Analysis of Lipids with the Agilent 385-ELSD and the Agilent 1260 Infinity LC

Sensitive detection through sub-ambient evaporation and nebulization

Application Note

Food Testing

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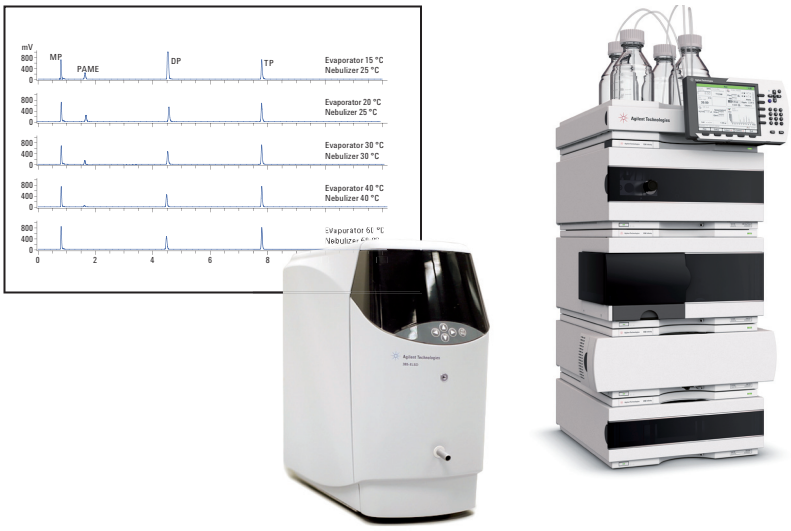

Abstract

This Application Note discusses how the Agilent 385-ELSD Evaporative Light Scattering Detector operates at various temperatures for a selection of lipid standards. The results clearly illustrate the benefit of sub-ambient evaporation and nebulization for certain (semi-)volatile compounds.

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Introduction

The Agilent 385-ELSD Evaporative Light Scattering Detector is equipped with a Peltier cooled evaporation tube which enables solvent removal at sub-ambient temperatures. This is an interesting feature for the detection of (semi-)volatile compounds. Evaporation at temperatures above ambient can lead to a loss of sensitivity, or even no detection at all for these compounds. This Application Note describes the analysis of lipid standard compounds with reversed-phase HPLC and ELSD. The detector temperature has a remarkable effect on the sensitivity for certain compounds.

Experimental

A standard mixture composed of 500 µg/mL 1-monopalmitin (MP), 1,2-dipalmitin (DP), tripalmitin (TP), and palmitic acid methyl ester (PAME) was made in mobile phase component B. This standard solution was injected under different detector temperature conditions. Five injections per condition were performed to evaluate the repeatability of injection and to obtain averaged/reliable results. The detectability, signal-to-noise ratio of PAME, and peak area of all compounds were compared for the different setpoints.

Results and discussion

Figure 1 shows the result for an injection of the standards at various temperatures. At sub-ambient conditions, that is evaporator temperature at 15 °C and nebulizer at 25 °C, all peaks are detected, and the signal for dipalmitin is outside the detector range.

Chromatographic conditions

An Agilent 1260 Infinity LC system with the following configuration was used:

- Agilent 1260 Infinity Quaternary Pump with integrated vacuum degasser (G1311B)
- Agilent 1260 Infinity Standard Autosampler (G4226A)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 385-ELSD Evaporative Light Scattering Detector (G4261A)

Method parameters

Column:	Agilent ZORBAX Eclipse XDB C18 RRHT, 2.1 mm L × 50 mm id, 1.8 µm d_p (p/n 927700-902)	
Mobile phase:	A = 0.1% acetic acid in methanol	
	B = isopropanol/hexane 50/40 v/v	
Flow rate:	1 mL/min	
Gradient:	0–1 min	0% B
	1–11 min	0–70% B
	11–12 min	70% B
	12–14 min	0% B (post-time)
Temperature:	25 °C	
Injection:	2.5 µL, needle wash (4 s, flushport, mobile phase B)	
Detection ELSD:	Nebulizer temperature	Varied (25–60 °C)
	Evaporator temperature	Varied (15–60 °C)
	Evaporator gas	1.6 SLM
	Detector rate	40 Hz
	Smoothing	3.0 s
	Gain	1

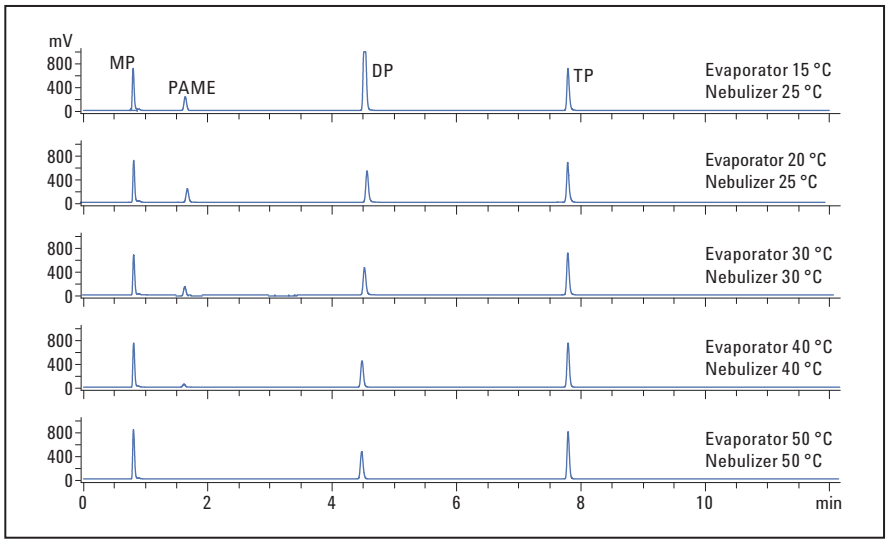

Figure 1
Analysis of the test mixture at various ELSD temperature settings.

When the evaporator temperature is slightly increased to 20 °C, the response for dipalmitin is within the detector range and remains relatively stable up to 60 °C (Table 1). The response for mono- and tripalmitin is significantly less dependent on the detector temperature.

The most striking influence of the evaporation/nebulization temperature is observed for the fatty acid methyl ester. Palmitic acid methyl ester (PAME) is easily detected at low temperatures, but contrary to the other compounds, the signal rapidly decreases when the ELSD is operated at temperatures above ambient. The signal-to-noise ratios (S/N) with the tested temperatures for this compound are shown in Table 1. Although the highest area is obtained at the lowest investigated temperature (15 °C), the signal-to-noise ratio, and consequently sensitivity, is maximal at ca. 25 °C (Figure 2). This is due to the slightly increased background noise observed with the low temperature settings. An overlay of a detail of the baseline for some representative temperatures are shown in Figure 3. This figure also illustrates the loss in signal intensity for PAME at higher temperatures. The compound is no longer detected at temperatures above 50 °C.

Conclusion

The advantage of low temperature nebulization and evaporation is demonstrated for a mixture of lipid standard compounds. The influence of the detector temperature on the detection of (semi-)volatile molecules can be impressive, therefore the option of a broad temperature range with sub-ambient capabilities is very useful for the analysis of such analytes.

Temperature		MP		TP		TP		PAME		
Evaporator	Nebulizer	Area	RSD%	Area	RSD%	Area	RSD%	Area	RSD%	S/N
15	25	1416	3.49	3931[1]	2.14	2032	3.38	735	1.26	398
20	25	1434	1.73	1637	3.30	1824	2.59	708	2.38	429
25	25	1362	1.74	1448	0.84	1869	0.49	607	1.41	530
30	30	1387	1.50	1403	0.97	1931	1.57	445	1.95	410
35	35	1473	1.13	1386	1.34	1882	1.57	270	3.41	267
40	40	1529	0.53	1408	2.84	1983	1.93	126	7.46	157
45	45	1602	1.68	1463	1.79	2031	1.08	50	2.82	48
50	50	1706	1.00	1527	0.90	2142	1.66	15	8.74	17
55	55	1759	1.65	1538	1.81	2196	1.58	N.D.	N.D.	< 3
60	60	1832	1.41	1574	1.46	2237	0.29	N.D.	N.D.	N.D.

(1) Out of range of the ELSD)

Table 1
Peak area and repeatability for the selected compounds and signal-to-noise ratio for PAME at various ELSD settings.

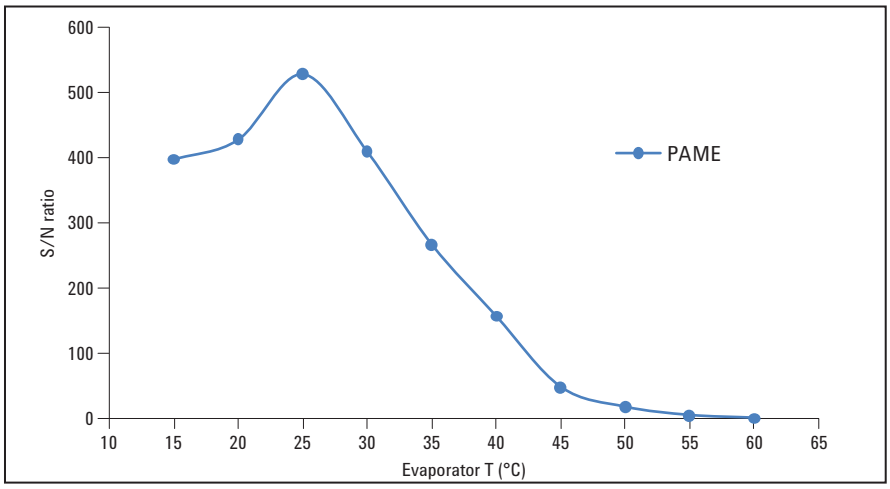

Figure 2
Influence of ELSD temperature on signal-to-noise ratio for PAME.

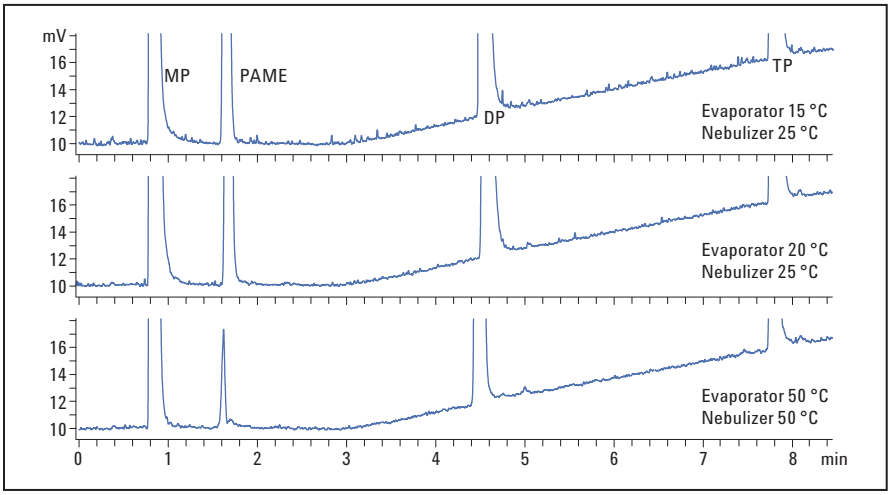

Figure 3
Detail on the baseline for the analysis of the test mixture at various ELSD temperature settings.

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