Agilent 1260 Infinity Analytical Hybrid SFC/UHPLC system

Shared use of Columns - Technical Note

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

Contents

Abstract 1

Introduction 2

Switching From HPLC to SFC 4

Switching from SFC to HPLC 6

Sequence Based Mode Switching 7

Usage with Mass Spectrometer equipped systems 8

Summary 8

Abstract

With the Agilent 1260 Infinity Analytical Hybrid SFC/UHPLC system columns are usually dedicated for either SFC or UHPLC use. 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 UHPLC operation is required. This shared operation can be accomplished by creating and utilizing intermediate (switch) methods that provide the proper conditioning of the column when switching modes. The methods required to perform the automated switching are described in this Technical Overview.

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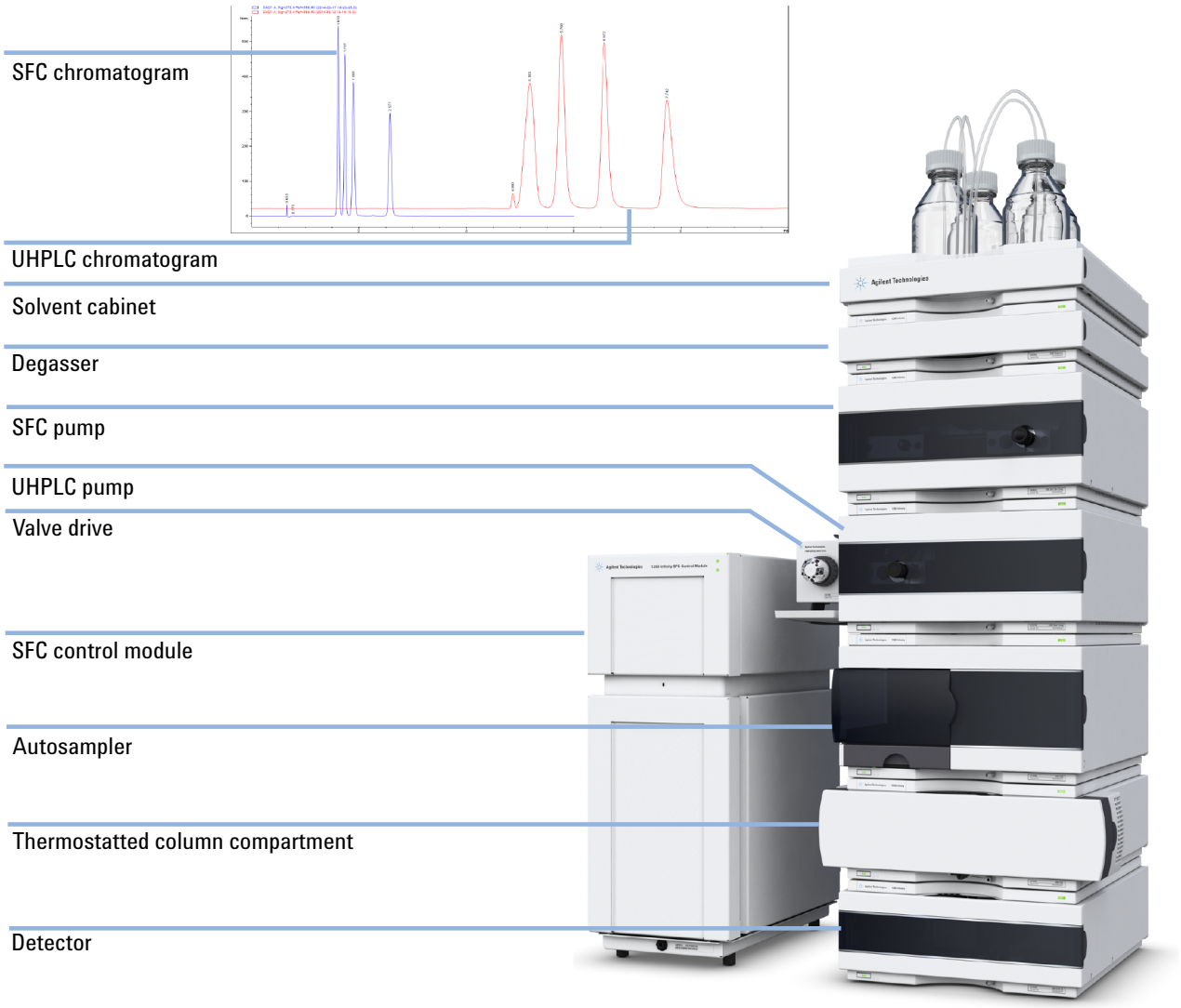

Figure 1 Agilent 1260 Infinity Analytical Hybrid SFC/UHPLC system - SFC (blue) and UHPLC (red) chromatograms attained from a shared column

Introduction

The Agilent 1260 Infinity Analytical Hybrid SFC/UHPLC allows chromatographic analysis in either SFC or UHPLC modes of operation. The system contains a *mode* selection valve to automatically place the system in the correct configuration for either UHPLC or SFC analyses. This valve configures the flow path and delivery of mobile phase fluids to the sampler, columns, and detectors. Normally, the columns are dedicated for either SFC or HPLC use as this provides the simplest, fastest, and most robust means for mode and column switching. When a single column is required for use in both SFC and UHPLC operation, a procedure must be followed to change the operating mode in order to ensure the column contains compatible fluids.

In SFC, the mobile phase is a mixture of dense, high pressure carbon dioxide (CO_2) and an organic modifier. The CO_2 in the mobile phase is kept dense by a backpressure regulator located after the detector. As the CO_2 decompresses from its dense state to a lower pressure gaseous state, it experiences significant decompressive cooling. In normal SFC operation (depicted in Figure 3 on page 4), this cooling is contained and mitigated by a back pressure regulator located after the detector in the SFC control module. In UHPLC operation (depicted in Figure 2 on page 3), this back pressure regulator is not required and the columns vent to waste. When an active column is switched from SFC to UHPLC mode, bypassing the back pressure regulator, decompressive cooling may occur within the column bed. Should the cooling of the column bed occur in the presence of low melting temperature liquids (such as water or acetic acid), the liquid may freeze resulting in formation of a solid blockage within the column. Flushing the column with a significant concentration of methanol prior to switching modes greatly reduces the probability of column blockage.

This process of switching modes while preventing freezing in a shared column can be automated and easily integrated into a sequence. Two methods are required, one for switching to SFC, and one for switching to UHPLC. The purpose of the methods is to provide procedures to alter mobile phase composition to be methanol rich in order to reduce water or modifiers in the column before the SFC/UHPLC mode valve changes position.

NOTE

For information, which modules are used in the stack, see Figure 1 on page 2.

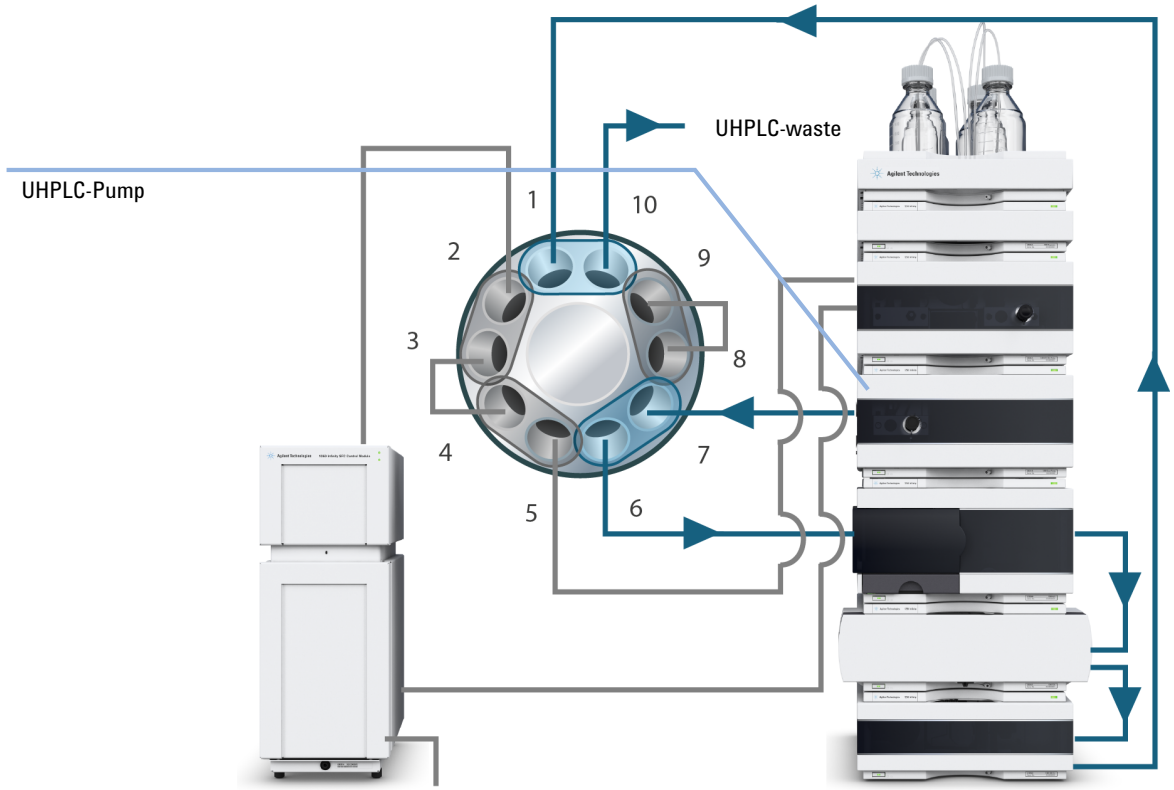

Figure 2 Hybrid SFC /UHPLC system in UHPLC mode (Mode valve in Position 1)

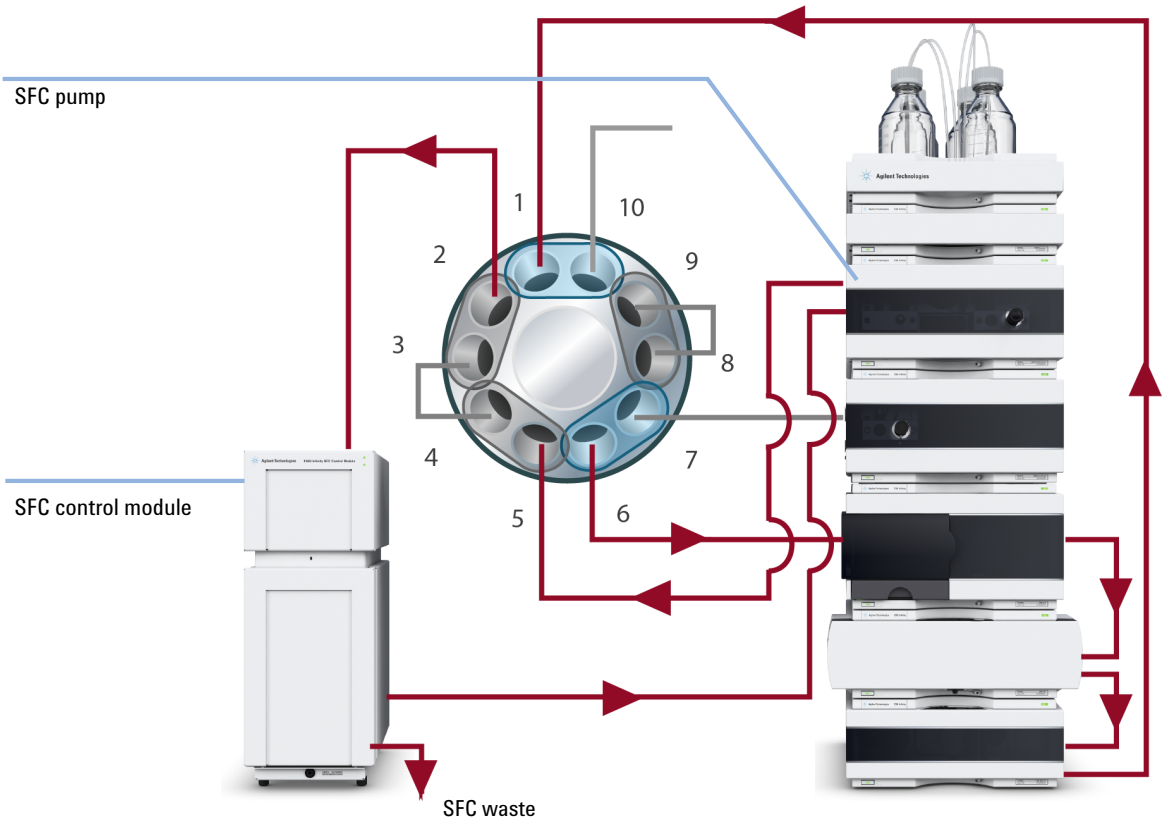

Figure 3 Hybrid SFC /UHPLC system operating in SFC mode (Mode valve in Position 2)

Switching From HPLC to SFC

When operating in UHPLC mode, the column may contain significant amounts of water. If not appropriately flushed when switching to SFC mode, the water could freeze in either the column or near the back pressure regulator. The solution is to flush the UHPLC flow path using the UHPLC pump flowing at 0.5 mL/min with 100 % methanol prior to switching modes. Simultaneously, the SFC pump will flow at 1.0 mL/min with 50 % methanol, effectively flushing the SFC flow path. After the period of time (specified by the valve timetable entry) a timetable event will switch the UHPLC/SFC mode valve, transitioning to SFC operation. Having displaced the water from both flow paths with the flushing, freezing should not occur. In the example shown in the method below, we have chosen this period of time to be 3.0 min to flush the flow paths prior to switching modes. With longer or larger diameter columns, the flushing time may be increased to ensure at least five column volumes of methanol traverse the column prior to the mode switch. This flushing time is adjusted by modifying the time of the valve timetable event.

This switch method should be run after an UHPLC method and would perform the necessary functions to seamlessly change to SFC mode. The settings and timetable of this method are shown in Table 1 on page 5. In this method, the UHPLC/SFC mode valve position initially remains unchanged. At the end of the

method, the valve is switched to SFC mode and the system flow is directed through the SFC flow path. By having the valve position initially remain 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 or operating mode will not matter.

Table 1 Method Settings for Switching from UHPLC to SFC

	Method Initial Settings	Method Setting at 3 min	Method Setting upon End of Run
Mode Valve	Use Current Position	Time Table Change Position 2 SFC Pumping	 Position 2 SFC Pumping
UHPLC Pump Flow	0.500 mL/min	0.000 mL/min	--
UHPLC Pump %B	100 % B(Methanol)	--	---
SFC Binary Pump Flow	1.0 mL/min	--	--
SFC Binary Pump %B	50 % B(Methanol)	Time Table Change 20 % Methanol	
SFC Method	Checked		
Run Time	4.0 min		

Setting the SFC Method checkbox with the SFC Control Module's Method Editor is required, and dictates that this method is to be operated in SFC Mode. This selection will allow the SFC control module to automatically turn on (if previously in standby) when the method is loaded. Accordingly, the SFC system will begin to pressurize and flow immediately upon the loading of the method. 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 UHPLC pump to flow methanol (flushing the column) prior to the start of the run.

Initially upon the loading of this method, the UHPLC pump is flowing to displace any water or mobile phase additives in the UHPLC flow stream. After the mode switch, the UHPLC flow stream is directed through the UHPLC bypass restrictor to waste and may be reduced to 0.0 mL/min. The SFC binary pump is setup to begin pumping at a reduced flow rate to avoid overpressures when the SFC flow stream is first directed through the column. Subsequent to the mode change, an additional timetable event may be created to adjust the SFC mobile phase flow rate, solvent, and composition to be compatible with the initial conditions of subsequent analyses.

Switching from SFC to HPLC

When switching from SFC to UHPLC operation, mobile phase from the UHPLC pump will easily displace the rapidly exiting SFC mobile phase remaining in the column. Provided sufficient methanol is present in the UHPLC mobile phase, blockages are unlikely. To ensure this, the solution is to begin the delivery of the UHPLC mobile phase with neat methanol to completely flush the UHPLC flow path. Having pumped enough methanol to displace any remaining SFC mobile phase within the column, the UHPLC mobile phase composition can be programmed (ramped) to a more aqueous composition. It is unnecessary to flush the SFC flow path after the mode valve is switched to UHPLC mode. Any entrained CO_2 will expand and expel through the back pressure regulator.

Table 2 Method Settings for Switching from SFC to UHPLC

	Method Initial Settings	Time Table at 1 min	Time Table at 3 min	Method Setting upon End of Run
Mode Valve	Use Position 1 HPLC Pumping			Position 1 HPLC Pumping
UHPLC Pump Flow	1.000 mL/min	1.00 mL/min	1.00 mL/min	--
UHPLC Pump %B	100 % B Methanol	100 % B Methanol	80 % A (water) 20 % B	---
SFC Binary Pump Flow	0.0 mL/min			--
SFC Binary Pump %B	50 % B Methanol			--
SFC Method	Not Checked			
Run Time	4.0 min			

Switching to UHPLC operation, does not require time table programming of the mode valve. The mode valve may be switched to UHPLC mode immediately upon method load.

The UHPLC pump will start pumping with a flow of 100 % methanol upon method load. The flow rate of 1.0 mL/min may be maintained throughout the method as specified by the method's run time. A time table entry for 1.0 min is utilized to specify the length of time the pump flushes with neat methanol.

In this example, a compositional gradient will gradually decrease the composition of methanol subsequent to the 1.0 min mark. A time table entry for 3.0 min specifies the result of a gradual change in the composition of the UHPLC flow.

In some instances it may be beneficial to provide additional column regeneration time at the composition specified in the 3.0 min time table entry. To do so, the method's run time may be increased to allow for the additional regeneration time at the composition specified in the 3.0 min time table entry. In this example, a 4.0 min run time provides 1.0 min after the 3.0 min time table entry to perform this regeneration.

With the mode valve switch to UHPLC mode occurring by the loading of this method, there is no need to maintain any SFC flow. The SFC binary pump will be set to a flow of 0.0 mL/min and placed in Standby by leaving the SFC Method checkbox in the SFC Control Module's Method Editor un-selected.

Sequence Based Mode Switching

Using the solution detailed by 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 UHPLC modes. An example is shown in the sequence in Table 3 on page 7. Within this sequence, the method named *SW_2_SFC* refers to the setpoints and method from Table 1 on page 5, and the method named *SW_2_UHPLC* refers to the setpoints and method from Table 2 on page 6.

Table 3 Sequence Table demonstrating mode switching

Vial	Sample Name	Method Name	Inj/Vial	Sample Type
		SW_2_UHPLC	1	Blank
Vial 1	UHPLC sample	UHPLC	1	Sample
		SW_2_SFC	1	Blank
Vial 1	SFC sample	SFC	1	Sample
		SW_2_UHPLC	1	Blank
Vial 1	UHPLC sample	UHPLC	1	Sample
		SW_2_SFC	1	Blank
Vial 1	SFC sample	SFC	1	Sample

In this example sequence, we will run a *blank* run with each switch method. By not specifying a vial position and selecting a sample type of *blank* within that sequence entry, the method will run without performing an injection. Subsequent to the mode switch, any number of method based runs for that mode may occur.

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

While this Technical Overview 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 as the first entry in a sequence prior to subsequent analytical runs to ensure that the proper column conditioning has taken place and to reduce the possibility of blockages.

Usage with Mass Spectrometer equipped systems

The Agilent 1260 Infinity Analytical Hybrid SFC/UHPLC/MS is described in the application note: *Analysis of antioxidants in vegetable oils using the Agilent 1260 Infinity Hybrid SFC/UHPLC System with MS detection* (5991-1546EN).

While the Hybrid SFC/UHPLC/MS system is not specifically shown in the examples described in this Technical Overview, the solution to shared column usage is equally well suited for MS hybrid systems. In an MS hybrid system, both valves pertaining to SFC or UHPLC operational modes should follow the same positioning guidelines and same time table switch settings as described for non-MS-setup.

Summary

Shared use of a single column for both UHPLC and SFC operation on the Agilent 1260 Infinity Analytical Hybrid SFC/UHPLC system is allowed, though use of individual columns for either mode is preferred. Directly switching between UHPLC and SFC with water or other mobile phase components in a shared column can cause unintended blockages. This is a consequence of the mobile phase freezing in the column and can be mitigated by flushing both flow paths with methanol rich streams prior to switching the mode valve. The flushing with methanol will displace the water preventing a blockage.

Two mode switching methods may be created that automate this flushing. The method parameters for valve position selections and timetable operation allow for easy automation of these operations. During the method, the position of the valve can be specified to turn when the method loads, when the method ends, or at various times within the method (timetable). The pumping system's flow rate and composition are easily adjusted within a run with the method's timetable events. The method for switching to UHPLC operation may also include additional timetable programming of the UHPLC pumps flow and composition. This allows the switching method to condition the column with a normal UHPLC flow prior to subsequent UHPLC runs. Appropriate conditioning will minimize any first run effects upon entering the UHPLC operation.

The utility of the switching methods are available for both manual operation and the combined operation within a sequence. The switching methods may also be incorporated into walk-up solutions such as Agilent's Mass Hunter Easy Access. Use of these method concepts will enhance the reliability and results of the Agilent 1260 Infinity Analytical Hybrid SFC/UHPLC system.

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