

# Agilent 1260 Infinity SFC/MS Solution

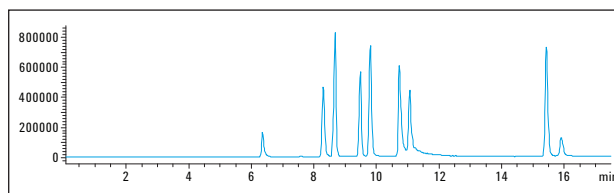
Superior sensitivity by seamlessly interfacing to the  
Agilent 6100 Series LC/MS system

## Technical Overview

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### Abstract

The applicability of Supercritical Fluid Chromatography (SFC) can be substantially enhanced by coupling SFC to Mass Spectrometric detection (MSD). In this Technical Overview, two instrument configurations are described that can be used to connect the Agilent 1260 Infinity Analytical SFC to a mass spectrometer either using an atmospheric pressure chemical ionization (APCI) or an electrospray (ESI) source. Tests were performed using an Agilent 6130 Single Quadrupole LC/MS, however all other Agilent Single Quadrupole LC/MS systems can also be used.

In the proposed configurations, no effluent splitting is used. Good repeatability (retention time RSD < 0.3% and peak area RSD < 10%) and sensitivity (10 × more sensitive than UV detection) are obtained while minimal peak dispersion is observed compared to UV/DAD detection.



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## Introduction

Supercritical Fluid Chromatography using packed columns is a valuable complementary technique to liquid chromatography. SFC has demonstrated its potential for chiral and normal phase separations. With the introduction of the Agilent 1260 Infinity Analytical SFC system, excellent performance in terms of resolution, sensitivity and sample throughput can be obtained. Increased performance of CO<sub>2</sub> flow control, by using a booster pump combined with an Agilent 1260 Infinity Binary Pump and high performance backpressure regulator (located after the detector), resulted in much lower noise in UV detection and enhanced sensitivity.

The application range of SFC can be widened by coupling SFC to MS detection. In this case, the effluent, mainly consisting of carbon dioxide, is decompressed before entering the MS source. The expanding CO<sub>2</sub> results in a significant cooling making the coupling of SFC to MS less straightforward as coupling LC to MS. For this reason, different approaches, including splitting of flow before or after the backpressure regulator, and adding liquid make-up flow have been applied.

Two configurations were found to result in excellent performance in terms of sensitivity, resolution and reproducibility. These configurations are described and illustrations are given of the SFC/MS performance.

## Experimental

### Solutions

Stock solutions of the individual pharmaceutical components were prepared in methanol (1–5 mg/mL, depending on solubility). These stock solutions were then mixed to obtain a 10-compound test mixture. Most experiments were performed using a 10 ppm solution; however, a dilution series was also

prepared from 0.1–100 ppm. Peak identification, chemical name, and formula weight are provided in Table 1. Negative ionization was also evaluated, and a separate test mixture was used (Table 2). Stock solutions of the individual components were prepared in methanol (1–5 mg/mL, depending on solubility), and the stock solutions were mixed to give a final concentration of 10 ppm.

Peak id	Chemical name	CAS	MW (g/mol)
1	Theobromine	83-67-0	180
2	Theophylline	58-55-9	180
3	Cortisone	53-06-5	360
4	Prednisone	53-03-2	358
5	Hydrocortisone	50-23-7	362
6	Prednisolone	50-24-8	360
7	Sulfaquinoxaline	59-40-5	300
8	Sulfamerazine	127-79-7	264
9	Sulfaguanidine	57-67-0	214
10	Cytosine	71-30-7	111

**Table 1**  
Ten-component test mixture.

Peak id	Chemical name	CAS	MW (g/mol)
1	Gemfibrozil	25812-30-0	250
2	Irgasan	9012-63-9	289
3	Ibuprofen	15687-27-1	206
4	Naproxen	22204-53-1	230
5	3,4,4'-Trichlorocarbanilide	101-20-2	314
6	Warfarin	81-81-2	308
7	Diclofenac	15307-79-6	296

**Table 2**  
Negative ionization test mixture.

## Experimental conditions

All experiments were performed using the same normal phase column - an Agilent ZORBAX RX-SIL, – 4.6 mm × 250 mm, 5 μm column (Agilent p/n: 880975-901) and using the same modifier (20 mM ammonium formate in methanol). Detection was performed at UV 254 nm, MS scan in positive mode, and MS SIM in positive mode using the M+1 ion.

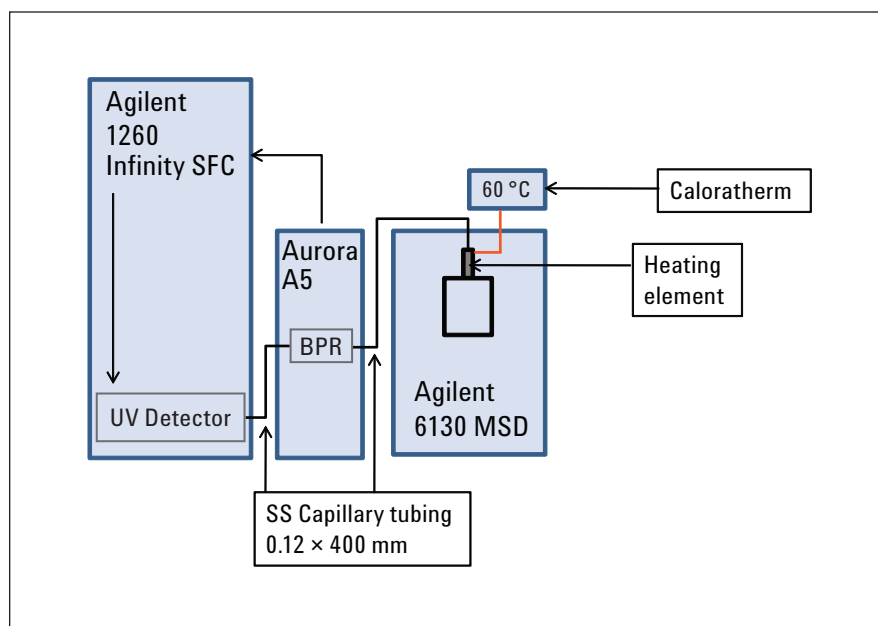
## System configurations

### SFC/MS Configuration 1

This SFC/MS configuration removes the waste line after the Back Pressure Regulator (BPR), and uses a stainless steel (SS) capillary tubing to connect the BPR to a Caloratherm heating device,<sup>1</sup> and then to the MSD. The heating device prevents freezing of the lines caused by the expansion of the CO<sub>2</sub> upon entering the source. As can be seen from Figure 1, two 0.12 mm × 400 mm SS capillaries (p/n: 5021-1823) were inserted into the system. One was used to replace the large bore SS tubing originally installed on the system that connected the UV detector to the BPR. The second was used to connect the outlet of the BPR to the Caloratherm heating device. The Caloratherm pre-heater sleeve was placed over an Agilent SS cap, 0.17 mm × 100 mm (p/n: 5061-3361). An Agilent SS zero dead volume union (p/n: 0100-0900) was used to connect the two pieces of tubing, and the tubing containing the pre-heater was connected directly to the inlet of the MSD. The diverter valve that directs the flow from the LC/SFC system to either waste or the source was bypassed in this set-up.

Conditions	
Column:	Agilent ZORBAX RX-SIL (4.6 id × 250 mm, 5 μm)
Supercritical fluid:	CO <sub>2</sub>
Modifier:	MeOH with 20 mM NH <sub>4</sub> COOH
Outlet pressure:	120 bar
Flow rate:	2.0 mL/min
Modifier gradient:	0-20 min: 5-40%
Temperature:	40 °C
Injection volume:	5 μL
Caloratherm heater:	60 °C
Make-up flow:	MeOH at 0.2 mL/min
Detection:	DAD, 254 nm MS scan 80 – 400 amu, SIM (M±1) APCI and ESI sources
APCI:	Capillary V ±3000V, Corona I = 4.0 μA (+), 15.0 μA (-) Drying gas = 12.0L/min at 325 °C Nebulizer = 50 psig Vaporizer = 350 °C
ESI:	Capillary V ±3000V Drying gas = 12.0L/min at 250 °C Nebulizer = 35 psig

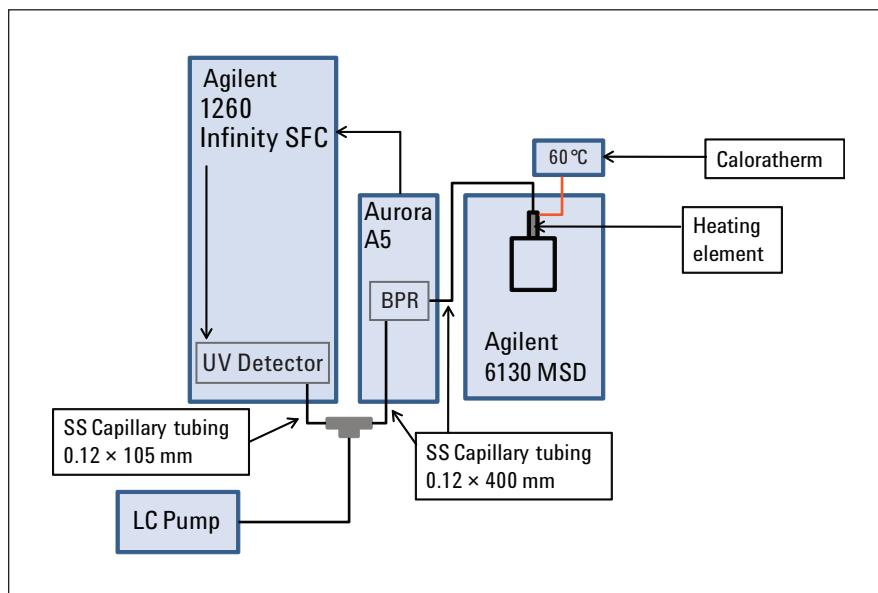
**Table 3**  
Experimental conditions.



**Figure 1**  
Schematic of SFC/MS Configuration 1.

## SFC/MS Configuration 2

This configuration is very similar to Configuration 1, however, a make-up flow is added to the system between the UV detector and the BPR (Figure 2). From the UV detector (p/n: 5021-1820), a 0.12 mm × 105 mm SS capillary is connected to an Agilent zero dead volume T-piece (p/n: 0100-0969). An Agilent 1260 Infinity Isocratic Pump was used to supply the make-up flow and was connected to the T using a 0.25 mm × 800 mm SS capillary (p/n: 5065-9930). A 0.12 mm × 400 mm SS capillary (p/n: 5021-1823) was used to connect the T to the BPR. After the BPR, all components remained the same as in SFC/MS Configuration 1 (Figure 1). All capillaries are available in the SFC/MS connection kit (p/n G4309-68000).



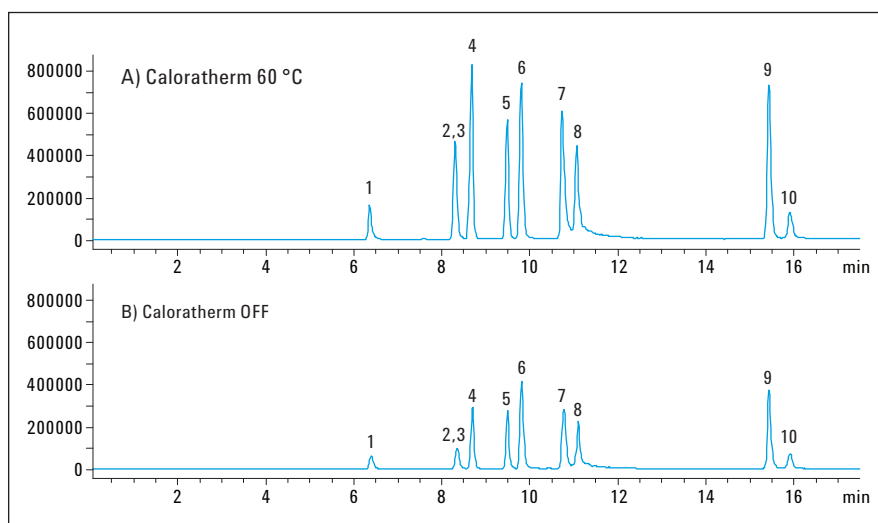
**Figure 2**  
Schematic of SFC/MS Configuration 2.

## Results and discussion

### Heating prior to MSD

For both SFC/MS Configurations 1 and 2, it was determined that heating of the lines prior to the MSD made a significant difference.

Figure 3 shows chromatograms obtained with and without the Caloratherm heater present. The two chromatograms are on the same scale, and it is easily seen that the signal is much lower when the heating device is not present. Noise and poor reproducibility are attributed to partial freezing of the lines and MS inlet due to the expansion of the CO<sub>2</sub>. With the heating device present in the configuration, freezing does not occur, and the MS reproducibility is significantly improved. Therefore, it is highly recommended that heating prior the MS inlet is performed. For all remaining experiments, the Caloratherm heating device was present.



**Figure 3**  
SIM chromatograms of the 10-compound test mixture using SFC/MS Configuration 2 (APCI) with (A) and without (B) the Caloratherm heating device present. The separation conditions and MS parameters are listed in Table 3.

## SFC/MS Configuration 1 versus Configuration 2

SFC/MS Configuration 1 is a lower-cost option than Configuration 2, because a separate LC pump to provide the make-up flow is not required. The best results, however, were obtained on Configuration 2.

SFC/MS Configuration 1 results in reproducible MS retention times (< 0.3% RSD in most cases). Larger variations are observed for MS peak area. This is due to the fact that the stream exiting the BPR may not be a uniform spray. It is recommended that SFC/MS Configuration 1 be used for qualitative information, general screenings, method development, and so forth. This configuration is not recommended for quantification purposes.

SFC/MS Configuration 2 gives MS retention time reproducibility of less than 0.3% RSD, and MS peak area reproducibility is better than 10% RSD. Due to the addition of the make-up flow, the stream exiting the BPR is most likely more uniform, allowing for more reproducible MS results. This configuration is more reproducible and can be used for quantification purposes.

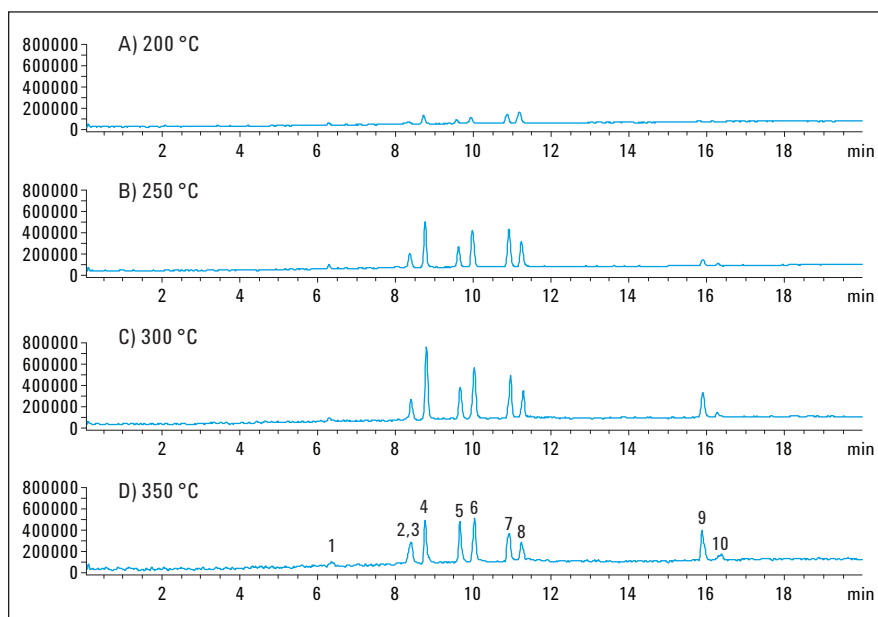
## MS source parameters

Both the APCI and ESI sources were used with SFC/MS Configuration 2. An experiment was designed in which the complete flow rate and temperature ranges of the sources were explored. For example, drying gas flow rates from 5–12.5 L/min were used in combination with drying gas temperatures ranging from 200–350 °C and vaporizer temperatures ranging from 200–450 °C. The 10-compound test mixture was injected

under generic SFC separation conditions, and the results were analyzed. It was determined that both the APCI and ESI sources can be used to perform SFC/MS. Just as in LC/MS, source choice will depend on sample ionization.

It was determined that for both the APCI and ESI sources, overall, MSD retention time reproducibility of the components was < 0.3% RSD, and the MSD peak area reproducibility was < 10% RSD. The APCI and ESI results were very similar, with the main difference being better ionization of certain

compounds using ESI compared to APCI. While the retention times of the compounds did not change, varying the source parameters did result in changes in peak areas. Figures 4 and 5 show the TIC obtained at varying source temperature for the APCI and ESI sources, respectively. In Figure 4, the y-axis scale is the same for all TICs presented, and as can be seen, at 200 °C, ionization was poor with the APCI source, however, as the source temperature increased, the ionization improved. This affect was also seen with the ESI source, but to less of an extent.

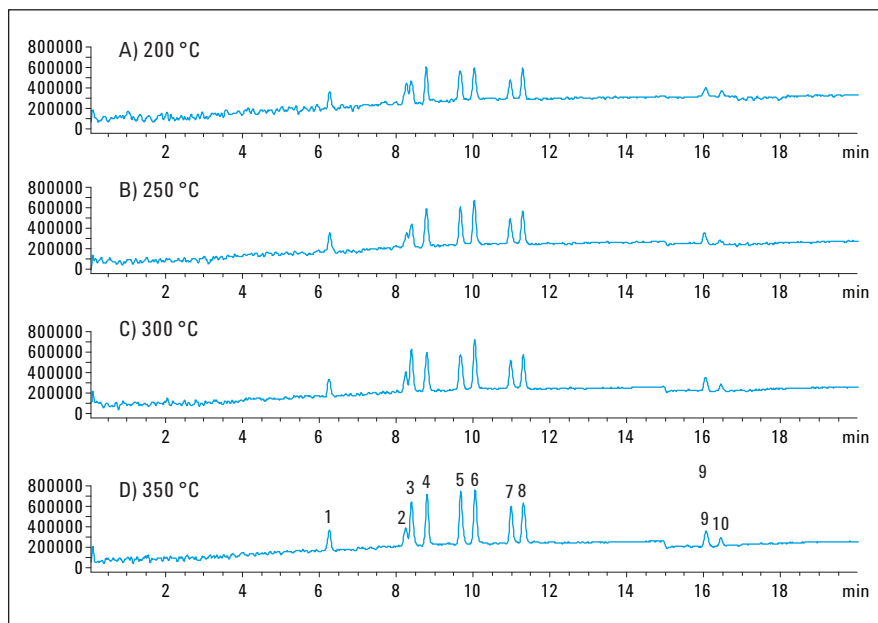


**Figure 4**  
TIC of the 10 component test mixture at varying source temperatures using the APCI source. In each case, the drying gas temperature was the same as the vaporizer temperature: A) 200 °C, B) 250 °C, C) 300 °C, and D) 350 °C. The drying gas flow was 5.0 L/min, and the nebulizer was 50 psig. The remaining separation conditions were the same as in Figure 3.

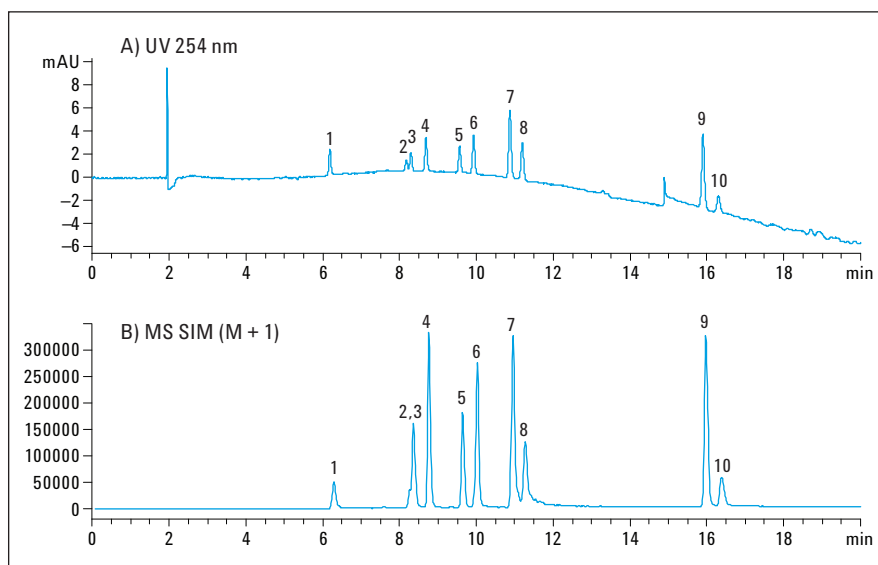
Figure 5 shows that peaks 9 and 10 do not ionize as well at a source temperature of 200 °C, but as the temperature increases, ionization of these two compounds improves. This is expected, as ionization may be better or worse depending on the drying gas flow rate and source temperatures. For this mixture, a source temperature of 300–350 °C provided the best results for both APCI and ESI ionization.

### SFC/MS sensitivity

Using the same 10-component test mixture, a dilution series from 0.1–100 ppm was analyzed using SFC/MS Configuration 2. Both UV and MS data (APCI) were collected and compared (Figure 6). Figure 6 shows the UV and MS traces of 5 ppm of the 10-component test mixture. Similar resolutions and peak widths for the UV and MS results were obtained. Linearity was good for both UV and MS detection with  $R^2$  values of 0.99 in most cases. The UV and APCI MS data was linear from 0.1–100 ppm, and the ESI MS data was linear from 0.1–10 ppm. The limit of detection (LOD) for each compound was calculated based on the calibration curves and are listed in Table 5. The MSD was approximately 10 times more sensitive than UV detection for all of the components of the test mixture.



**Figure 5**  
TIC of the 10 component test mixture at varying source temperature using the ESI source: A) 200 °C, B) 250 °C, C) 300 °C, and D) 350 °C. The remaining separation conditions were the same as in Figure 4.



**Figure 6**  
A) UV and B) APCI SIM traces of the 10 compound test mixture at 5 ppm. The separation conditions are the same as in Figure 3.

## SFC parameters

The SFC parameters were examined to determine if limitations would exist when using MS detection. The flow rate and backpressure of the SFC system were varied; flow rates from 1–5 mL/min and back pressures from 100 to 250 bar were analyzed using the 10-compound test mixture.

The flow rate of the SFC system was varied from 1–5 mL/min in increments of 1 mL/min. It was determined that the maximum flow rate for SFC/MS in combination with a makeupflow was 3 mL/min.

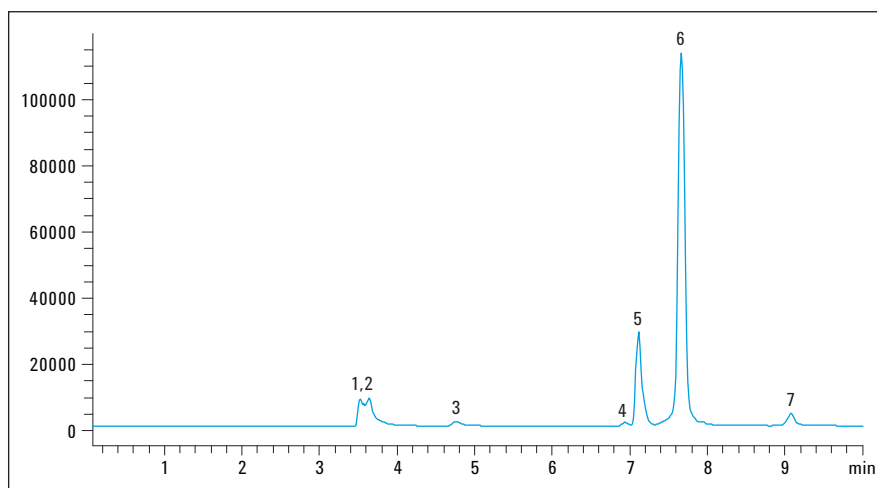
The back pressure of the SFC system was varied from 100–250 bar in increments of 50 bar. It was determined that the SFC/MS operated well under all of the backpressures tested. Baseline noise and peak area reproducibility was slightly worse at 100 bar and improved as the backpressure increased.

## Negative ionization

Lastly, negative ionization was examined using SFC/MS Configuration 2 with the APCI source present. Figure 7 shows the chromatogram of the test mixture for negative ionization at 10 ppm using the APCI source.

Peak ID	Name	UV	APCI MS		ESI MS	
		LOD (pg)	LOD (pg)	R <sup>2</sup>	LOD (pg)	R <sup>2</sup>
1	Theobromine	500	50	0.9925	50	0.9978
2	Theophylline	1250	200	0.9887	50	0.9912
3	Cortisone	500	25	0.9977	20	0.9942
4	Prednisone	375	20	0.9977	35	0.9978
5	Hydrocortisone	500	25	0.9989	20	0.9984
6	Prednisolone	375	25	0.9977	63	0.9957
7	Sulfaquinoxaline	375	25	0.9991	53	0.9968
8	Sulfamerazine	500	50	0.9982	50	0.9991
9	Sulfaguandine	375	20	0.9982	250	0.9869
10	Cytosine	500	50	0.9988	165	0.9977

**Table 5**  
LOD values for UV and MS detection using SFC/MS Configuration 2.



**Figure 7**  
SIM chromatogram of the test mixture for negative ionization. The separation conditions are listed in Table 3.

## Conclusions

The Agilent 1260 Infinity Analytical SFC system can be coupled to MS detection. Both APCI and ESI sources can be used and positive and negative ion detection modes can be applied. It was determined that heating prior to the MSD is necessary in order to prevent freezing upon the expansion of the CO<sub>2</sub> and to obtain stable/reproducible results.

Configuration 2, where a make-up flow is added before the backpressure regulator, results in the best retention time and peak area reproducibility. Good sensitivity, linearity and high robustness are obtained. This configuration is recommended for qualitative and quantitative analyses.

Configuration 1, without liquid make-up flow, can be used but the MS response shows higher variability.

There is no limitation concerning source temperature or drying gas flow rate; the entire ranges can be used. As for the SFC parameters, the entire pressure and temperature range can be used. However, only flow rates of up to 3.0 mL/min can be used with the current Configuration 2 setup.

## References

1. The Caloratherm (p/n: AG1) and pre-heater (p/n: AG004) are products from SandraSelerity Technologies and are available through RIC.

For more information, contact [info@richrom.com](mailto:info@richrom.com).

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