Use of Hardware Advancements to Enhance Applications

Ronald Guilliet, Jennifer Van Anda and Rick Wikfors
Agilent Technologies, Inc., 2850 Centerville Rd. Wilmington, DE 19808

Agilent Technologies

Introduction

Recent advances in hardware for the Agilent 1260 Infinity Supercritical Fluid Chromatography System (SFC) have enhanced the application areas currently using SFC. The development of a new splitter for interfacing the SFC flow stream to a mass selective detector or an evaporative light scattering detector increases the robustness and reliability of the split. In addition the splitter may be adapted, allowing all the flow to go into the detector for improved sensitivity. The capability of a solventless injection (SPE) has been demonstrated using a Flexible cube allowing aqueous injections. The use of columns in series with a detector between columns expands the separation capabilities.

MS Interfacing

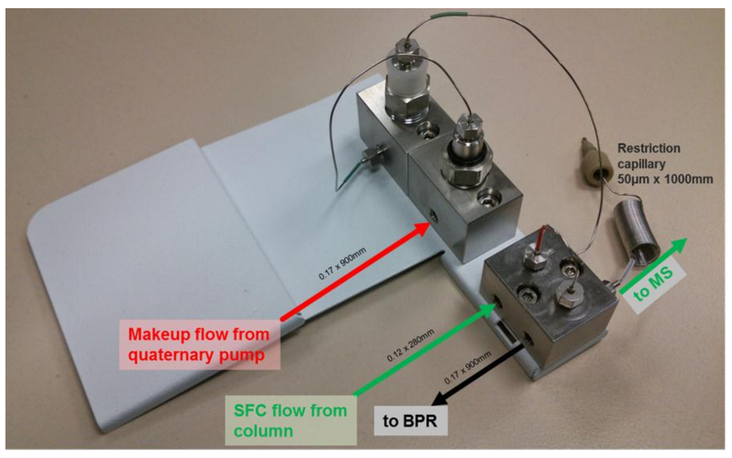

Figure 1. G4309-68715 flow splitter

The new G4309-68715 SFC flow splitter is a robust solution for partitioning mobile phase flow to a MSD, mass selective detector or an ELSD, evaporative light scattering detector. The addition of a check valve in the flow from the makeup pump to the second block prevents back flush of CO2, which allows purging without the loss of prime. A replaceable filter has been added for further increase robustness. Figures 2 and 3 demonstrate a comparison of injections of caffeine and theophylline using either the splitter or a full flow interface with a TOF mass detector.

In Figure 2, the both UV and MS signals are shown relative to each other. The chromatograms are scaled to maximum heights and presented on a percent scale. This view demonstrates the minimized time delay associated with the two interfaces. A comparison of resolution between the mass and UV peaks demonstrate excellent resolution with both interface techniques. It is readily apparent that the split flow interface, with its minimum volume, provides better time alignment, and slightly narrower mass peaks than the full flow interface. However, while slightly broader than the split interface, the full flow interface retains most all of the resolution of adjacent peaks without excessive merging.

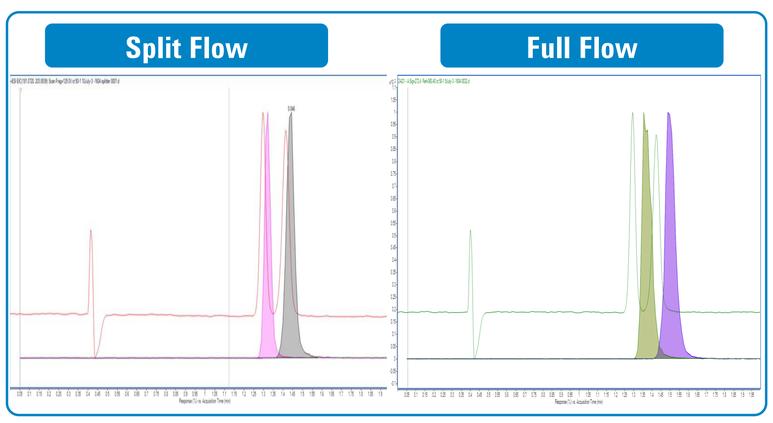

Figure 2: Overlaid UV and extracted mass chromatograms (scaled) illustrate time alignment and relative broadening of the two interfaces.

In Figure 3, below, the mass chromatograms are shown on an absolute (counts) scale. This view demonstrates the improvement in height and area for a full flow (less loss) interface. In comparison, the splitter reports areas of approximately 60% of the full flow interface in this example using a near minimum achievable total flow. Since flow through the splitter restrictor is largely fixed by back pressure, higher levels of total flow would necessarily result in larger split ratios. The net effect of a flow splitting interface is loss of sensitivity that is not seen in a full flow interface. This could be important when analyzing trace level compounds near the limits of the mass detector. The full flow interface additionally obviates the need to calculate a split ratio when quantitating peaks.

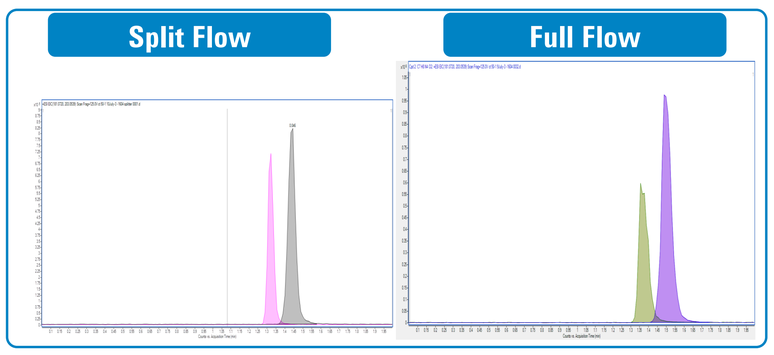

Figure 3: Unscaled, extracted mass chromatograms illustrate height and area response of split and full flow interfaces.

There are two mass detector interfaces available with the Agilent 1290 Infinity SFC system, full flow and split flow. Each interface has distinct advantages and differentiation from the other. The choice of which interface is best is application dependent. With the new flow splitter interface, conversion to a full flow interface is simply achieved with minor plumbing alteration.

Aqueous Sample Introduction

Demonstrated in 1995 by Berger, Sandra[3,4], and others, the Solventless Injection technique allows for both sample enrichment and use of non-traditional (aqueous) sample solvents. Solventless injection, a variant of SPE, is a technique where a sample is loaded onto a pre-column, the sample solvent is removed, and the pre-column is inserted into the mobile phase flow stream to complete the injection. The G4227A Flexible Cube combined with an Agilent SFC system can provide a technical demonstration of the solventless injection technique.

The Flexible Cube hosts two switching valves, a liquid metering pump with solvent selection. The switching valves and pump are fully controlled though the time table interface of the Flexible Cube method editor. Within the Flexible Cube, a 10 port, 2 position valve directs either a solvent or a purging gas through the system. A 6 port, 2 position, valve directs the positioning of a pre-column in either a load/dry (offline) position or in an elute (online) position in the mobile phase flow stream. A G4303A SFC AutoSampler provides sample 'injection' and is fitted with a large volume sample loop via the needle and seat to allow use of sample volumes of 90uL programmable via an injection program.

The process of solventless injection begins with the AutoSampler drawing the sample to be analyzed. Subsequently, the sample is moved through the pre-column, as depicted in Fig 4, by the Flexible Cube's metering pump. The next step is to remove the solvent from the pre-column with a flow of gaseous N2 as depicted in Fig 5. Lastly, the injection process is completed with the Flexible Cube's 6 port valve switching to the elute position placing the pre-column on-line. Once injected, the pre-column may be taken off-line for rinsing and conditioning for the next injection.

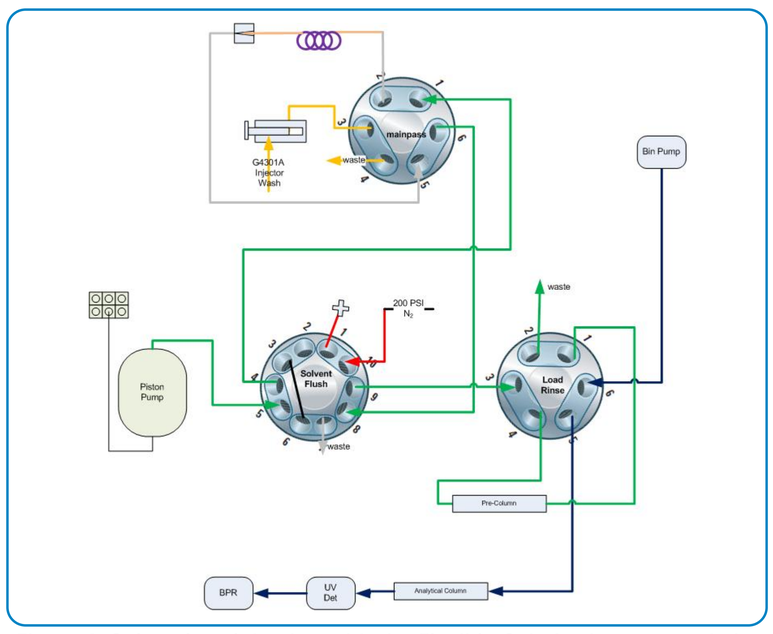

Figure 4: Solventless Injection with the Flexible Cube's piston pump being directed through the AutoSampler loop to load the sample onto the pre-column.

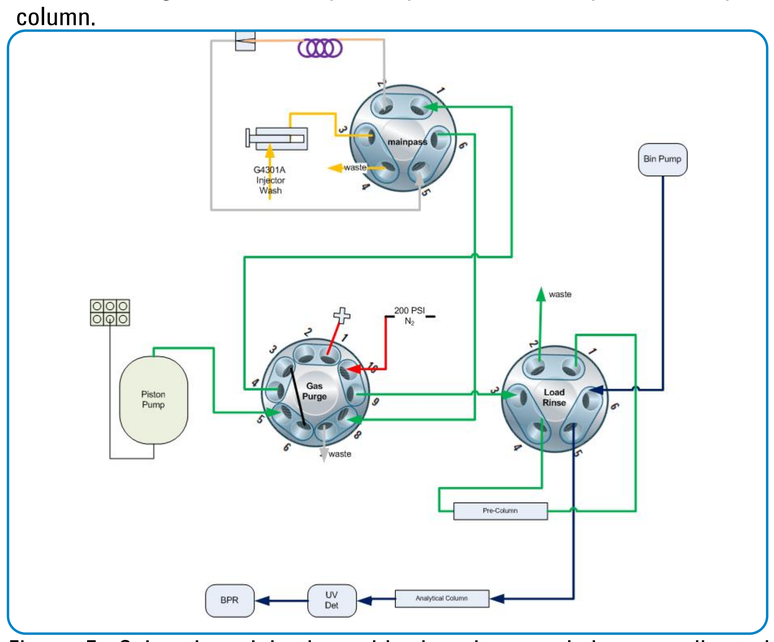

Figure 5, Solventless Injection with the nitrogen drying gas directed through the pre-column to eliminate sample solvents

The results of 5 sulfonamides in water were analyzed using the system described above. A C18 pre-column was used after being conditioned with methanol and water and dried for approximately 3.5 minutes with nitrogen at ambient temperature. The primary analytical column was a 4.6x150mm 5u Rx-Sil column. The chromatogram shown in Figure 6 demonstrates the separation of the compounds without the broadening effects of the aqueous sample solvent.

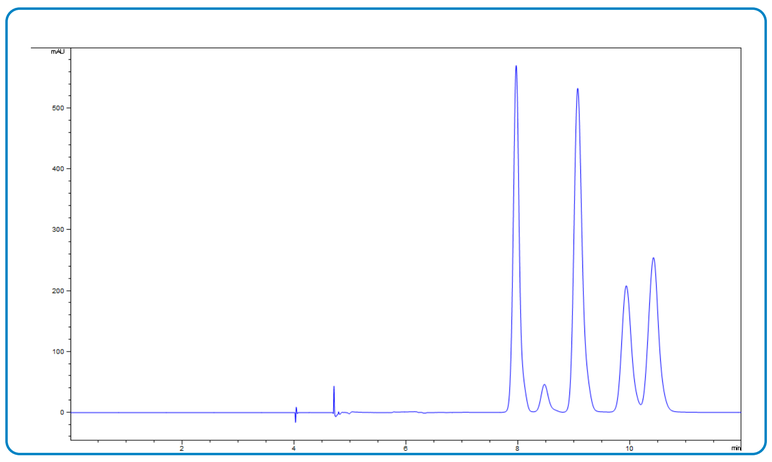

Figure 6: A 65ul aqueous injection of sulfonamides using the solventless injection interface.

Serial Column Selection

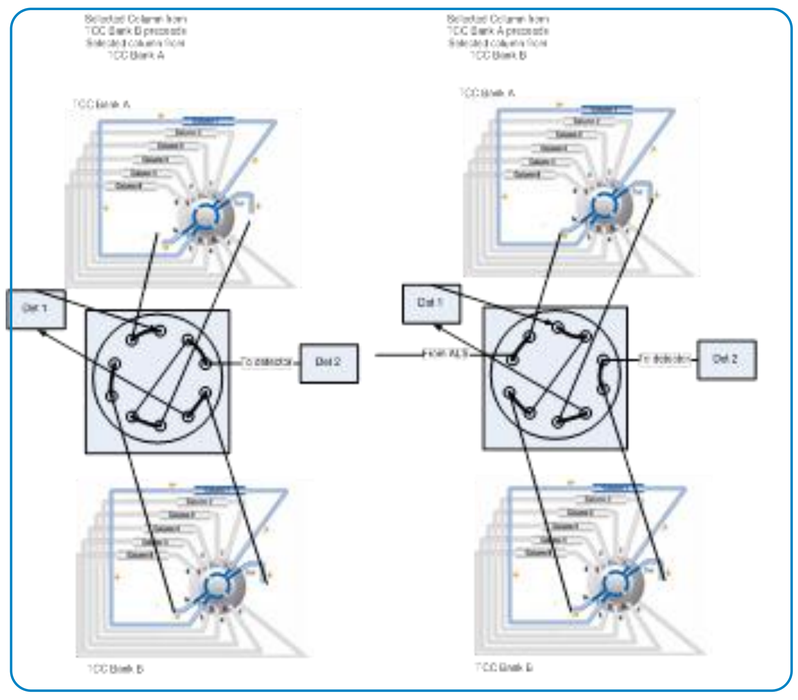

Figure 7: Valving diagram for using two columns in series.

The demands of chiral screening often require direct access to a large number of individual columns. An Agilent G1316 Theromostatted Column Compartment fitted with a G4234A 6 column selection valve meets this need. Chaining multiple TCCs using one position to address a second valve can further extend the range of columns. The use of an external switching valve could also be used to select an individual TCC and a specific column.

Some separations are more difficult, or require combinations of an achiral separation and a subsequent chiral separation. Often, the performance of a chiral column in performing a separation is changed when it is placed in advance of a second column. To address all of these needs, the system shown in Fig 7 can allow selection of any individual column (when each column selection valve contains a bypass position), or any pair of columns, one from each bank, in series, in either order. The system additionally provides for a detector positioned after the first column, and a second detector located after the second column.

The use of two columns in series allows the possibility for the analysis of complex mixtures of similar compounds such as the analysis of estrogen metabolites[1] or phthalates is salvia[2]. These columns may be of similar phases or different phases to enable complete or adequate separation of the compounds of interest. In Figure 8 the use of two columns in series is illustrated. The first column was an achiral silica column and the second column was a chiral ADH column. The test compounds were ibuprofen and flurbiprofen. The results of the first column are shown with the peaks eluting shown in blue. The results of second chiral column are shown in red. There four peaks after the first column, with ibuprofen eluting first followed by flurbiprofen.

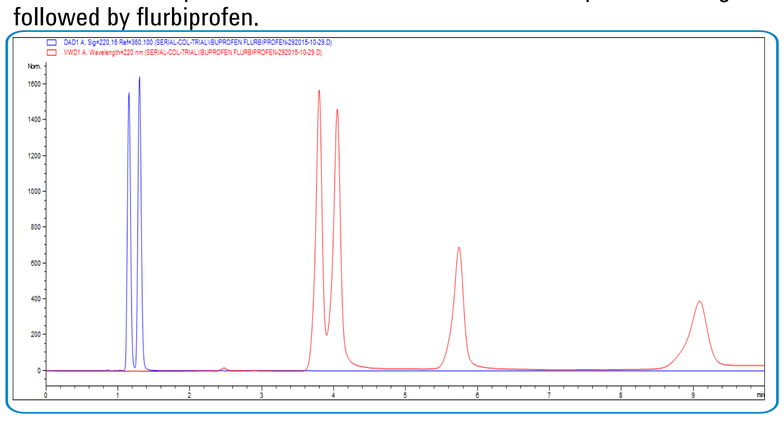

Figure 8: Use of valves to separate chiral compounds on an achiral columns followed by a separation on a chiral column in a single analysis.

Results and Discussion

The recent advances have expanded the capabilities of the Agilent SFC allowing for additional applications. The use of full flow into mass spectrometers will increase sensitivity. The solventless injection has enabled the injection of water, increasing the quantity of sample injected resulting in an increase in the sensitivity of the analysis. The ability to couple columns of the same or different phases is easily achieved with the CO2 as the mobile phase and the use of a number of valves. Finally the Agilent flow splitter is a robust and easy solution to easily and rapidly switch between full flow and split flow in to mass spectrometer or light scattering detectors.

Conclusions

- Introduction the new G4309-68715 flow splitter for split or full flow to mass spectrometers or ELSDs.
- The Flexible Cube introduces a solventless injection for the Agilent SFC to increased sensitivity and allow for injections of water.
- The use of valving to couple multiple columns, maximizing separation capabilities.

References:

1. Feasibility of Screening Large Aqueous Samples for Thermally Unstable Pesticides using High Efficiency Packed Column Supercritical Chromatography and Multiple Detectors. Terry Berger, Chrom. 41,no. 7/8, Oct. 1995.

2. Selected applications of the use of supercritical fluids in coupled systems. Pat Sandra et al.. J.Chrom. A,703, 467, 1995.

3.Analysis of Fifteen Estrogen Metabolites Using Packed Column Supercritical Fluid Chromatography-Mass Spectrometry. Xia Xu et al. Anal.Chem. 2006.78, 1553-1558.

2. Agilent Pub. No. 5990-9597 EN.