Enhanced Analysis of Semi-volatile Compounds using Sub-ambient ELSD and HPLC

Application Note

Author

Stephen Ball
Agilent Technologies, Inc.

Introduction

Evaporative light scattering detection (ELSD) is a powerful tool for detecting any sample that is less volatile than the mobile phase in HPLC applications, irrespective of the optical properties of the compounds of interest. ELSD can therefore offer distinct advantages over the more conventional UV or DAD detection, particularly for gradient separations. In recent years, the analysis of non-UV absorbing semi-volatile analytes (eg drug candidates) has required ELS detectors to operate at increasingly lower temperatures in order to maximize their detection. Most of today's ELSDs are capable of evaporating highly aqueous eluents at ambient temperature. However, for low molecular weight, low boiling point compounds, operating at ambient temperature is still too high and sensitivity to these analytes is very poor. To improve the detection of these types of compounds, sub-ambient ELSD is required.

The Agilent 385-ELSD is specifically designed to perform under these exacting conditions. The 385-ELSD contains a Peltier cooled evaporation tube that provides sub-ambient ELS detection down to 10 °C. The removal of solvent at sub-ambient temperatures is facilitated by the addition of dry nitrogen gas during the evaporation step. This 'evaporation gas' reduces the relative humidity (ie saturation ratio) inside the evaporation tube, thus enabling the complete evaporation of highly aqueous mobile phase. As the evaporator temperature is lowered below ambient temperature, the evaporation gas can be increased to compensate for the decrease in vapor loading (ie increase in relative humidity) of the surrounding gas, while at higher operating temperatures, the evaporation gas is not required and can be turned off.

Instrumentation

Figure 1
Column: Pursuit C18 5 µm, 150 x 4.6 mm (p/n A3000150X046)
Detection: 385-ELSD (neb=30 °C, gas & evap as shown)

Figure 2
Column: PLRP-S 100Å 5 µm, 150 x 4.6 mm p/n PL1111-3500)
Detection: 385-ELSD (neb=30°C, gas=1.8 SLM, evap as shown)

Materials and Reagents

Figure 1
Eluent A: 0.1% TFA in Water
Eluent B: 0.1% TFA in ACN

Figure 2
Eluent: 10% ACN in Water

Sample Preparation

Figure 1
Sample: 250 µg/mL Acetanilide

Figure 2
Sample: 1 mg/mL PEG 106

Conditions

Figure 1
Flow Rate: 1.0 mL/min
Injection Volume: 20 µL
Gradient: 60-90% B in 5 min

Figure 2
Flow Rate: 1.0 mL/min
Injection Volume: 10 µL

Results and Discussion

Operating below room temperature provides maximum sensitivity for low molecular weight semi-volatile compounds, as shown in Figure 1.

However, sub-ambient ELSD not only improves the detection of small pharmaceutical molecules, but other low molecular weight compounds, such as polyethylene glycol (MW 106) can benefit, as shown in Figure 2.

PEG 106 shows a 40-fold increase in sensitivity when the temperature is reduced from 30 °C to 20 °C.

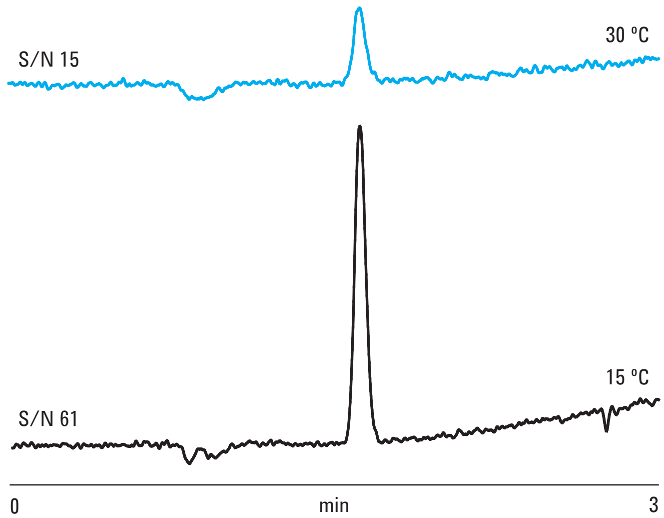

Figure 1. Enhanced detection of acetanilide (MW 135) using the Agilent evaporative light scattering detector for sub-ambient ELSD.

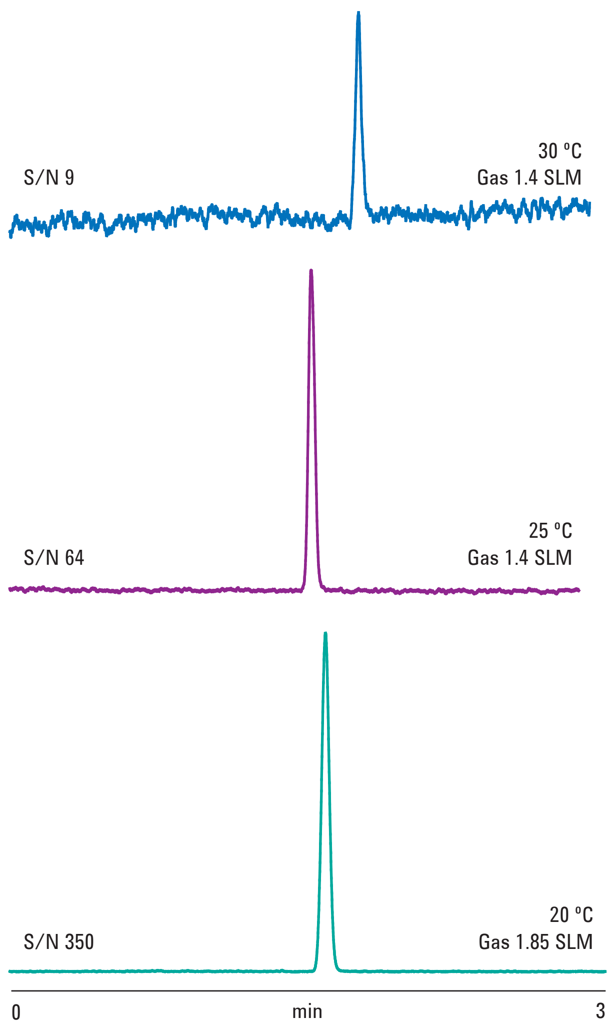

Figure 2. 40-fold improvement in detection of PEG 106 using the Agilent evaporative light scattering detector for sub-ambient ELSD.

Conclusion

Pharmaceutical analyses demand low temperature operation in order to detect the low molecular weight compounds, such as acetanilide. At ambient temperature (30 °C), the lowest operating temperature of other ELS detectors, the response of acetanilide is limited due to its volatility. However, by operating the 385-ELSD at 15 °C, thermal degradation of the compounds is minimized and the response improves by a factor of four.

Even a temperature difference of 5 °C has a significant effect on a compound's response. Therefore, it is vital that the evaporator temperature on an ELSD is accurately controlled, especially at ambient temperature and below. The accurate temperature control of the evaporation step in the 385-ELSD therefore provides excellent reproducibility for semi-volatile compounds, even in unstable thermal environments.

www.agilent.com/chem

This information is subject to change without notice.

Published in UK, May 20, 2011

5990-8212EN

Agilent Technologies