Optimize the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 1290 Infinity ELSD

Technical Overview

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Abstract

This Technical Overview gives a detailed description of an optimized configuration of an instrument for supercritical fluid chromatography (SFC) when used with an evaporative light scattering detector (ELSD). The influence of preheating the SFC effluent on the signal quality of the ELSD is shown. This was done by means of a heat exchanger in an additional thermostatted column compartment. The effect of additional make-up flow prior to the ELSD is also discussed, supported by statistical data.

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Introduction

Modern supercritical fluid chromatography (SFC) instruments offer huge performance advantages compared to classical HPLC instruments. Compared to HPLC mobile phases, SFC's mobile CO_2 phase has lower viscosity, increased diffusion, and better mass transfer capabilities. This enables higher speed separation at lower backpressure by means of columns with small inner diameter and small particle size packing material. Both methods, SFC and HPLC, are comparable in terms of sensitivity and stability, but provide orthogonal selectivity for the separation. This makes SFC a valuable complementary technique to classical HPLC, as well as modern UHPLC.

The application range of SFC can be widened by coupling with other detectors, for example, evaporative light scattering (ELSD). However, effects such as expansion cooling from decompressed CO_2, the need for splitting, and a make-up flow to equal flow rates make it less straightforward.

This Technical Overview describes a configuration to connect an ELSD to an SFC instrument. The effect of CO_2 expansion cooling and preheating of the column effluent on the peak performance are shown and discussed. The introduction of a make-up flow is included in the instrument configuration.

Experimental

Instruments

Agilent 1260 Infinity Analytical SFC Solution (G4309A) with:

- Agilent 1260 Infinity SFC Control Module
- Agilent 1260 Infinity SFC Binary Pump
- Agilent 1260 Infinity High Performance Degasser
- Agilent 1260 Infinity SFC Standard Autosampler
- Agilent 1260 Infinity Thermostatted Column Compartment (TCC)
- Agilent 1260 Infinity DAD with high pressure SFC flow cell
- Agilent 1260 Infinity Isocratic Pump (G1310B)
- Agilent 1290 Infinity Thermostatted Column Compartment (TCC)
- Agilent 1290 Infinity ELSD (G4261B)

Instrument setup

The recommended configuration of the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 1290 Infinity ELSD is shown in Figure 1. The exit capillary of the DAD flow cell is directly connected to a splitter assembly (p/n G4309-68715), which contains two combined splitters (and an additional check valve to prevent backflush of CO_2 into the make-up pump and a solvent filter). At the first splitter, the make-up flow coming in from an isocratic pump is introduced into the flow path. This splitter is connected to the second one by a short 0.12-mm id capillary (both splitters could also be used independently). Here, the flow is split into the part going to the ELSD, and the other part going to the backpressure regulator (BPR) of the SFC module. The connection from the second splitter to the heat exchanger in the second TCC for preheating of the effluent stream is made by a newly developed 50-µm id stainless steel capillary, 1 m in length. The connection from the heat exchanger in the TCC to the ELSD is made by another 0.12-mm id capillary. The split ratio depends on the backpressure generated by this restriction capillary and the pressure set by the BPR.

Column

Agilent ZORBAX Rx-SIL,
4.6 × 150 mm, 5 µm (p/n 883975-901)

Software

Agilent OpenLAB CDS ChemStation Edition for LC & LC/MS Systems, Rev. C.01.05

Standards

A solution of the following compounds was used: 1) caffeine, 2) theophylline, 3) cortisone, 4) prednisone, 5) hydrocortisone, 6) prednisolone, 7) sulfamerazine, 8) sulfaquinoxaline (stock solution at 1 mg/mL each in methanol). This solution was diluted with methanol to a final concentration of 10 µg/mL.

Chemicals

Methanol was purchased from J. T. Baker, Germany. Chemicals were purchased from Sigma Aldrich, Corp., Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak).

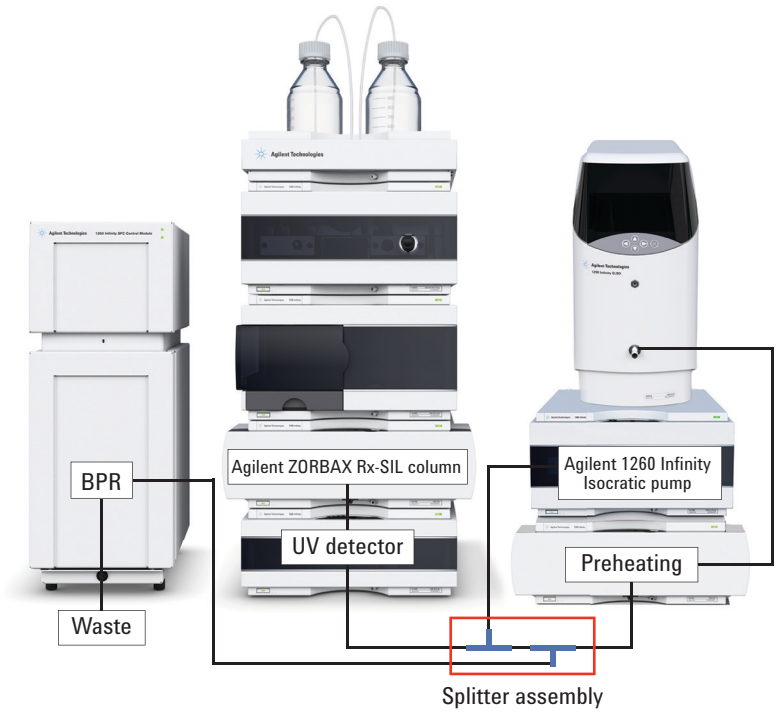

Figure 1. Configuration of the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 1290 Infinity ELSD. The DAD flow cell is directly connected to the splitter assembly containing two splitters, a check valve, and a solvent filter (BPR = backpressure regulator, splitter assembly p/n G4309-68715). Capillary from splitter assembly to preheating TCC: 0.05 × 1,000 mm.

SFC conditions

Agilent 1260 Infinity Analytical SFC Solution conditions	
Solvent A	CO_2
Modifier B	Methanol
SFC flow	3 mL/min
Gradient	5 % B – 0 minutes, 25 % B – 10 minutes
Stop time	10 minutes
Post time	2 minutes
Backpressure regulator (BPR) temperature	60 °C
BPR pressure	290 bar
Column temperature	40 °C
Injection volume	5 µL, 3 × loop over fill
Needle wash in vial with methanol	
Make-up flow	0.2 mL/min, methanol
DAD	254 nm/band width 4 nm; ref. 360 nm/bandwidth 100 nm data rate: 20 Hz
Agilent 1290 Infinity ELSD conditions	
Evaporator temperature	40 °C
Nebulizer temperature	55 °C
Gas flow rate	1.15 SLM
Data rate	10 Hz
Smoothing	2 s
PMT gain	5

Results and Discussion

The SFC flow was split behind the DAD flow cell and before the BPR to the ELSD. The BPR was adjusted to produce a higher backpressure in the system to direct a large part of the flow through the splitter towards the ELSD for improved detection. In the initial experiment, the transfer capillary was connected directly to the ELSD and allowed decompression directly at the nebulizer in the ELSD. This is also the point where decompression cooling occurs. This effect is known to compromise ELSD signal intensity. Under the given conditions, the chromatography showed clear separation of the eight compounds in the test mixture, where the first two compounds were separated early in the run with a valley between these peaks and the other compounds were baseline separated. The separation was maintained from DAD detection during splitting and transfer through the 50-µm id × 1 m capillary to the ELSD (Figure 2). To avoid the negative effect of expansion cooling, a heat exchanger in another TCC was connected to the 50-µm id capillary for preheating the SFC effluent before entering the ELSD.

To demonstrate the effect, heat exchangers with different internal volumes were kept at regulated ambient temperature (25 °C) and connected to the 50-µm id capillary. Comparison of the signals immediately showed an increase in signal height and signal area when the SFC effluent was preheated before entering the ELSD (Figure 3). There was also a slight increase in peak width, but due to the much higher peak intensity and peaks area, they were in an acceptable range. Because there was not much difference in peak width for the different heat exchanger volumes (6, 3, and 1.8 µL), the 3-µL heat exchanger was used for further experiments.

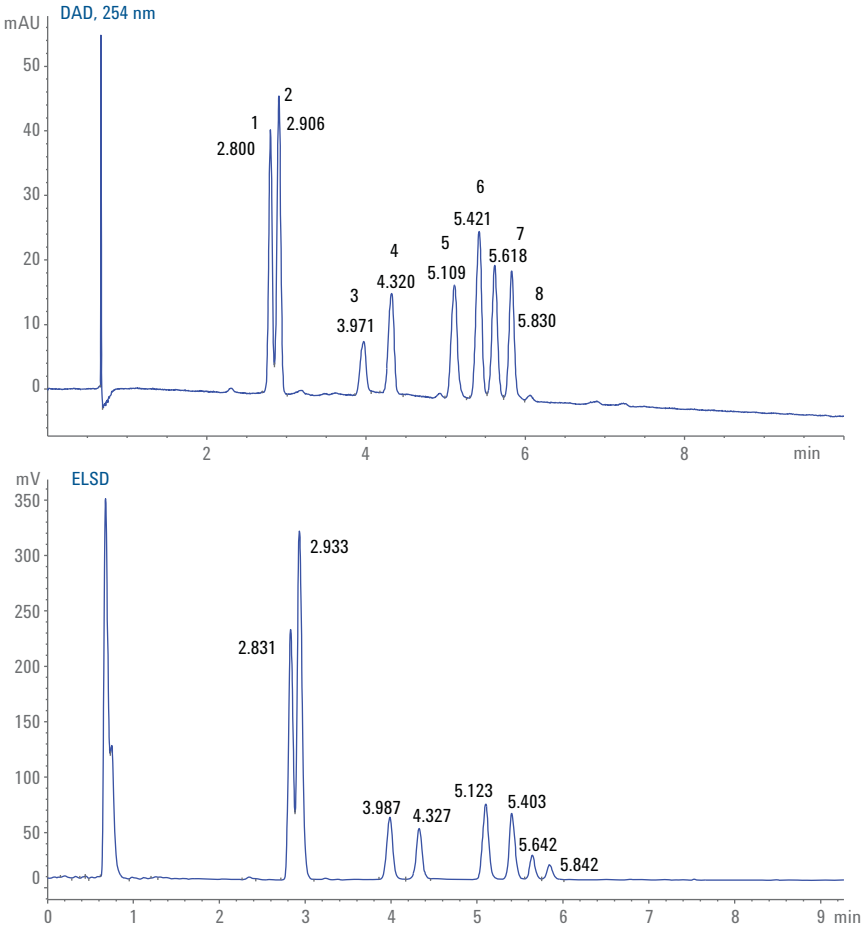

Figure 2. Separation of an eight-compound mix with SFC and DAD/ELSD detection. The splitting connection to the ELSD was located in the flow path behind the DAD and before BPR by means of a T-piece and a 50 µm × 1 m capillary.

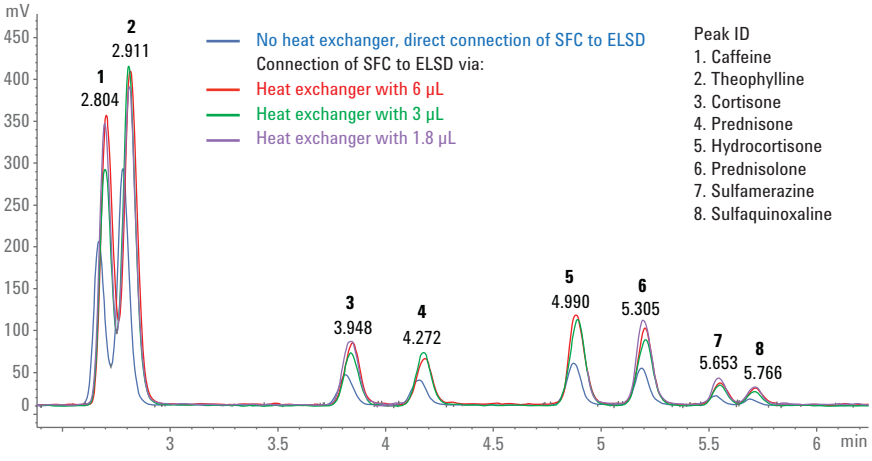

Figure 3. Effect of the introduction of a heat exchanger in the SFC effluent before entering the ESLD. Peak broadening and peak height effects are shown for different heat exchanger volumes at regulated ambient temperature of 25 °C.

The effect of preheating the SFC effluent on the signal performance at the ELSD was examined in more detail. The signals of six compounds were compared at 30, 40, and 60 °C to a direct connection of SFC to ELSD without preheating for signal area, signal height, and signal-to-noise (S/N) ratio. The experiments were repeated 12 times for a proper statistical evaluation. The dependence of the peak area showed a clear increase for all compounds for preheating of the SFC effluent to 30 °C before entering the ELSD. Further increase in preheating to 40 and 60 °C decreased the signal area again (Figure 4). The statistical evaluation showed that relative standard deviations (RSD) were 8 to 10 % for the directly connected ELSD and approximately 12 % for preheating at 30 °C, with a tendency to decrease again at higher preheating temperatures.

The examination of the dependence of peak height on preheating temperature showed a similar result (Figure 5). From the direct connection of SFC to ELSD without preheating, to preheating with a heat exchanger temperature of 30 °C, the signal height showed a clear increase. Further heating to 40 and 60 °C decreased the signal again. The signal height RSD values were approximately 10 % for the direct connection and 10 to 12 % for the preheated examples at 30 °C.

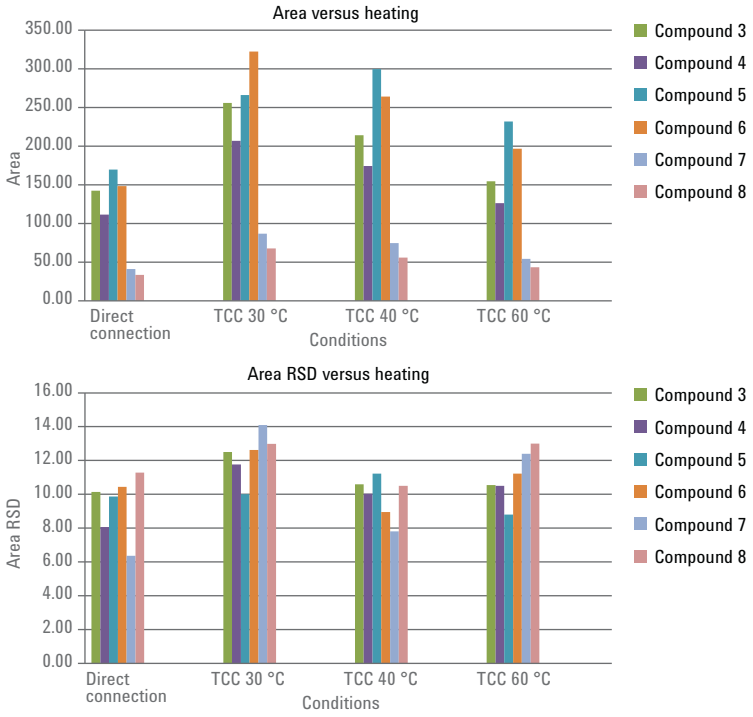

Figure 4. Peak area and peak area RSD % versus preheating temperature of split SFC effluent. The maximum peak areas were obtained at a preheating temperature of 30 °C with an RSD of approximately 12 %.

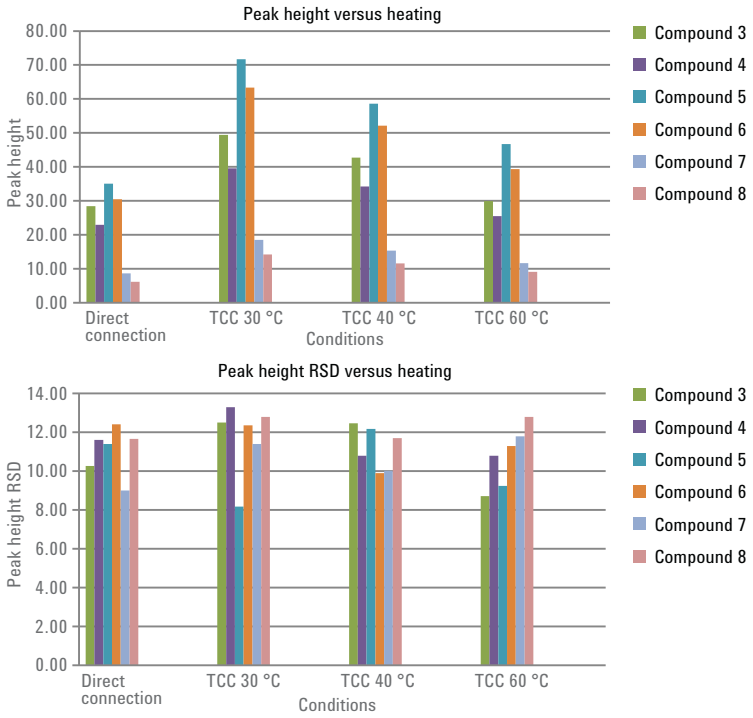

Figure 5. Peak height and peak height RSD % versus preheating temperature of split SFC effluent. The maximum peak heights were obtained at a preheating temperature of 30 °C with an RSD of approximately 10 to 12 %.

The S/N ratios were at approximately the same level for direct connection of SFC to ELSD without preheating compared to preheating at 30 °C (Figure 6). The S/N ratio decreased with preheating temperature at 40 and 60 °C.

Starting from the optimized preheating temperature at 30 °C, the influence of an additional make-up flow and its dependence from the flow rate was examined. The peak areas decreased during the addition of a make-up flow due to the dilution, but the area RSD achieved a minimum at approximately 5 % for a make-up flow rate of 0.2 mL/min (Figure 7).

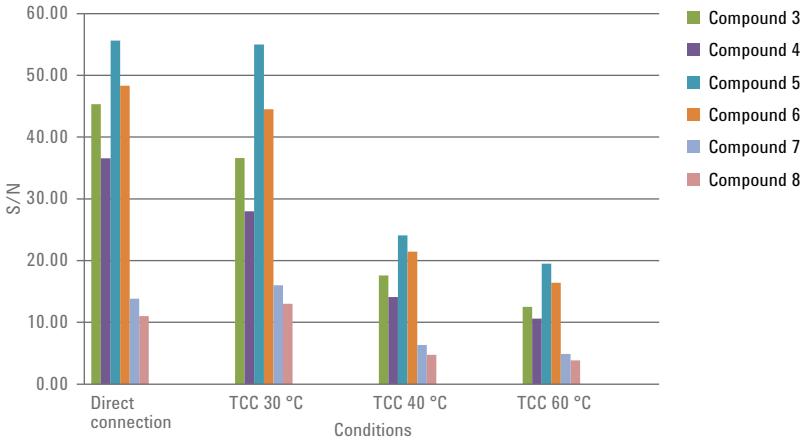

Figure 6. S/N ratio versus preheating temperature of split SFC effluent. The S/N ratios were at the same level for direct connection and a preheating temperature of 30 °C.

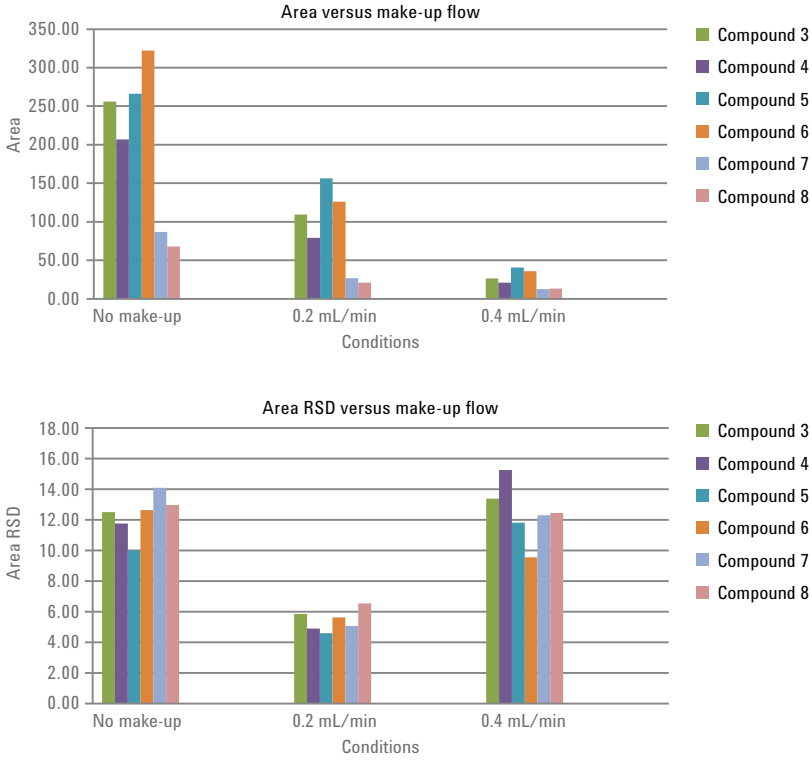

Figure 7. Peak area and peak area RSD % versus make-up flow rate with split SFC effluent at 30 °C. The peak areas decreased during the addition of a make-up flow, but the area RSD achieved a minimum at approximately 5 % for a make-up flow rate of 0.2 mL/min.

The peak heights showed the same behavior. They decreased during the addition of a make-up flow, but the peak height RSD achieved a minimum at approximately 4 to 6 % for a make-up flow rate of 0.2 mL/min (Figure 8).

The S/N ratios remained almost constant during the addition of a make-up flow of 0.2 mL/min and started to decrease for higher make-up flow rates (Figure 9). When considering the retained S/N at a preheating temperature of 30 °C, the connection from the SFC to the ELSD was optimum at 30 °C for preheating with 0.2 mL/min make-up flow.

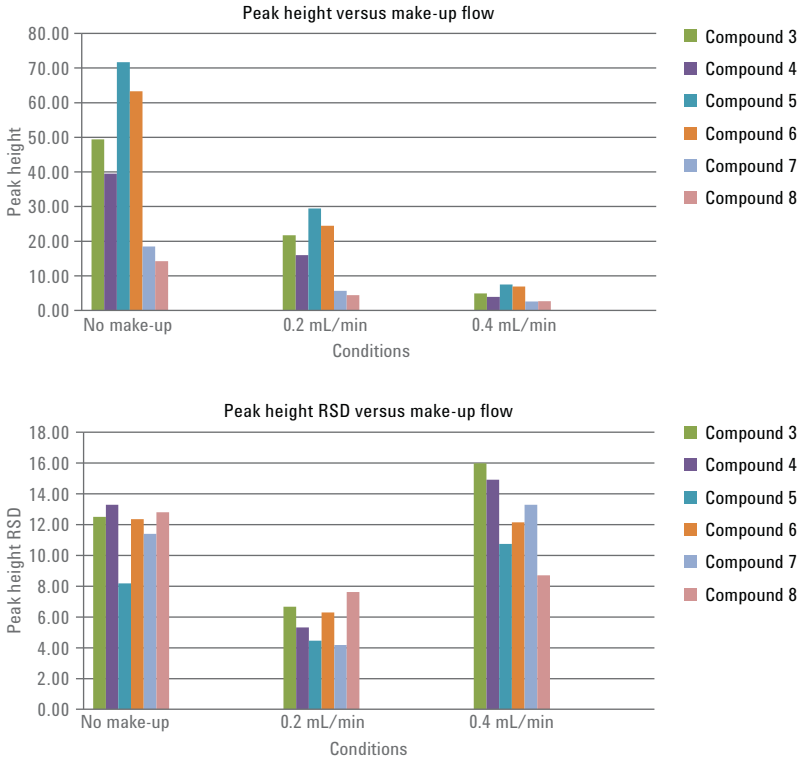

Figure 8. Peak height and peak height RSD % versus make-up flow rate with slit SFC effluent at 30 °C. The peak heights decreased during the addition of a make-up flow but the peak height RSD achieved a minimum at about 4 to 6 % for a make-up flow rate of 0.2 mL/min.

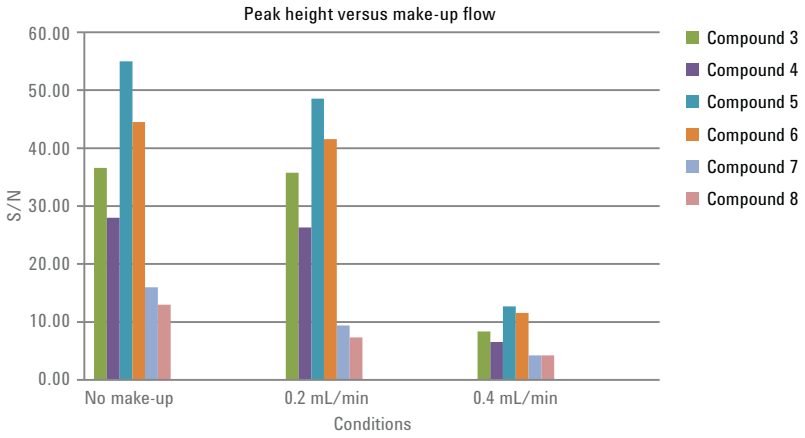

Figure 9. S/N ratio versus make-up flow rate with split SFC effluent at 30 °C. The S/N ratios remained almost constant during the addition of a make-up flow of 0.2 mL/min and started to decrease for higher make-up flow rates.

Conclusions

This Technical Overview describes the connection of the Agilent 1260 Infinity Analytical SFC Solution to an ELSD detector by a specially designed splitter. The effects of preheating the SFC effluent after the split identified an optimum preheating temperature to get the best performance in peak height, peak area, S/N ratio, and their relative standard deviations. Additionally, the introduction of a make-up flow at the optimized temperature and the dependence of the parameters was examined. Finally, an optimized preheating temperature and make-up flow rate was derived from the data.

The combination of SFC and ELSD is recommended, with additional modules and a splitting assembly to obtain optimum performance. To obtain the optimized performance, a second TCC for preheating the SFC effluent before entering the ELSD is required. An additional pump for introduction of a make-up flow is highly recommended. The preheating temperature, as well as the make-up flow rate, must be optimized for each analytical SFC separation.

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