# Optimize the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 6150 Single Quadrupole MS

## Technical Overview


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### Abstract

This Technical Overview gives a detailed description of an optimized configuration of an SFC instrument with MSD detection. The influence of preheating the SFC effluent on the signal quality of the MSD is shown. Preheating is done by means of a heat exchanger in an additional thermostatted column compartment. The effect of an additional make-up flow for enhanced signal performance and ionization prior to the MSD is demonstrated.


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## Introduction

Supercritical fluid chromatography (SFC) is complementary to classical HPLC and modern UHPLC. Both techniques, SFC and HPLC, typically provide orthogonal selectivities, but are comparable in sensitivity and robustness. In comparison to classical HPLC instruments, SFC offers performance advantages in terms of higher separation speed at lower backpressure. This is due to the $CO_2$ mobile phase used for SFC, which has a lower viscosity, increased diffusion, and better mass-transfer capabilities compared to classical HPLC mobile phases. Coupling the SFC instrument to other detectors, such as mass spectrometers, enables access to more applications. However, effects of expansion cooling occurring while $CO_2$ is decompressed, splitting, and make-up flow have to be considered.

This Technical Overview discusses an instrument configuration that connects a single quadrupole MS equipped with an Agilent Jet Stream source to an SFC instrument. This configuration can also be used for other mass spectrometers such as triple quadrupole and time-of-flight mass spectrometers. The effects of $CO_2$ expansion cooling, and preheating of the column effluent on peak performance are shown and discussed. The introduction of a make-up flow for enhanced ionization 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 TCC
- Agilent 1260 Infinity DAD with high pressure SFC flow cell
- Agilent 1260 Infinity Isocratic Pump (G1310B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 6150 MSD (G6150B)

### Instrument setup

The recommended configuration of the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 6150 Single Quadrupole MS 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 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 in two; one part goes to the MSD, and the other part goes to the backpressure regulator (BPR) of the SFC module. The connection from the second splitter to the heat exchanger in the second thermostatted column compartment (TCC) for preheating of the effluent stream is made by a 50-µm id stainless steel capillary, 1 m long. The connection from the heat exchanger in the TCC to the MSD 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.

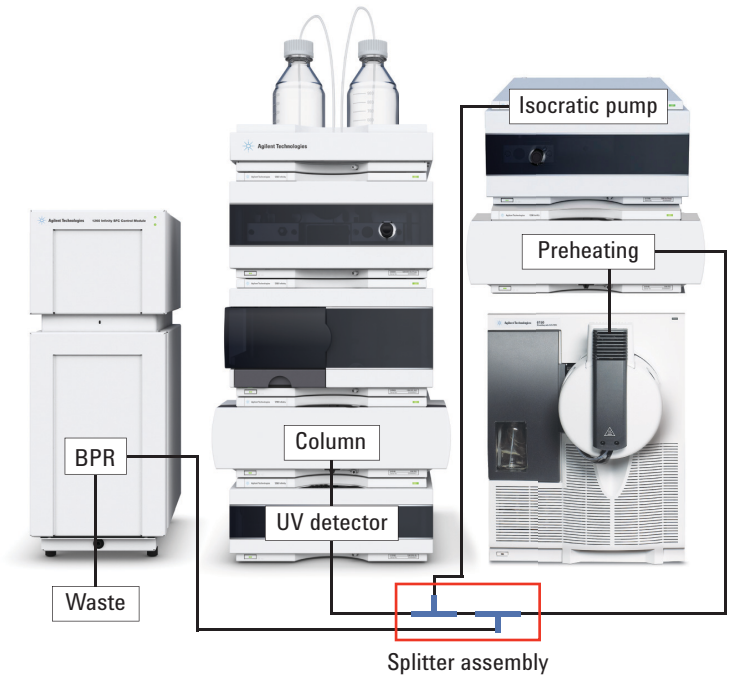


Figure 1. Configuration of the Agilent 1260 Infinity Analytical SFC Solution with the Agilent 6150 Single Quadrupole MS. The DAD flow cell is directly connected to the splitter assembly containing two splitters, a check valve, and a solvent filter (BPR = backpressure regulator, splitter assembly (p/n G4309-68715)).

### Standards

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

## Results and Discussion

### Influence of temperature by preheating the SFC effluent stream

In an initial experiment, the SFC column was directly connected to a splitter and the effluent was split between the mass spectrometer ion source and SFC backpressure regulator. If the capillary (50-µm id × 100 cm) from the splitter was directly connected to the sprayer at the MS source, icing at the outside of the sprayer could be observed. This was due to decompression cooling, which occurs at the connection from the 50-µm capillary to the sprayer. To avoid the decompression cooling that compromises MS detection performance, the heat exchanger of a thermostatted column compartment was connected to the 50-µm capillary from the splitter. The end of the heat exchanger was connected to the sprayer. With this setup, no icing could be observed for preheating temperatures above 30 °C, and six out of eight compounds of the mixture could be separated (Figure 2). The ionization reagent ammonium formate was added directly to the organic modifier for proper ionization.

### Instrument conditions

| | |
|---|---|
| **Column and software** | |
| Column | Agilent ZORBAX Rx-SIL, 4.6 × 150 mm, 5 µm (p/n 883975-901) |
| Software | Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS Systems, Rev. C.01.05 |
| **SFC conditions** | |
| Solvent A | $CO_2$ |
| Solvent modifier | A) Methanol + 10 mM ammonium formate<br>B) Methanol |
| Flow rate | 3 mL/min |
| Gradient | 5 % B, 0 minutes, 25 % B, 10 minutes |
| Stop time | 10 minutes |
| Post time | 2 minutes |
| BPR temperature | 60 °C |
| BPR pressure | 120 bar |
| Column temperature | 40 °C |
| Injection volume | 5 µL, 3 × loop over fill |
| Needle wash | In vial with methanol |
| Make-up flow | 0.2, 0.4, 0.6 mL/min, methanol + 10 mM ammonium formate |
| DAD | 254 nm, band width 4 nm; ref. 360 nm, band width 100 nm, data rate, 20 Hz |
| **MS conditions** | |
| Ionization mode | Positive |
| Capillary voltage | 2,000 V |
| Nozzle voltage | 300 V |
| Gas flow | 5 L/min |
| Gas temperature | 250 °C |
| Sheath gas flow | 9 L/min |
| Sheath gas temperature | 280 °C |
| Nebulizer pressure | 50 psi |
| Fragmentor | 100 V |
| Scan range | 150 to 500 *m/z* |
| Peak width | 0.05 minutes |

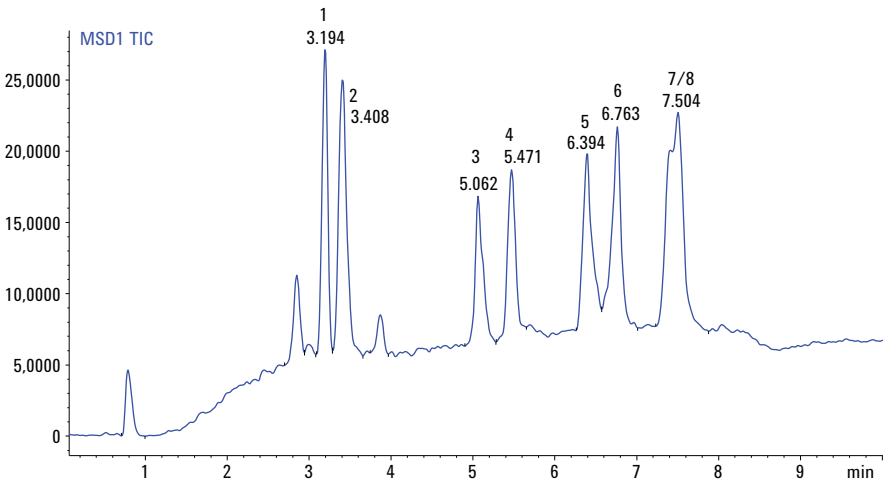


Figure 2. Separation of an eight-compound mix with SFC and single quadrupole MS detection. The splitting connection to the MS was located in the flow path behind the column and before the backpressure regulator by means of one T-piece of the splitter assembly and a 50 µm × 100 cm capillary.

Temperature settings of 30, 40, 50, and 60 °C were used for the following experiments. The results demonstrate the influence of preheating of the SFC effluent on detection performance.

The peak areas showed an increase when preheating of the SFC effluent was applied compared to the direct connection (Figure 3). For a statistical evaluation, the sample was injected 12 times for every temperature setting. The optimum peak areas were found by preheating with a heat exchanger at 30 to 40 °C. At higher temperatures, the area declined again. Preheating at 30 to 40 °C improved peak area by approximately 25 %. The relative standard deviation of the peak area declined from approximately 12 % for the direct connection to approximately 8 to 10 % when preheating at 30 to 40 °C.

The peak height showed a similar pattern (Figure 4), increasing by approximately 15 to 20 % for the optimum temperature range between 30 and 40 °C. The relative standard deviation values declined for the optimum temperature range from approximately 13 to 16 % down to 10 to 12 %. The major effect of preheating was observed on the signal-to-noise (S/N) value, by lowering the noise level (Figure 5). For direct connection, the S/N was around 10. This improved to an S/N of 20 to 25 in the optimum preheating range of 30 to 40 °C.

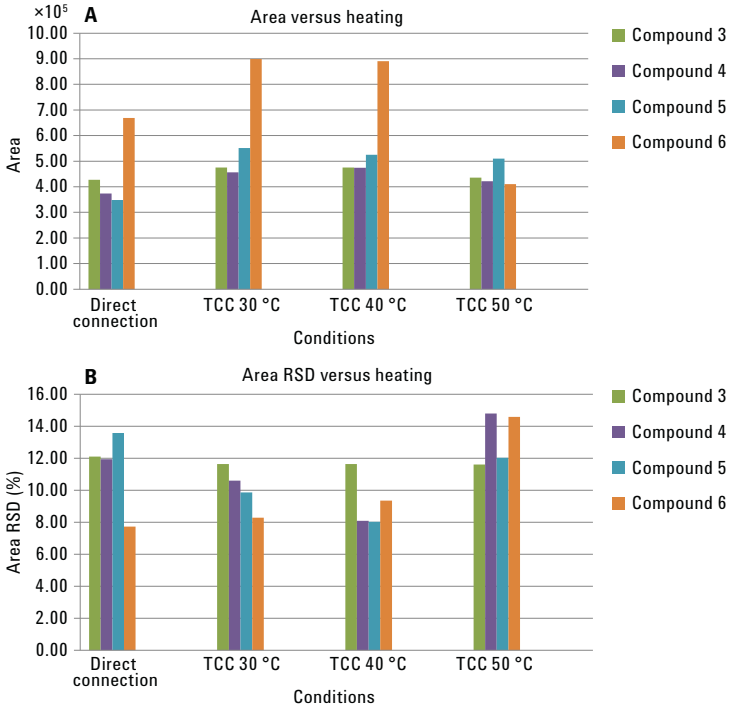


Figure 3. Peak area and peak area RSD % versus preheating temperature of split SFC effluent. The maximum peak areas were obtained at a preheating temperature of 30 to 40 °C with an RSD of approximately 8 to 10 %.

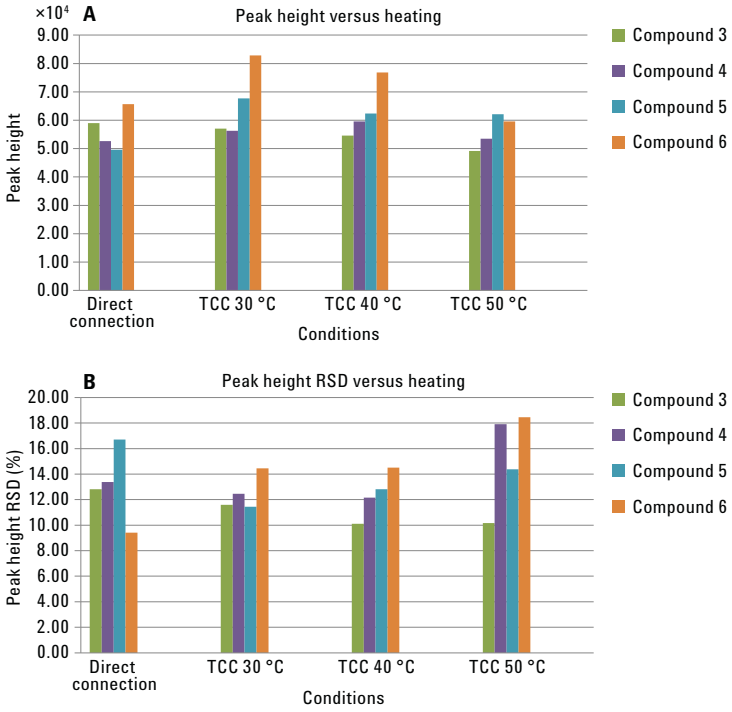


Figure 4. Peak height and peak height RSD % versus preheating temperature of a split SFC effluent. The maximum peak heights were obtained at a preheating temperature of 30 to 40 °C with an RSD of approximately 10 to12 %.

## Influence of a post-column make-up flow on the SFC effluent

The approach described above, coupling the SFC either directly or with preheating to a mass spectrometer, has potential for improvement. To obtain proper ionization of the analytes in this approach, the ionizing agent must be added to the $CO_2$ modifier (here methanol) as an additive. This means, at low modifier concentration and typically at the beginning of a gradient run or for isocratic separations with low modifier, there is only weak or even no ionization of early eluting analytes. Additives to the modifier are typically used in SFC because of their influence on the separation. The need for an ionizing agent could, in the worst case, have an unavoidable negative influence on separation. In addition, additives can change the column's separation behavior permanently.

To turn these negative aspects of coupling SFC to mass spectrometers into more flexibility, the ionizing agent can be added to the SFC effluent by a splitter assembly (as described in the experimental section) after the column. The solvent to dissolve the additive can be different from the modifier; it is possible to use the one achieving best ionization. To retain the positive influence of preheating, the splitter assembly was connected to the heat exchanger after adding the ionizing agent. The mass spectrometric detection of the standard compounds was demonstrated by means of the splitter assembly to introduce the ionizing agent (Figure 6A). Without ionizing agent in the modifier and no post-column addition, no ionization occurred in the ion source of the MS (Figure 6B).

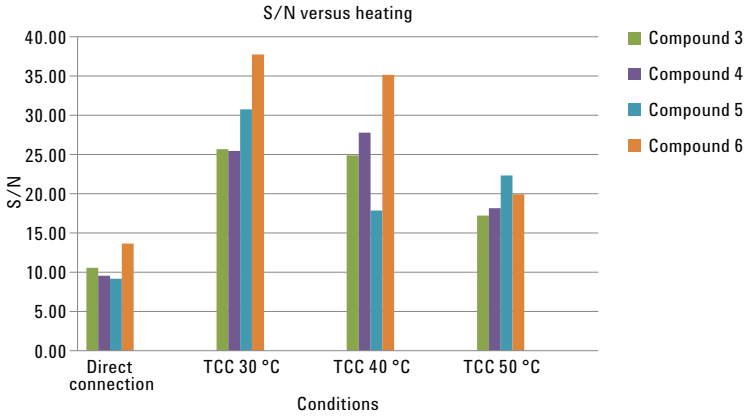


Figure 5. Signal-to-noise ratio versus preheating temperature of a split SFC effluent. The S/N ratios were at a higher level for a preheating temperature of 30 to 40 °C compared to direct injection.

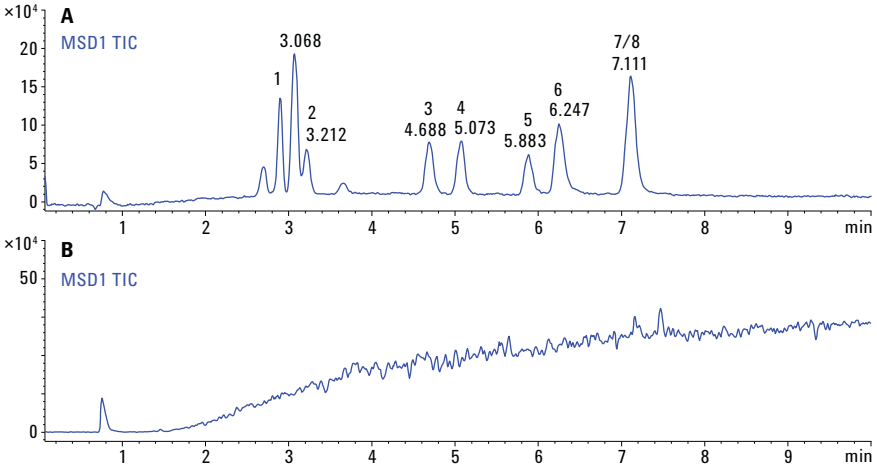


Figure 6. Separation of an eight-compound mix with SFC and single quadrupole MS detection. A) The splitter assembly was located in the flow path behind the column and to introduce the ionizing agent (0.4 mL/min) before backpressure regulation, a 50 µm × 100 cm capillary was used to connect to the thermostatted column compartment (30 °C); B) direct connection of column effluent to the mass spectrometer ion source without ionizing agent in the modifier or post-column addition.

To determine the influence of adding the ionizing agent after the column with the splitting approach, the experiment was repeated using different solvent flow rates and multiple runs for statistical evaluation. First, the influence of the flow rate of the added solvent containing the ionizing agent on the MS peak area was tested (Figure 7). The peak areas and their standard deviation at different make-up flow rates at 30 °C were compared to the SFC/MS connection with preheating at 30 °C without make-up flow (reagent for ionization dissolved in the modifier, as above). The peak areas were almost unaffected during the addition of a make-up flow, but the area RSD achieved a minimal value at approximately 6 to 8 % for a make-up flow rate of 0.4 mL/min. There was no negative influence on peak heights and their relative standard deviation by adding a make-up flow to the SFC effluent compared to the preheated example. The peak height remained constant up to a make-up flow rate of 0.4 mL/min. The peak height RSD achieved a minimum of approximately 8 to 10 % for a make-up flow rate of 0.2 to 0.4 mL/min (Figure 8). The S/N ratio, in comparison to the preheated example without make-up flow, decreased when adding make-up flow, but stayed constant for different make-up flow rates (Figure 9).

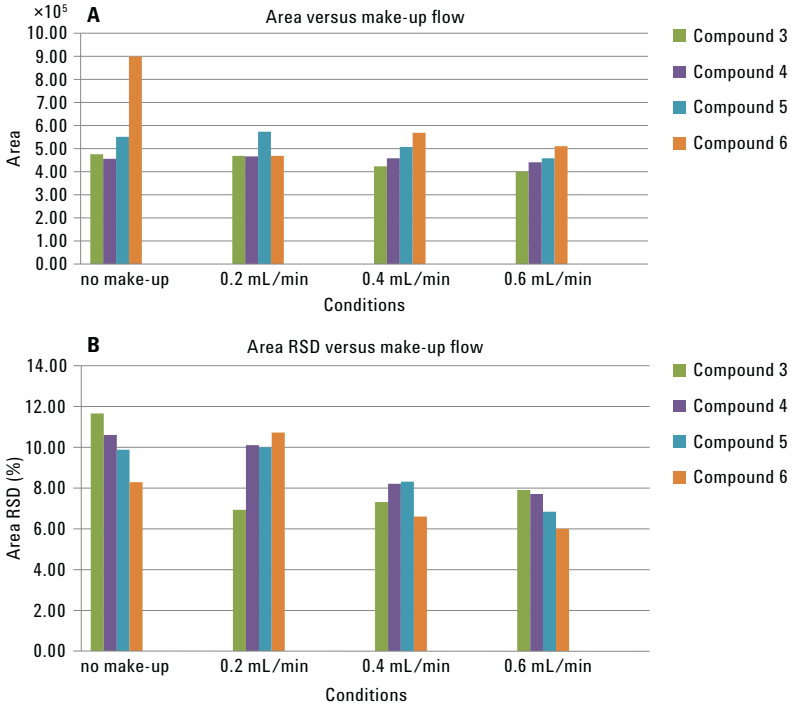


Figure 7. Peak area and peak area RSD % versus make-up flow rate at 30 °C temperature of a split SFC effluent. The peak areas were almost unaffected during the addition of a make-up flow but the area RSD achieved a minimal value at approximately 6 to 8% for a make up flow rate of 0.4 mL/min.

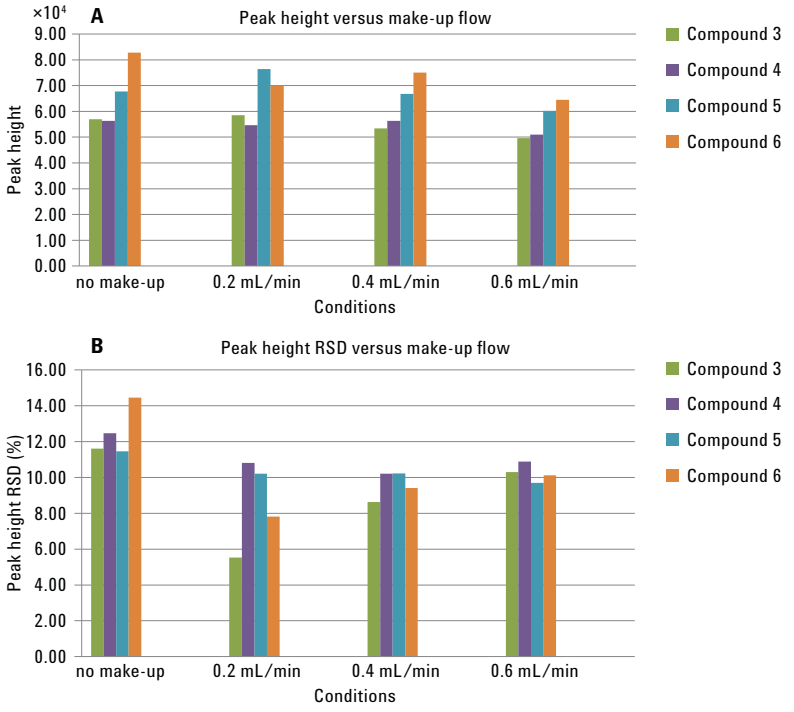


Figure 8. Peak height and peak height RSD % versus make-up flow rate at 30 °C temperature of a split SFC effluent. The peak height remained constant up to a makeup flow rate of 0.4 mL/min. The peak height RSD achieved a minimum at approximately 8 to 10 % for a makeup flow rate of 0.4 mL/min.

## Conclusions

This Technical Overview demonstrates the capability of coupling the Agilent 1260 Infinity Analytical SFC Solution to an Agilent 6150 Single Quadrupole MS with a Jet Stream Technology ion source. The results could be enhanced by adding another thermostatted column compartment and preheating the SFC column effluent before it enters the ion source of the MS. This prevents icing at the source and sprayer needle, improving peak area, peak height, and S/N values. The ionizing agent could be introduced by a special splitting assembly, which is specially designed for use with mass spectrometers and other additional detectors. This avoids having ionizing additives in the $CO_2$ modifier and offers additional flexibility without loss in performance.

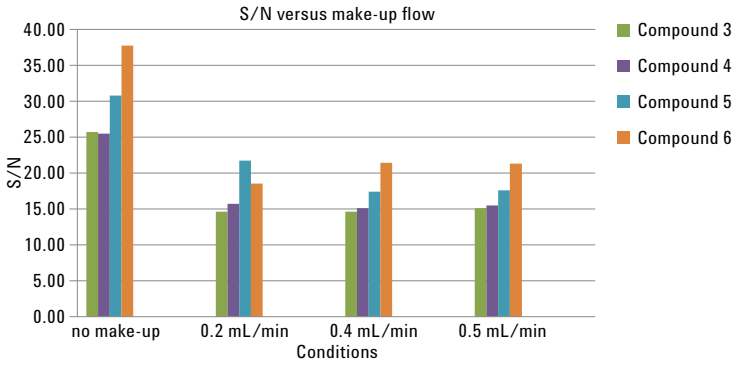


Figure 9. S/N ratio versus make-up flow rate at 30 °C temperature of a split SFC effluent. The S/N ratio, in comparison to the preheated example without make-up flow, decreased to a lower level when adding make-up flow, but remained constant for different make-up flow rates.

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