

# Purity and Concentration in High Throughput Screening

## Varian ELSD

*Advantage Statement:* The value of the Varian Evaporative Light Scattering Detector lies in its ability to non-UV chromophore samples, such as are encountered in high throughput screening of combinatorial libraries.

Pharmaceutical and biotechnology companies routinely use automated compound synthesis to produce DMSO combinatorial libraries of structurally related compounds. The subsequent requirement is to screen these compounds for potential pharmaceutical activity. Associated with this is the need for high throughput screening laboratories to perform qualitative and quantitative analysis of purity and concentration. This is done using HPLC that has been optimized to reduce the experimental time by using short columns with high flow rates and steep gradients. For gradient HPLC analysis the most universally applicable means of detection is by UV. However, to be successful the compounds must be UV active and, for quantification without the use of reference materials, they must have similar extinction coefficients. For high throughput screening this does not always apply and so an alternative detector is required. To this end, evaporative light scattering detection is rapidly replacing RI for the analysis of solutes which do not have a UV chromophore. The advantages of the Varian ELSD for the analysis of a DMSO combinatorial library is demonstrated here.

The HPLC system utilized a short column with a rapid gradient and 2.0 mL/min flow rate to give a total analysis time, including re-equilibration, of three minutes. This enabled the determination of 480 samples to be achieved in 24 hours. To screen this library, a UV detector operating at 254 nm and the Varian ELSD were connected in series to obtain the purity and concentration information.

Column: C18 3  $\mu$ m, 50 x 2.1 mm  
Eluent A: 0.05 % Formic acid in water  
Eluent B: ACN  
Flow Rate: 2.0 mL/min  
Gradient: Linear 5-95 % B in 2 min, 1 min reset  
Detection: Varian ELSD (neb=85 °C, evap=50 °C, gas=1.8 SLM)

Example chromatograms of two samples from this library are shown in Figure 1. The advantages of using the evaporative light scattering detector can be seen clearly. In the Varian ELSD trace of Sample 8020, a second component can easily be identified which was not detected by the UV at 254 nm. By comparing the two traces for Sample 8014, the improved baseline stability obtained with the Varian ELSD is evident. This enables a minor component to be identified which was obscured by the poor baseline in the UV trace.

The Varian ELSD is thus well suited to monitoring purity and concentration of a DMSO library under high throughput screening conditions. A stable baseline is obtained with the 2.0 mL/min flow rate and rapid gradients, thus permitting several hundred samples to be analyzed per 24 hours, and compounds which have little UV activity can be detected in the presence of a UV active solute.

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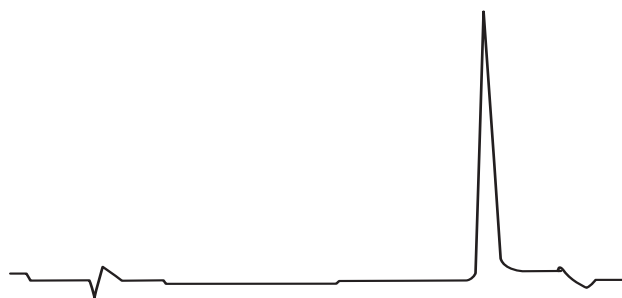


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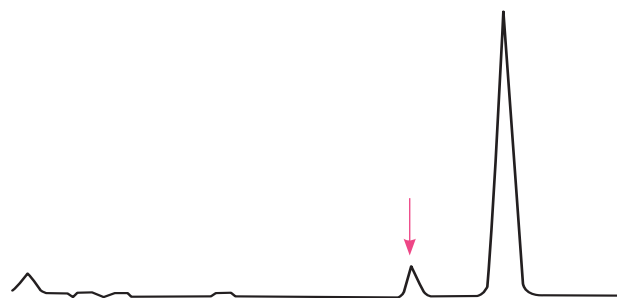
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Sample 8020

UV, 254 nm

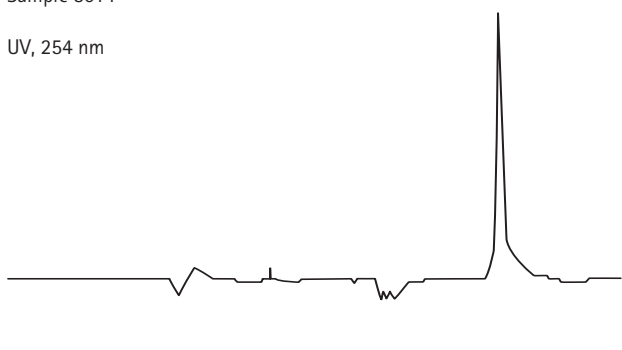


Varian ELSD



Sample 8014

UV, 254 nm



Varian ELSD

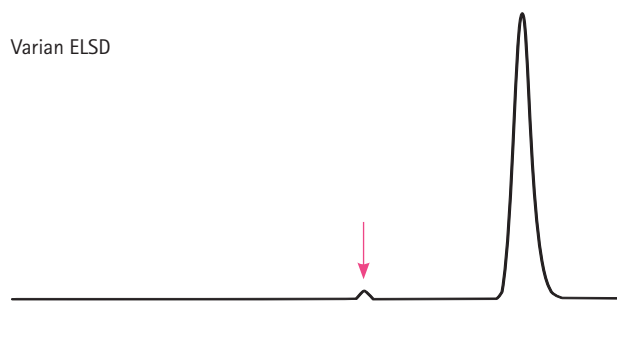


Figure 1. Chromatograms of two samples. The arrows indicate non-chromophore compounds detected only by the Varian ELSD (separations courtesy of Mr S Elliot, Rhone-Poulenc Rorer Ltd, Dagenham, UK).

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



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