



# Determination of the Limit of Detection for Caffeine by HPLC with ELSD

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### Introduction

Caffeine is widely used in the food and drink industry as well as being incorporated into some nutraceutical formulations. HPLC is often employed in assays for the quantification of caffeine. In this particular example, a gradient HPLC method was used to analyze caffeine at low concentrations to determine the on-column limit of detection (LOD). The Varian evaporative light scattering (ELS) detector was employed because of its superior performance, as the detection method is independent of the optical properties of the compound under consideration.

### Instrumentation

Column: C8 3  $\mu$ m, 50 x 4.6 mm

Detector: Varian ELSD (neb=50 °C, evap=50 °C, gas=1.4 SLM)

### Materials and Reagents

Eluent A: 5 mM Ammonium acetate in Water

Eluent B: 5 mM Ammonium acetate in Acetonitrile

### Conditions

Gradient: 10–100 % B in 5 min, 1 min hold at 100 % B

Flow Rate: 1.0 mL/min

Injection Volume: 10  $\mu$ L

### Results and Discussion

In this case, the LOD is defined as a peak height of greater than three times the baseline noise. The chromatograms in Figure 1 illustrate the signal to noise ratio (S/N) obtained for injections of 97 ng and 19 ng on column. The 19 ng loading gave a S/N = 15 and so, for this application, the LOD is well below 19 ng on-column.

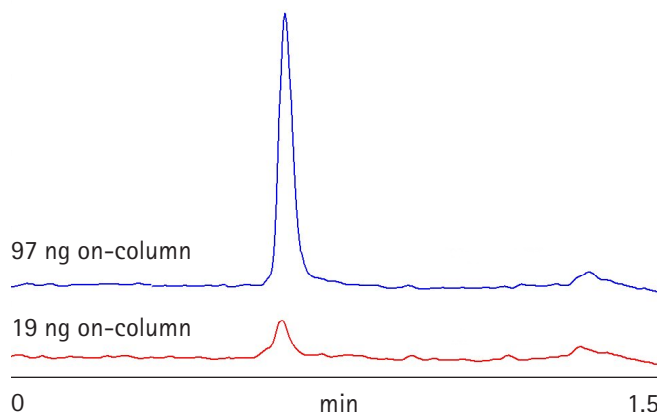


Figure 1. Very low signal to noise ratios achieved by Varian ELSD.

### Conclusion

The Varian ELS detector successfully revealed an LOD for caffeine below 19 ng on-column. The Varian ELS detector surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELS detector's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian ELS detector provides superior performance compared to detectors from other vendors for the analysis of semi-volatile compounds.

*These data represent typical results. For further information, contact your local Varian Sales Office.*

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