Enhanced Detection of Semi-volatile Compounds using Sub-ambient ELSD

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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. Over 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 after 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 PL-ELS 2100 Ice 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.

Operating below room temperature provides maximum sensitivity for low molecular weight, semi-volatile compounds, as shown in Figure 1. 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 PL-ELS 2100 Ice at 15°C, thermal degradation of the compounds is minimized and the response improves by a factor of four.

Sample: 250µg/ml Acetanilide
Column: Pursuit C18 5µm, 150x4.6mm
Eluent A: 0.1% TFA in Water
Eluent B: 0.1% TFA in Acetonitrile
Gradient: 60-90% B in 5 minutes
Flow Rate: 1.0mL/min
Inj Vol: 20ul
Detector: PL-ELS 2100 Ice (neb= 30°C, gas=1.8 SLM, evap as shown)

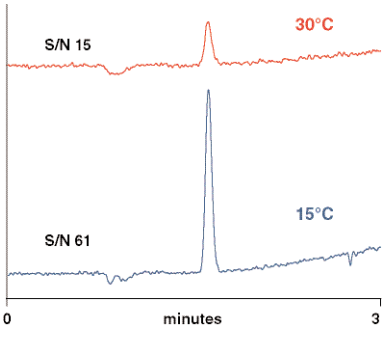

Figure 1: *Enhanced Detection of Acetanilide (MW 135) using the PL-ELS 2100 Ice*

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 lowered from 30°C to 20°C.

Sample: 1 mg/ml PEG 106
Column: PLRP-S 100Å 5µm, 150x4.6mm
Eluent: 10% ACN in Water
Flow Rate: 1.0mL/min
Inj Vol: 10µl
Detector: PL-ELS 2100 Ice (neb= 30°C, gas & evap as shown)/t1

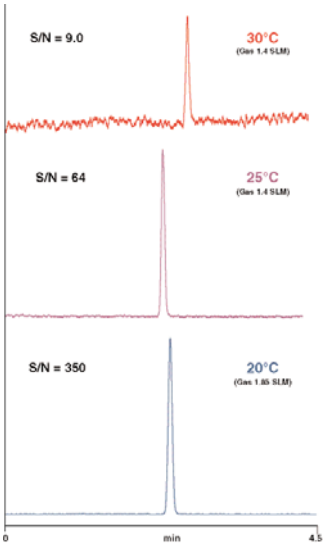

Figure 2: *Improved Detection of PEG 106 using Sub-ambient ELSD*

Even a temperature difference of 5°C has a significant effect on the 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 PL-ELS 2100 Ice therefore provides excellent reproducibility for semi-volatile compounds, even in unstable thermal environments.