Overcoming Compound Volatility with Sub-ambient ELSD

Application Note

Author

Stephen Ball
Agilent Technologies, Inc.

Introduction

The success of evaporative light scattering detection relies on evaporating the eluent without destroying the analyte particle. Where the analyte is non-volatile, evaporator temperatures of 50-100 °C can be used without compound degradation, thus giving maximum sensitivity. However, when compounds have high vapor pressures and the eluent has a high boiling point (e.g. water), detection of semi-volatile compounds is problematic, because the evaporation temperature needs to be set at 30 °C or above in order to evaporate the solvent.

Most of today's ELSDs are only capable of evaporating aqueous eluents at 30 °C, or above, but for low molecular weight, low boiling point compounds even 30 °C is too high, and these compounds will be destroyed in the evaporation process, resulting in poor sensitivity.

To improve the detection of thermally sensitive compounds, evaporation at sub-ambient temperature is required. However, evaporating water at increasingly lower temperatures requires exponentially longer evaporation times. Therefore, temperature alone cannot be used to evaporate water at sub-ambient temperature.

The Agilent 385-ELSD has been specifically developed to operate at sub-ambient evaporation temperatures, using a Peltier cooled evaporation tube. The removal of solvent is achieved using a secondary stream of dry nitrogen gas that is added at the evaporation stage. When the evaporation temperature is lowered below ambient temperature, the "evaporation gas" is increased to compensate. At higher evaporation temperatures, the evaporation gas can be reduced or even turned off, to maximize signal-to-noise ratios. Using this sub-ambient technology, eluent can be evaporated as low as 10 °C.

This note demonstrates the value of sub-ambient evaporative light scattering detection in the analysis of semi volatile compounds.

Conditions (Figure 1)

Sample:	Parabens
Column:	Pursuit C18 5 µm, 150 x 2.1 mm (p/n A3000150X020)
Eluent A:	Water
Eluent B:	Acetonitrile
Gradient:	5-70% B in 5 min; 70-95% B in 12 min
Flow Rate:	0.2 mL/min
Inj Vol:	10 µL
Detection:	385-ELSD (neb=30 °C, evap temp. as shown, gas=1.4 SLM)

Conditions (Figure 2)

Samples :	Acetanilide MW 135
Column:	C18 5 µm, 150 x 2.1mm
Eluent A:	Water + 0.1% TFA
Eluent B:	ACN + 0.1% TFA
Gradient:	60-90% B in 5 min
Flow Rate:	0.2 mL/min

Results and Discussion

The benefit of a cooled evaporation zone that provides sub-ambient operation to detect low molecular weight compounds is shown in the analysis of parabens (Figure 1). The lower the temperature, the more parabens detected.

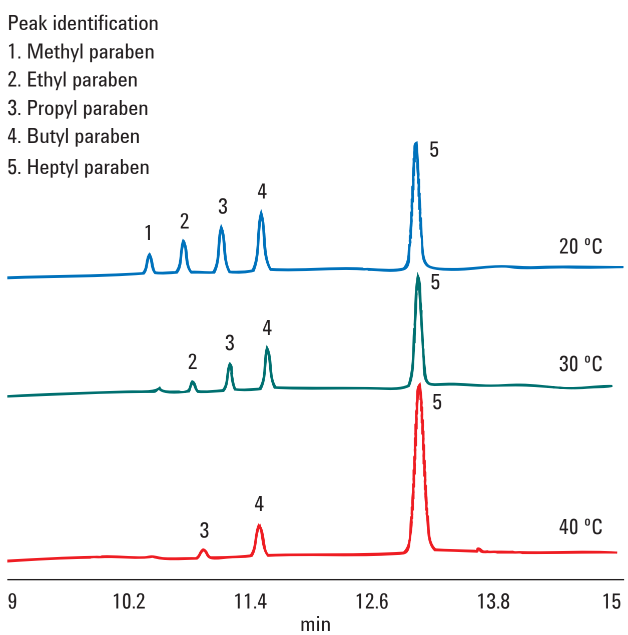

Figure 1. Improved detection of parabens using sub-ambient evaporative light scattering detection (parabens mixture injected at 100 ng except heptyl at 160 ng).

Figure 2 shows the sub-ambient ELS detection of low molecular weight and semi volatile acetanilide, a precursor in penicillin synthesis and other pharmaceuticals including painkillers and intermediates.

In this example the signal-to-noise ratio increases four-fold at 15 °C compared to 30 °C. Sub-ambient ELSD at 20 °C not only increases the number of semi-volatile compounds detectable by ELSD, but also minimizes any response variation between compounds due to volatility differences. Consequently, sub-ambient ELSD can improve the accuracy of compound quantification and uniformity of response.

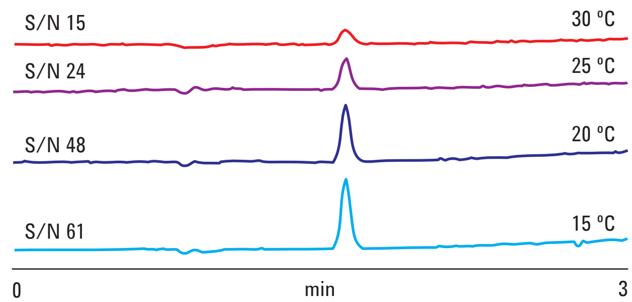

Figure 2. Improved detection of acetanilide at sub-ambient temperatures using evaporative light scattering detection.

Conclusion

In the past, quantification and purity measurements using evaporative light scattering detection showed poor accuracy due to compound volatility and response variation across a solvent gradient. However, sub-ambient ELSD at 20 °C, as delivered by the 385-ELSD, increases the accuracy of measurement by minimizing compound volatility effects.

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