Agilent 1260 Infinity Hybrid SFC/UHPLC System

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The Agilent 1260 Infinity Hybrid SFC/UHPLC system is a unique solution that is capable of performing both supercritical fluid chromatography (SFC) and ultrahigh performance liquid chromatography (UHPLC). This Technical Overview describes the system configuration in detail and demonstrates the excellent reproducibility and method robustness for both SFC and UHPLC during a sequence in which a 16-compound polyaromatic hydrocarbon (PAH) mixture was analysed by switching automatically between the two techniques.

The deployment of orthogonal separation techniques has gained significant importance in liquid phase separations – especially for purity analyses, impurity profiling, and for separation of complex mixtures – to obtain comprehensive results that are increasingly demanded by regulatory agencies. Supercritical fluid chromatography (SFC) is a normal phase technique which provides different selectivity compared to reversed phase separation. This makes SFC an ideal complement to reversed phase separation, eliminating the inherent disadvantages of standard normal phase chromatography such as low analysis speed and long equilibration times. Furthermore, SFC known as a green technique minimises the consumption of organic solvents, avoiding the generation of large amounts of toxic waste and consequently inflicting minimal or no harm on the environment.

The Agilent 1260 Infinity Analytical SFC system represents state-of-the-art, packed-column SFC, providing HPLC-like sensitivity, 600-bar power range, and high instrument and method robustness – all achieved on a truly modular and flexible LC-based system.

In this Technical Overview, we describe a hybrid system, which facilitates analyses in SFC or UHPLC mode up to pressures of 600 bar on a single system. With this unique hybrid solution, it is possible to obtain orthogonal data on analyte mixtures in a single sequence of runs by simply switching between SFC and UHPLC modes. This eliminates the need to invest in two individual systems, excludes system-to-system variability, and saves significant cost and laboratory space. Existing Agilent HPLC and SFC systems can be easily upgraded to a hybrid SFC/UHPLC system.

System configuration

The flow path for the Agilent 1260 Infinity Hybrid SFC/UHPLC solution is shown in *Figures 1a* and *1b*. An Agilent 1260 Infinity Analytical SFC system can be converted into a 600-bar power-range hybrid SFC/UHPLC system by simple addition of a 2-position/10-port valve comprising universal valve drive with valve head, a second pump, and the hybrid SFC/UHPLC capillary kit. Furthermore, earlier Agilent 1100 Series or Agilent 1200 Series systems can also be converted to 400 bar power-range hybrid systems with the analytical SFC upgrade bundle (G4308A), a 2-position/10-port valve and the hybrid SFC/UHPLC capillary kit. The converted systems can be run in SFC mode *(Figure 1a)* or in UHPLC mode *(Figure 1b)*.

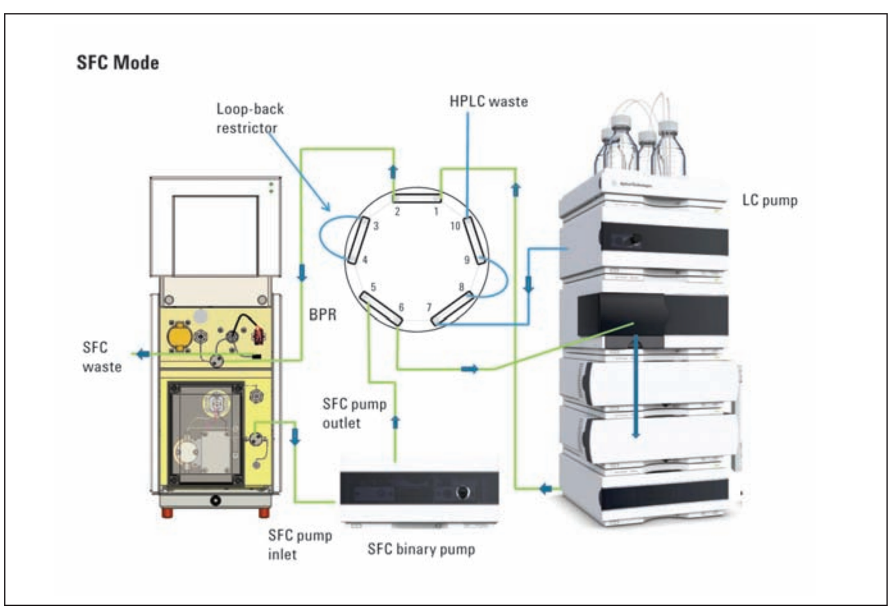

Figure 1a. Agilent 1260 Infinity SFC/UHPLC in SFC mode.

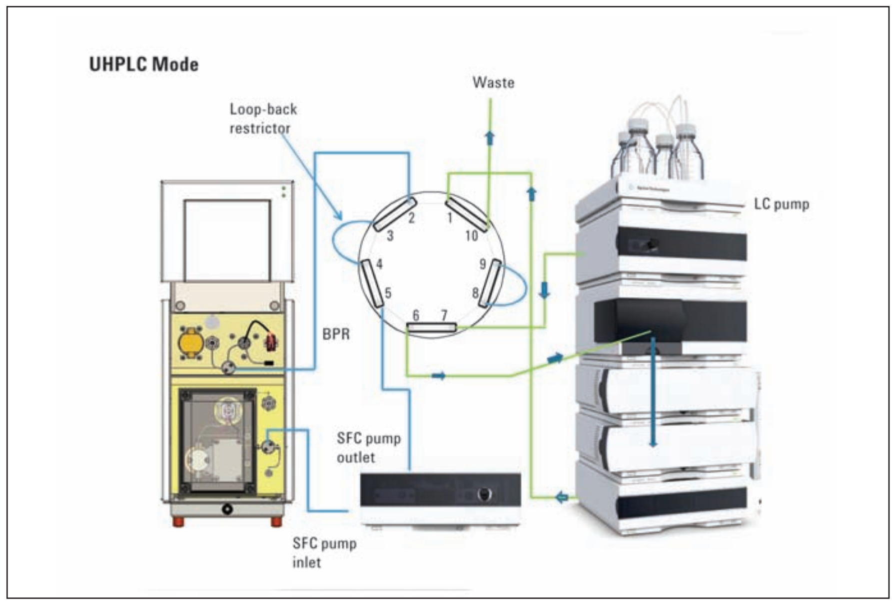

Figure 1b. Agilent 1260 Infinity SFC/UHPLC Hybrid System in UHPLC mode.

Alternating between modes is accomplished by simply switching the 2-position/10-port valve, which can be programmed as a method parameter at the beginning of the respective method.

In the Agilent 1260 Infinity Hybrid SFC/UHPLC system, the autosampler, column compartment and detector are shared modules and used in both modes. The Aurora SFC Fusion A5 module is used exclusively in SFC mode. The different modes have dedicated pumps. This provides highest flexibility for UHPLC and allows you to use either an Agilent 1260 Infinity Isocratic, Binary, or Quaternary Pump, or an Agilent 1290 Infinity Quaternary Pump.

It has to be noted that an injector program has to be applied for both UHPLC and SFC methods, since the autosampler is shared by both UHPLC and SFC methods and was converted to fixed loop injection. *Table 1* shows a typical injector program. The default injection volume is 15 µL for a 5 µL loop capillary because the fixed loop injection procedure requires a threefold overfill for high repeatability. In general, we recommend starting with the default hybrid SFC method (SFC_Hybrid_def) and default hybrid UHPLC method (LC_Hybrid_def) to create a new method. Both default methods are available in the user contributed library (UCL) of Agilent OpenLAB CDS ChemStation Edition.

Equilibration time is minimal when switching between SFC and UHPLC and only needed to ramp up or ramp down to the different backpressure settings in the different modes. To achieve a short equilibration time we recommend maintaining the backpressure settings of the backpressure regulator of the A5 module at 90 bar in LC mode (150 bar in SFC mode). Pump flow of the SFC binary pump should be also maintained at 1mL/min CO_2 during UHPLC method to avoid complete depressurisation and to minimise re-equilibration times. Method parameters used in the separation of the 16-component PAH mixture are given in *Table 2*.

Results and Discussion

A 16-component PAH mix was analysed to demonstrate the ease of use and performance of the Agilent 1260 Infinity Hybrid SFC/UHPLC system. The objective of the experiment was to show proof of principle that, within a sequence of alternating UHPLC and SFC runs, high separation power and good reproducibility can be achieved with both techniques for a complex mixture of analytes. In both modes Agilent ZORBAX Eclipse Plus C18 reversed phase stationary phases were used and therefore comparable modes of separation can be expected. Mobile phases were H2O (A)/acetonitrile (B) for the UHPLC separation and CO_2(A)/methanol + 2% H20 (B) for SFC. Detailed chromatographic conditions are given in *Table 2*. When applying different gradients and flow rates for the two different separation techniques most compounds could easily be resolved and detected without any further method optimisation on the hybrid system *(Figure 2)*. Comparable resolution was achieved when the separation was performed on separate instruments [1,2]. Equilibration times of less than one minute were typically needed to reach the necessary backpressure of the backpressure regulator when the system was switched from UHPLC to SFC.

High precision was achieved for retention times and areas even when the mode of separation was switched after every run. This is demonstrated by four overlaid analyses obtained within a sequence of alternating SFC and UHPLC methods *(Figure 3)*. Relative standard deviations (%) for retention times and areas were calculated for all eluting peaks, see *Table 3*, demonstrating high method robustness and the ease of use of the Agilent 1260 Infinity Hybrid SFC/UHPLC solution.

Table 1. Recommended injector program for SFC and LC methods.

Function	Parameter
Eject	Eject maximum volume to seat with default speed using default offset.
External contacts	Close external contact B.
Wait	Wait 0.1 min.
External contacts	Open external contact B.
Valve	Switch valve to "Bypass".
Draw	Draw 1.5 µL from air with default speed.
Draw	Draw default volume from sample with default speed using default offset.
Draw	Draw 5 µL from air with default speed.
Eject	Eject maximum volume to seat with default speed using default offset.

Table 2. Method parameters.

Function	Parameter UHPLC mode	Parameter SFC mode
Injection volume	15 µL	15 µL
Injector program	yes	yes
BPR	90 bar	150 bar
SFC flow rate	1 mL/min	3 mL/min
UHPLC flow rate	1.5 mL/min	0 mL/min
UHPLC gradient	0 min 40% B, 20 min 95% B,21 min 95 % B, 21.5 min 40% B	-
SFC gradient	5% B (to waste)	0 min 5%B, 10 min 22.5% B, 11 min 60% B, 11.5 min 5% B
Detection	254/16 nm (Ref 360/100 nm) 40 Hz acquisition rate	254/16 nm (Ref 360/100 nm) 40 Hz acquisition rate
Thermostatted column compartment solvent preheating	40 °C	37.5 °C
Thermostatted column compartment solvent post conditioning	Not controlled	40 °C
Columns	Agilent ZORBAX Eclipse PAH, 150 x 4.6 mm, 5 µm	Agilent ZORBAX Eclipse Plus C18, 150 x 4.6 mm, 5 µm

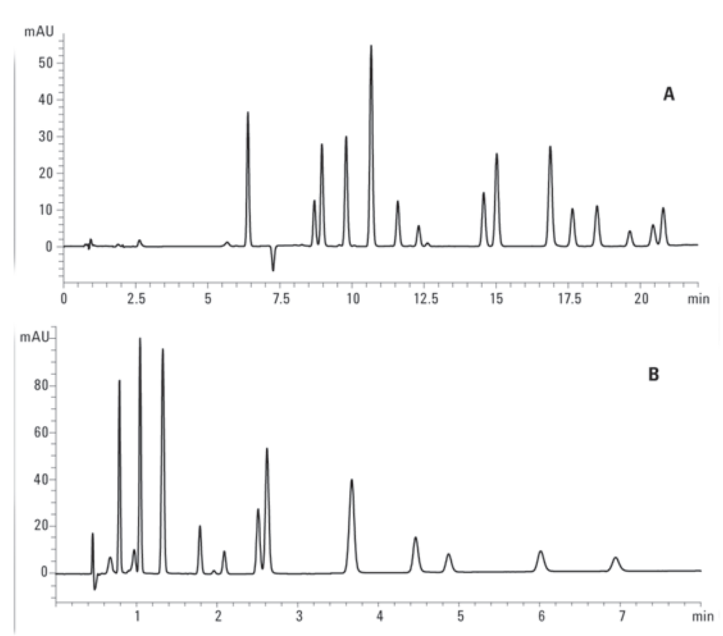

Figure 2. Separation of a complex PAH mix by UHPLC (A) and SFC (B).

Table 3. %RSD for alternating repeated SFC/UHPLC runs.

Mode	%RSD
UHPLC retention times	0.19%
SFC retention times	0.12%
UHPLC areas	0.51%
SFC areas	2.34%

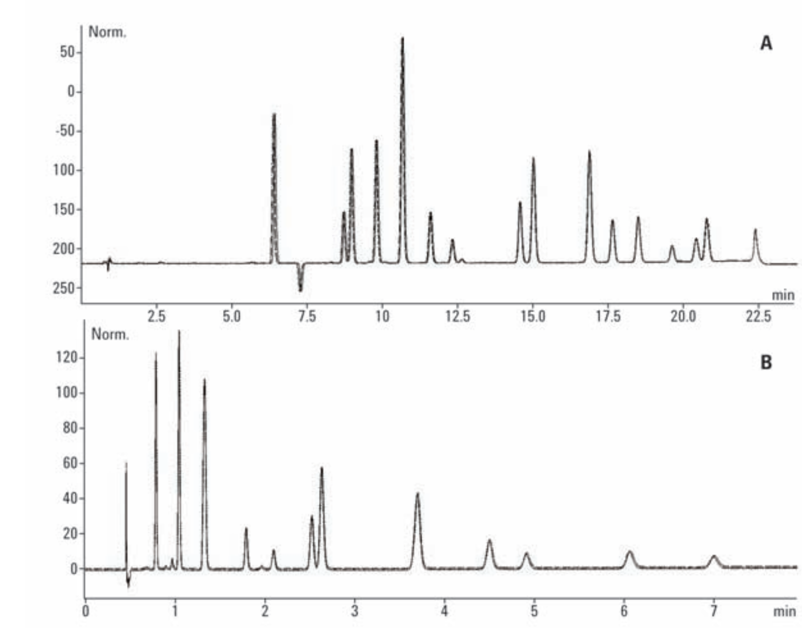

Figure 3. Overlays of four alternating LC (A) and SFC (B) runs generated in an automated sequence.

Conclusion

The Agilent 1260 Infinity Hybrid SFC/UHPLC system offers a single, highly robust and easy to use system to perform both SFC and UHPLC in an automated sequence without any hardware or software modifications. The system combines the superior UHPLC performance of the Agilent 1260 Infinity LC System and the sensitivity and unique power range of Agilent 1260 Infinity Analytical SFC system. This facilitates screening of complex samples automatically with orthogonal methods while saving capital budget, bench space, and overall analysis time.

References

1. Comparison of UV Detection Limits Between the Agilent 1260 Infinity Analytical SFC System and an Agilent 1200 Series LC System, Agilent Application Note Pub No 5990-9195EN

2. Analysis of PAHs in soil according to EPA 8310 method with UV and fluorescence detection, Agilent Application Note Pub No 5990-8414EN

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