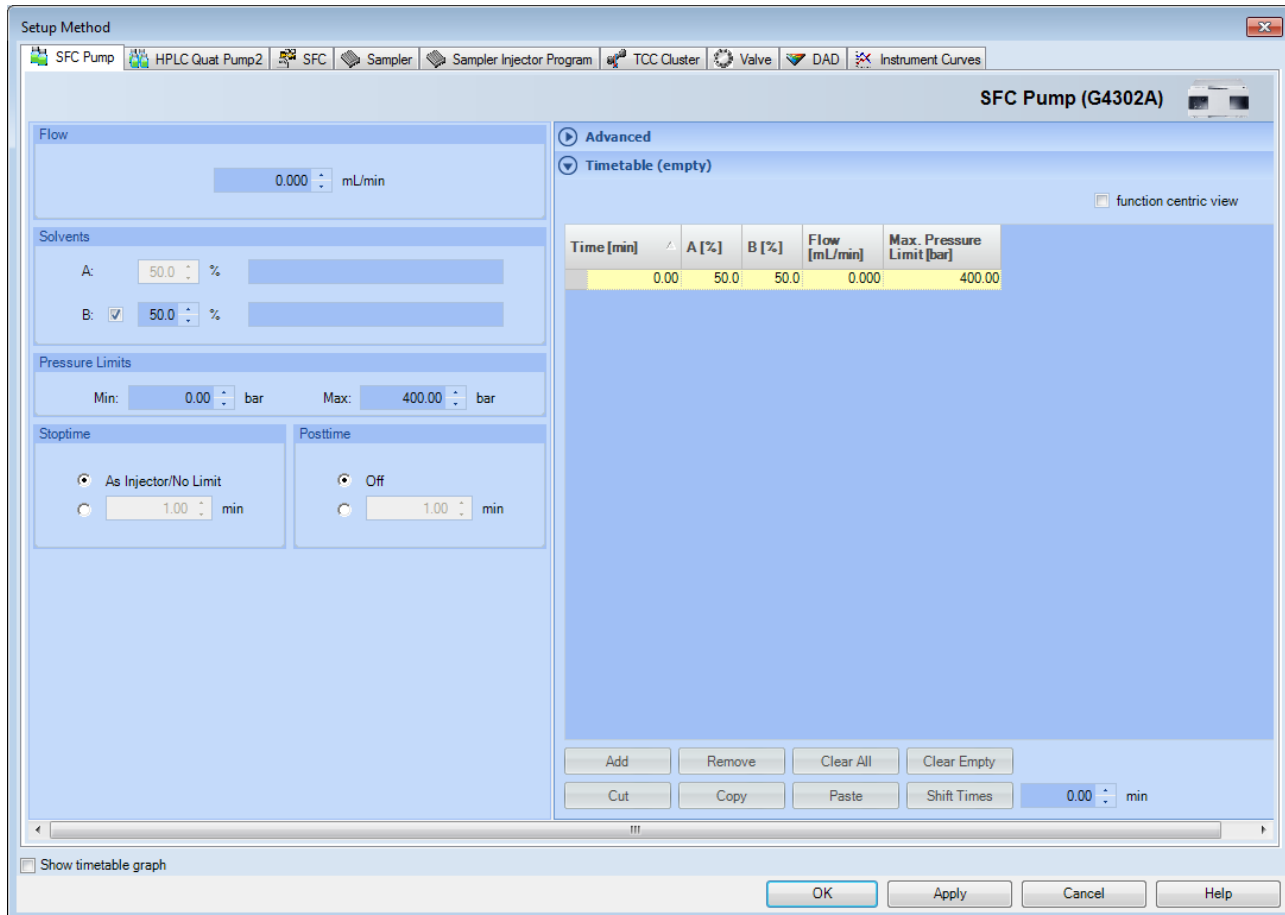
The screenshot shows the 'Setup Method' dialog for the 'HPLC Quat Pump (G1311A)'. The 'Flow' is set to 1.000 mL/min. Under 'Solvents', solvent B is selected with a concentration of 100.0%. The 'Pressure Limits' are set to a minimum of 0.00 bar and a maximum of 400.00 bar. The 'Stoptime' is set to 'As Injector/No Limit' and the 'Posttime' is set to 'Off'. The 'Timetable (empty)' table contains one entry:

Time [min]	A [%]	B [%]	C [%]	D [%]	Flow [mL/min]	Max. Pressure Limit [bar]
0.00	0.0	100.0	0.0	0.0	1.000	400.00

Buttons at the bottom include 'Add', 'Remove', 'Clear All', 'Clear Empty', 'Cut', 'Copy', 'Paste', 'Shift Times', and a '0.00 min' field. The 'OK', 'Apply', 'Cancel', and 'Help' buttons are at the bottom right.

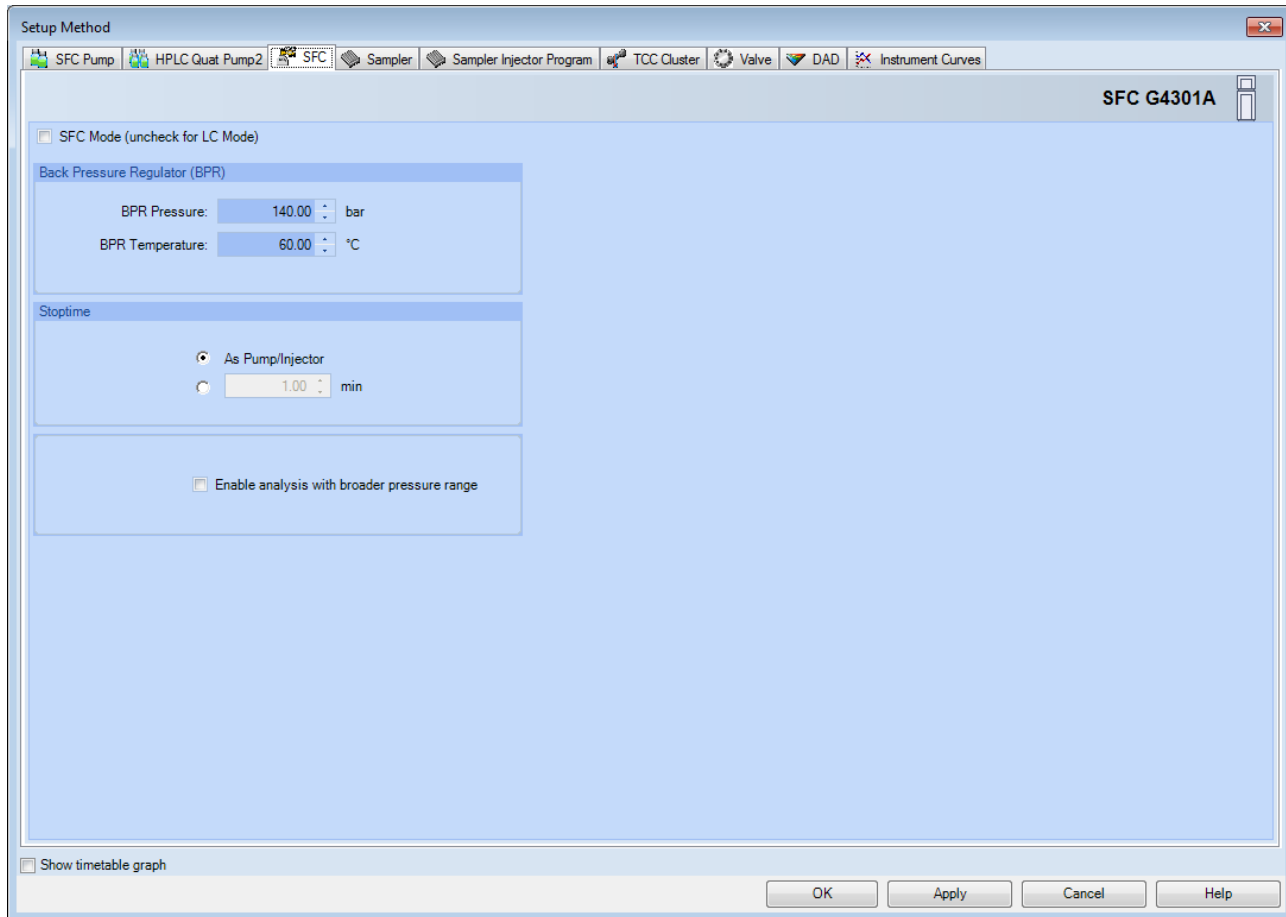
The HPLC pump will begin flowing 100 % methanol upon method load. This flow rate will be maintained throughout the run.

In our example, we will operate isocratically through the duration of the run. However, it is possible to create a compositional gradient that gradually decreases the composition of methanol after 1.0 min within the run. After 1.0 min, a series of timetable entries can be placed to gradually increase the flow and composition of the normal HPLC solvents. In some instances, this may be beneficial to provide additional column regeneration time. This can be accomplished by increasing the runtime of this method.



Our SFC binary pump will be set to a flow of 0.0 mL/min. As the valve switch to HPLC mode occurs with the loading of this method, there is no need to maintain any SFC flow.

Switching from SFC to HPLC Background



Without requiring SFC flow in this switch to HPLC method, this method is operated in LC mode. Thus, the **SFC Mode** is not selected. This will place the SFC control module in standby upon method load.

Sequence Based Mode Switching

Using the strategy of the switch methods developed above, a sequence can be created to automatically alter the operating mode while using the same column for both SFC and HPLC modes. An example is shown below:

Sequence Table: Hybrid

Currently Running
Line: Method: Vial: Inj:

Sample Info

Line	Vial	Sample Name	Method Name	Inj/Vial	Sample Type	Cal Level	Update RF	Update RT	Interval	Sample Amount	ISTD Amount	Multiplier
1		switch	SW-SFC-2-HPLC	1	Blank							
2	VIAL 1	ttc	SW-HPLC	1	Sample							
3		switch	SW-HPLC-2-SFC	1	Blank							
4	VIAL 1	ttc	SW-SFC	1	Sample							
5		switch	SW-SFC-2-HPLC	1	Blank							
6	VIAL 1	ttc	SW-HPLC	1	Sample							
7		switch	SW-HPLC-2-SFC	1	Blank							
8	VIAL 1	ttc	SW-SFC	1	Sample							
9		switch	SW-SFC-2-HPLC	2	Blank							
10	VIAL 1	ttc	SW-HPLC	1	Sample							
11		switch	SW-HPLC-2-SFC	2	Blank							
12	VIAL 1	ttc	SW-SFC	1	Sample							

Insert Cut Copy Paste Append Line Undo All Reprocess Sequence

Insert/FillDown Wizard Undo Wizard Custom Fields OK Cancel Help

C:\CHEM32\1\DATA\SWITCH\SWITCH 2013-07-26 13:08:44\SW-HPLC.M Configure Table

In this example sequence, we will run a 'blank' run with each switch method. Subsequent to the mode switch, normal method processing in that mode may occur.

For manual operation outside a sequence, the switch methods can be loaded and blank runs initiated.

While this note has dealt with a single column being shared, the same strategy can be applied to multiple columns within a TCC Cluster. In such a setup, a switch method will need to be created for each column. It is recommended to run a switch method in a sequence prior to subsequent analytical runs to ensure that the proper column conditioning has taken place to prevent blockages.

Sequence Based Mode Switching
Background



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