Coupling the Agilent 1260 Infinity Analytical SFC System to an Agilent 1260 Infinity Evaporative Light Scattering Detector

Technical Overview

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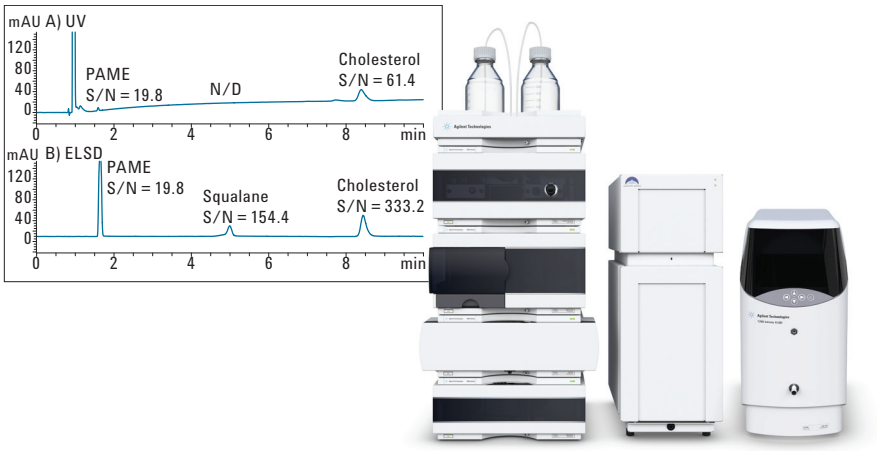

Abstract

This Technical Overview describes the coupling of the Agilent 1260 Infinity Analytical SFC System and the Agilent 1260 Infinity ELSD. A make-up flow pump and an external heating device were incorporated in the system configuration. It was shown that both heating and make-up flow are necessary to obtain a stable baseline and to avoid condensation of solutes in the transfer line between the back pressure regulator (BPR) and evaporative light scattering detector (ELSD). Good repeatability (retention time RSD < 0.5% and peak area RSD < 3.0%) and sensitivity were obtained, allowing the system to be used for qualitative as well as quantitative analysis.

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Introduction

Supercritical fluid chromatography (SFC) using packed columns is a valuable complementary technique to liquid chromatography. SFC has demonstrated its potential particularly for chiral and normal phase separations. Excellent performance in terms of resolution, sensitivity, and sample throughput can be obtained using the Agilent 1260 Infinity Analytical SFC System. Using a booster pump combined with an Agilent Infinity Binary Pump and high performance backpressure regulator (located after the UV detector), to increase performance of CO_2 flow, resulted in low noise in UV detection and enhanced sensitivity.

The applicability of SFC can be further enhanced by coupling to an evaporative light scattering detector (ELSD). In this case, however, the effluent, mainly consisting of carbon dioxide, is decompressed before entering the ELSD nebulizer. The expanding carbon dioxide resulted in a significant cooling, making the coupling of SFC to ELSD less straightforward as coupling LC to ELSD. For this reason, different approaches, including adding a liquid make-up flow and an external heating device have been applied.

To evaluate the need for additional make-up flow and heating after the BPR, two apolar test mixtures were used. Mix 1 contained compounds with different volatility, which should give differences in ELSD response. Mix 2 contained triglycerides of similar volatility, which is a typical SFC-ELSD application.

Experimental

Solutions

Mix 1 contained cholesterol, squalane, and palmitic acid methyl ester (PAME), which were purchased from Fluka (Switzerland). These compounds range in volatility, with PAME being most volatile, and cholesterol the least volatile of the three. The mixture was prepared at 1,000 ppm in chloroform.

Mix 2, the triglyceride mix, contained tripalmitin (PPP), triolein (OOO), and trilinolein (LLL), which were purchased from Sigma-Aldrich (Bornem, Belgium). Individual stock solutions were prepared at 5,000 ppm in chloroform, except OOO, which was delivered at 5,000 ppm in pyridine. The mixture was prepared at 1,000 ppm in chloroform.

System configuration

Different evaluation tests were performed on a 1260 Infinity Analytical SFC System combined with an Agilent 1260 Infinity ELSD. The Evaporative Light Scattering Detector was coupled to the SFC module using a similar procedure as used for SFC-MS [1]. The system components are summarized in Table 1. Figure 1 shows a schematic of the configuration.

Part number	Description	
G4309A	Agilent 1260 Series Analytical SFC System	
G1310B	Agilent 1260 Infinity Isocratic Pump (Make-up Flow)	
G4260B	Agilent 1260 Infinity Evaporative Light Scattering Detector	
AG1	Caloratherm	Available through RIC[1,2]
AG004	Preheater	Available through RIC[1,2]

[1]Contact info@richrom.com for more information.
[2] Alternatively, the heat exchanger of a G1316A can be used for heating the post BPR transfer line to the ELSD.

Table 1
System modules.

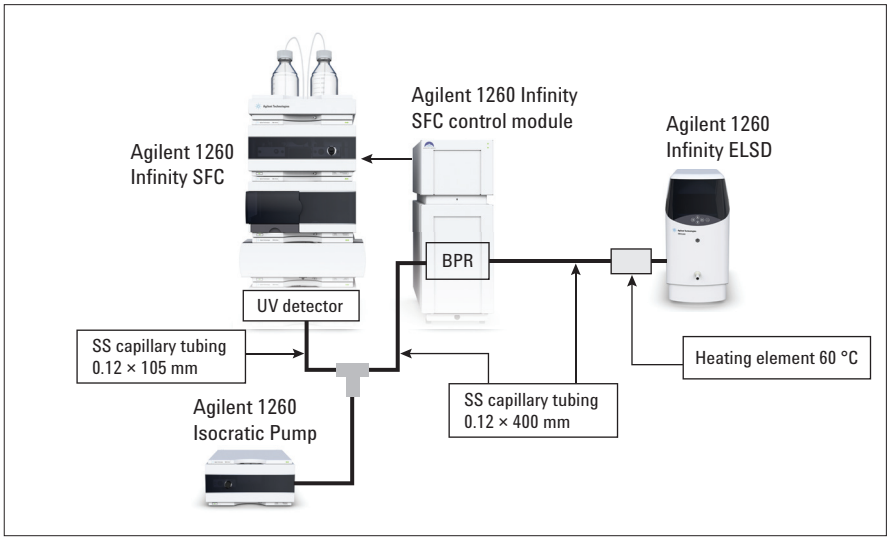

Figure 1
Schematic of the SFC-ELSD configuration.

Experimental conditions

Two different mixtures were analyzed, Mix 1 containing cholesterol, squalane, and PAME, and Mix 2 containing OOO, LLL, and PPP. The experimental conditions are summarized in Table 2.

Results and discussion

Influence of heating prior to ELSD

It was determined that heating the capillaries connecting the BPR to the ELSD made a substantial difference. Without an external heating device, the CO_2 exiting the BPR causes the inlet of the ELSD to freeze, which in turn, causes the ELSD to give a leak error due to condensation on the leak sensor.

A capillary heating device was installed just before the ELSD inlet. Temperatures ranging from 30 °C up to 80 °C (for example, 30, 40, 50, 60, and 80 °C) were applied for the analysis of Mix 1. It was determined that a minimum temperature of 50 °C was required for the system to run properly. Lower temperature settings might result in a leak error. Figure 2 shows the chromatograms obtained at different temperatures, and Table 3 contains the reproducibility data.

Conditions	
Column:	Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm (p/n 880975-902)
Supercritical fluid:	CO_2
Modifier:	9:1 ACN/MeOH
Outlet pressure:	150 bar
Flow rate:	3 mL/min
Gradient;	0–10 minutes: 5%-10% (Mix 1) 0–20 minutes: 3 %-15% (Mix 2)
Column temperature:	25 °C
Injection volume:	5 µL
Make-up flow:	IPA at 0.6 mL/min
Transfer line heating:	60 °C
DAD:	210/4 nm, Ref. 360/100 nm
ELSD:	Evap 30 °C, Neb 30 °C, 1.60 SLM, Gain 1, Smoothing 5 seconds

Table 2
Experimental conditions.

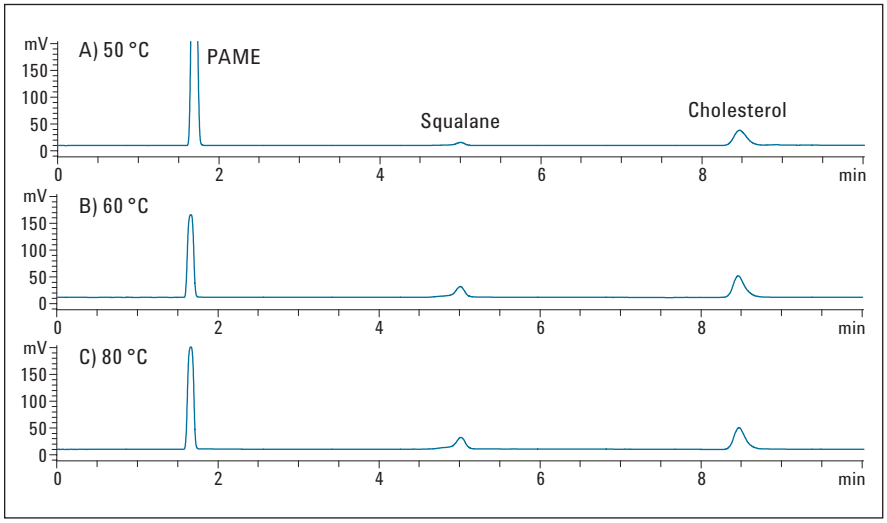

Figure 2
Separation of Mix 1 at varying transfer line heating temperature: A) 50 °C, B) 60 °C, and C) 80 °C. The separation conditions are listed in Table 2; a make-up flow at 0.6 mL/min was used.

From these experiments, it was determined that retention time and retention time reproducibility were little affected by the heating temperature. However, peak area and peak area reproducibility were greatly affected. It was determined that a temperature of 60 °C was optimal, as this gave the best results in terms of peak area reproducibility, which was less than 2.5% for all components of Mix 1. Since the heating device is placed just before the inlet of the ELSD, the temperature of the effluent entering the nebulizer will be dependent upon the capillary heating temperature, which can then affect nebulization. As can be seen in Table 3, the peak area of PAME, the most volatile component of the test mixture, is much higher at a temperature of 50 °C than at 60 °C or 80 °C; however, the opposite is seen for squalane (and cholesterol), which shows a much lower peak area at 50 °C than at higher temperatures. This is probably an indication of solute loss due to condensation. In addition, the peak area RSDs at 50 °C are quite high. At 60 °C and 80 °C, peak areas were relatively similar while good RSDs were obtained. Since the peak area RSDs for all components were best at 60 °C (that is < 5%), this value was selected for the remaining experiments

Figure 3 shows the separation of Mix 1 using both UV and ELSD detection at the optimized temperature (60 °C) and using 0.6 mL/min make-up flow rate. As seen, the ELSD is much more sensitive than UV detection, especially for the detection of methyl palmitate and squalane.

		PAME	Squalane	Cholesterol
50 °C	t_R Avg (min)	1.69	4.99	8.46
	RSD% t_R	0.56	0.21	0.11
	Area Avg (mV)	2495.8	64.1	344.0
	RSD% Area	15.0	13.7	6.68
60 °C	t_R Avg (min)	1.66	5.01	8.46
	RSD% t_R	0.33	0.20	0.18
	Area Avg (mV)	853.2	219.1	458.1
	RSD% Area	2.16	2.09	2.26
80 °C	t_R Avg (min)	1.65	5.01	8.45
	RSD% t_R	0.51	0.19	0.17
	Area Avg (mV)	961.7	225.6	430.6
	RSD% Area	3.74	2.08	5.58

Table 3
Reproducibility data for varying Caloratherm temperature.

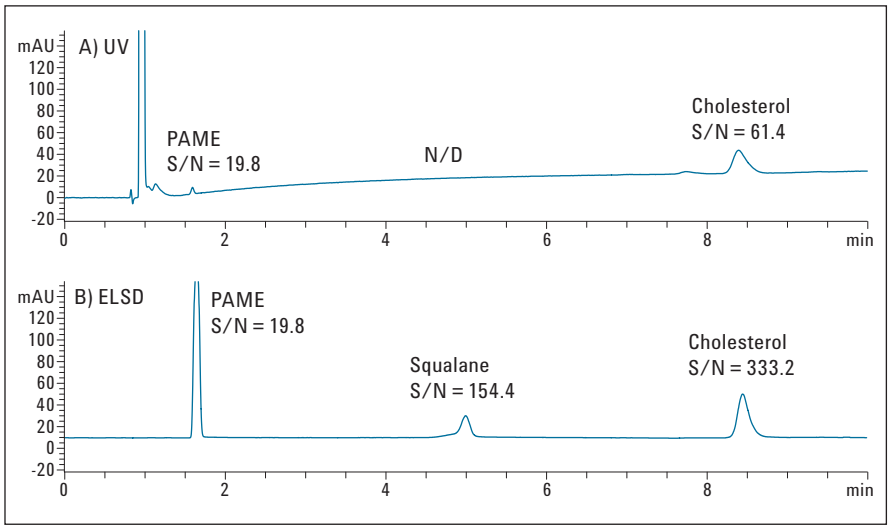

Figure 3
Separation of Mix 1 with A) UV detection and B) ELSD detection. The separation conditions are listed in Table 2. The heater was set to 60 °C, and the make-up flow was 0.6 mL/min.

Influence of make-up flow

In order to determine which make-up flow rate gave the best results, the make-up flow rate was varied from 0 – 0.8 mL/min in increments of 0.2 mL/min. Note that when no make-up flow was applied, the system was replumbed, so that the make-up pump was not included in the configuration that is, the outlet of the UV detector was connected directly to the BPR. This was done so that no effects from turbulent flow could be attributed to the results observed. Table 4 shows the results obtained for Mix 1. It was observed that, as the make-up flow rate was increased, the baseline noise, S/N, and reproducibility all improved, up to 0.6 mL/min. After 0.6 mL/min, these values began to fall off. Due to the addition of the make-up flow, the stream exiting the BPR is most likely more uniform, resulting in more reproducible ELSD results. This is seen when comparing the peak area RSDs in Table 4. Without make-up flow, the area RSDs are >10%, but once a make-up flow is applied, the values are below 5% in most cases.

Additionally, a small decrease in retention time is observed from 0.2 to 0.8 mL/min make-up flow. Since the make-up flow is a portion of the total flow rate of the system, as this value is increased, the total flow rate is increased, which explains a small loss of retention. For this application, the optimum make-up flow rate was 0.6 mL/min, however, as this is a portion of the total flow rate, if the flow rate for the separation is changed, then the make-up flow will need to be optimized. Typically, a make-up flow between 10–20% of the total flow was shown to give the best results.

		PAME	Squalane	Cholesterol
No make-up flow	t_R Avg (min)	1.68	4.98	8.45
	RSD% t_R	0.46	0.49	0.15
	Area Avg (mV)	1168.1	255.4	382.4
	RSD% Area	22.10	12.88	10.54
	S/N	927.2	146.4	147.0
	Noise (6.0 – 7.0)	0.1972 (6*SD)		
0.2 mL/min	t_R Avg (min)	1.68	5.04	8.47
	RSD% t_R	0.54	0.22	0.13
	Area Avg (mV)	1206.6	240.8	478.2
	RSD% Area	4.97	7.89	5.88
	S/N	881.5	99.1	185.8
	Noise (6.0 – 7.0)	0.2191 (6*SD)		
0.4 mL/min	t_R Avg (min)	1.66	5.02	8.46
	RSD% t_R	0.35	0.21	0.09
	Area Avg (mV)	924.3	242.6	522.4
	RSD% Area	2.97	3.90	1.81
	S/N	1144.7	157.8	307.3
	Noise (6.0 – 7.0)	0.1520 (6*SD)		
0.6 mL/min	t_R Avg (min)	1.66	5.01	8.46
	RSD% t_R	0.33	0.20	0.18
	Area Avg (mV)	853.2	219.1	458.1
	RSD% Area	2.16	2.09	2.26
	S/N	1304.1	171.7	337.7
	Noise (6.0 – 7.0)	0.1182 (6*SD)		
0.8 mL/min	t_R Avg (min)	1.65	4.99	8.44
	RSD% t_R	0.50	0.18	0.12
	Area Avg (mV)	894.3	203.9	422.7
	RSD% Area	5.66	2.37	1.76
	S/N	1119.0	115.4	233.2
	Noise (6.0 – 7.0)	0.1708 (6*SD)		

Table 4
Reproducibility and S/N at different make-up flow rates for Mix 1.

This experiment was also performed using Mix 2, with similar trends observed. Figures 4 and 5 show the separation of the triglycerides with no make-up flow, and varying the make-up flow rate (0.2 – 0.8 mL/min), respectively. When no make-up flow was applied, the system was replumbed, so that the make-up pump was not included in the configuration (that is, the outlet of the UV detector was connected directly to the BPR), so that no affects from turbulent flow could be attributed to the results observed.

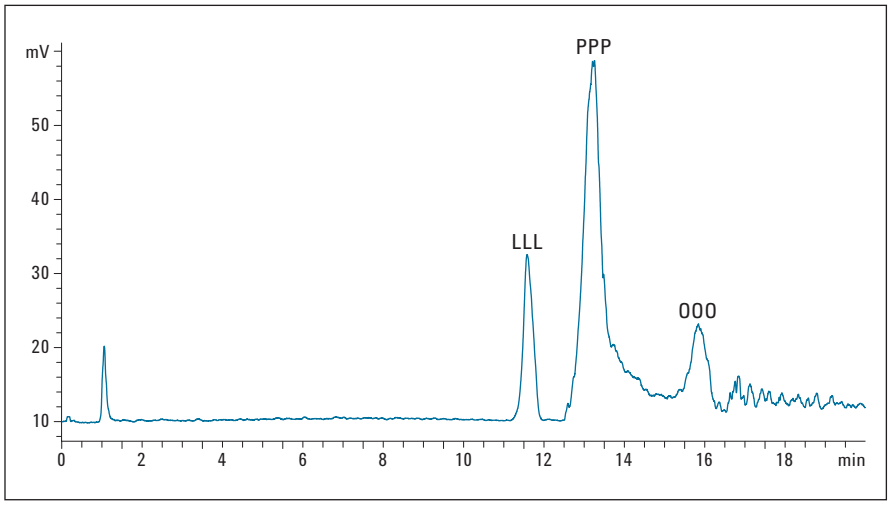

Figure 4
Separation of the triglyceride mix when no make-up flow was applied.

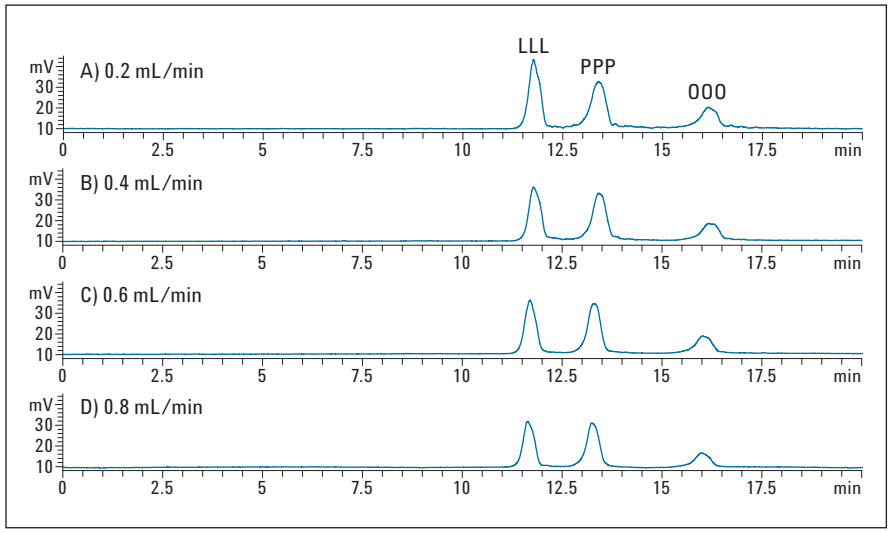

Figure 5
Separation of the triglyceride mix at varying make-up flow rates.

Table 5 demonstrates the reproducibility, S/N, and baseline noise values obtained for Mix 2. Just as was seen with Mix 1, as the make-up flow is added and the flow rate increased, the baseline noise, S/N, and peak area reproducibility improve up to 0.6 mL/min. Above 0.6 mL/min, these values fall off. This is seen when comparing the peak area RSDs in Table 5. Without make-up flow, the area RSDs are > 15%, but once a make-up flow is applied, these values are below 6% in most cases. With Mix 2, the decrease in retention is more easily observed. As the make-up flow rate is increased from 0.2–0.8 mL/min, the retention times decreased due to the increase in total flow rate.

		LLL	PPP	000
No make-up flow	t_R Avg (min)	11.59	13.18	15.89
	RSD% t_R	0.43	0.69	0.63
	Area Avg (mV)	591.4	1974.9	336.1
	RSD% Area	48.48	25.5	15.48
	S/N	7.6	16.0	3.8
	Noise (17.6 – 18.6)	2.975 (6*SD)		
0.2 mL/min	t_R Avg (min)	11.78	13.41	16.18
	RSD% t_R	0.13	0.16	0.21
	Area Avg (mV)	677.8	611.6	315.7
	RSD% Area	2.23	1.49	4.90
	S/N	83.2	56.3	24.8
	Noise (17.6 – 18.6)	0.3991 (6*SD)		
0.4 mL/min	t_R Avg (min)	11.73	13.38	16.14
	RSD% t_R	0.15	0.39	0.18
	Area Avg (mV)	562.9	572.4	265.9
	RSD% Area	1.98	1.87	6.01
	S/N	151.2	130.9	46.9
	Noise (17.6 – 18.6)	0.1717 (6*SD)		
0.6 mL/min	t_R Avg (min)	11.65	13.29	16.01
	RSD% t_R	0.16	0.22	0.07
	Area Avg (mV)	534.6	597.9	245.9
	RSD% Area	2.73	1.52	2.95
	S/N	185.7	171.0	59.8
	Noise (17.6 – 18.6)	0.1393 (6*SD)		
0.8 mL/min	t_R Avg (min)	11.62	13.25	16.00
	RSD% t_R	0.12	0.14	0.18
	Area Avg (mV)	444.2	488.2	205.1
	RSD% Area	2.87	3.68	4.15
	S/N	112.5	108.3	34.8
	Noise (17.6 – 18.6)	0.1959 (6*SD)		

Table 5
Reproducibility and S/N at different make-up flow rates for Mix 2.

Conclusion

The Agilent 1260 Infinity Analytical SFC System can be coupled successfully to an evaporative light scattering detector (ELSD). It was determined that heating prior to the ELSD is necessary to prevent freezing upon expansion of the CO_2 and to obtain stable/reproducible results. Adding a make-up flow before the backpressure regulator is required to obtain the best retention time and peak area reproducibility. Good sensitivity and high robustness are obtained, allowing this configuration to be recommended for qualitative and quantitative analyses.

References

1.
M. Dunkle, G. Vanhoenacker, F. David, P. Sandra, M. Vollmer, The Agilent 1260 Infinity SFC/MS Solution, Agilent Technologies Publication Number 5990-7972EN, **2011**.

www.agilent.com/chem/sfc

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