



The Determination of Glycerol in Wine by HPLC with Low Temperature Evaporative Light Scattering Detection

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This application note describes the benefit of near-ambient drift tube temperatures with an Evaporative Light Scattering Detector. Detector response to the moderately volatile glycerol in a wine sample is observed as a function of systematically varied drift tube temperatures.

Since its inception, Evaporative Light Scattering (ELS) detection has emerged as a valuable complement to spectroscopic detectors for HPLC (1,2). For some applications, such as carbohydrates, the ELSD is the preferred detector for the majority of needs (3,4). The ELSD is compatible with all useful LC solvents and numerous volatile mobile phase modifiers. It is also blind to gradients and offers sensitivity at the high picogram to low nanogram levels.

The detector's response is limited only by the volatility of the analyte — nonlabile analytes may be detected with good sensitivity over a wide range of drift tube temperatures. Some analytes, however, are more volatile and may be lost to evaporation at traditionally high drift tube temperatures (70–250 °C). An example is glycerol, or 1,2,3-trihydroxypropane, commonly found in wine as a byproduct of the fermentation process (5). Glycerol evaporates readily at high drift tube temperatures (~70 °C) and is not “seen” by the detector under those conditions (6). High evaporation temperatures may preclude the formation of light scattering particle

aggregates in the optical cell, a condition necessary for detector response. Here, we examine the effect of drift tube temperature on an ELS detector response to glycerol in a White Bordeaux, a personal favorite of the author.

Method

The Shimadzu ELSD-LT is capable of low (near ambient) drift tube temperatures (28–40 °C). For the study, 5 µL of neat White Bordeaux was injected and an isocratic separation was established using a normal phase approach. Peaks were identified by authentic injection of standards. With each run, the ELSD-LT drift tube temperature was elevated incrementally to illustrate the effect of evaporation temperature on detector response. The detector sensitivity (Gain 5) was not varied. Drift tube temperatures of 28, 32, 35, 40, 45, 50, 55, 70, and 80 °C were used.

Results and Conclusion

As shown in Figure 1, the detector response to glycerol was excellent at 28 °C (peak amplitude 700 mV at Gain 5), then dropped by 84% over an increase of only 4 °C in evaporation temperature. Response continued to decline with each increase in temperature until the peak was nearly absent at 45 °C. Further work at the highest detector sensitivity (Gain 12) showed that glycerol was completely lost at drift tube temperatures above 55 °C. The detector's response to carbohydrates did not change with evaporation temperature.

This simple example illustrates one of the advantages of low-temperature ELS detection using the Shimadzu ELSD-LT. With it, more volatile analytes may be determined, extending the range of useful analyses to those compounds that may be lost to evaporation in traditional high-temperature ELSDs.

References

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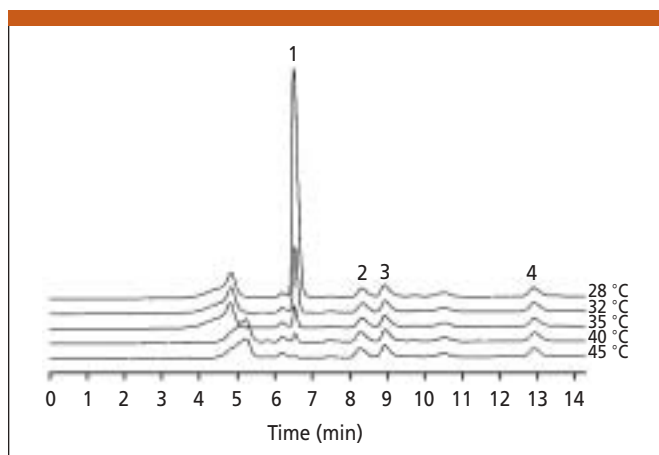


Figure 1: Chromatograms obtained at various drift tube temperatures from near ambient to 45 °C. Detector: Shimadzu ELSD-LT (Gain 5; pressure 250 kPa). Column: Asahipak NH₂P-50, 5 µm, 250 × 4.6 mm; mobile phase = acetonitrile–0.0004N NH₄OH(aq) (80:20). Peaks: 1 = glycerol, 2 = fructose, 3 = glucose, 4 = sucrose.

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