Varian 385-LC and Varian 380-LC

Real-time Gas Control Overcomes Solvent Gradient Effects

Advantage Statement: The Varian 380-LC series is a range of advanced evaporative light scattering detectors from Polymer Laboratories, now a part of Varian, Inc. The Varian 385-LC delivers sub-ambient evaporation down to 10 °C, providing maximum sensitivity for compounds with significant volatility below ambient temperature. The Varian 380-LC is the instrument of choice for non-volatile compounds, where temperatures of 100 °C or above are required. Both instruments benefit from fast data output rates and extremely low dispersion for fast LC, and deliver a universal response down to the low-nanogram range for truly representative analysis. Reproducibility is less than 2 % for improved consistency of results. Real time gas management eliminates solvent effects to give a constant response across a gradient. Control and digital data collection come as standard for multi-vendor platforms and so there is no need for an analog to digital convertor. On-the-fly adjustment of light source intensity saves time during a run, too - all this in the smallest footprint around. Being complementary to LC/MS, and offering unrivalled flexibility and sensitivity, the Varian 380-LC series are the ELSDs of choice for the most demanding applications.

The response of an ELS detector is dependent on the composition (ie viscosity) of the chromatographic eluent. The solvent viscosity greatly affects the number and size of droplets formed during nebulization. Hence, at a fixed nebulizer gas flow, low viscous solvents (eg organic) produce greater numbers of droplets than solvents of a higher viscosity (eg water). Consequently, for reversed phase gradient elution, the ELSD response will continually increase due to the continuous decrease in mobile phase viscosity that occurs as the organic composition increases. The effect of this phenomenon can be highlighted by injecting a compound of fixed concentration at intervals across an aqueous-organic solvent gradient (Figure 1).

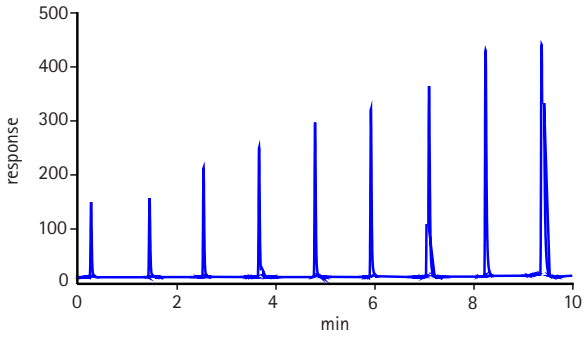

Figure 1. Typical ELSD response change across a solvent gradient.

For a typical reversed phase HPLC gradient, the ELSD response can change by a factor of ten, from beginning to end, depending on the mobile phase program. This non-uniform response across a solvent gradient has several implications for ELSD quantification:

1. Large errors are observed if the concentration of a compound is calculated solely on peak area. The enhancement effect of the mobile phase has to be considered.

2. A shift in a compound's retention time means a shift in mobile phase composition, which will lead to poor reproducibility.

Errors in quantification can be reduced if the response change across the gradient is accounted for. One method is to generate a 3-D calibration model of ELSD response with respect to compound concentration and retention time (ie eluent composition) across a gradient. The 3-D model can subsequently be used to quantify unknowns by correcting for any response enhancement based on the retention time. With this method the level of quantification error can be reduced significantly[1].

NOTICE: This document contains references to Varian. Please note that Varian, Inc. is now part of Agilent Technologies. For more information, go to **www.agilent.com/chem.**

Agilent Technologies

Another approach to controlling ELSD response change across a solvent gradient is to compensate for the changing solvent mixture using a second HPLC pumping system[2]. By combining the exact inverse solvent gradient with the liquid flow after the column (ie just before the ELSD inlet) the mobile phase composition can be controlled to a constant 50/50 mixture. This ensures that the ELSD only experiences an isocratic mixture of solvent throughout the analysis and so the signal shows no enhancement during the gradient. This approach also reduces errors in quantification, but at the same time introduces other system errors from the additional pumping system, which need to be accounted for. While this approach eliminates the need to generate a 3-D calibration, the investment cost is high. Both methods provide a means of correcting for the ELSD signal enhancement during gradient elution, but an alternative option is available with the Varian 380-LC series.

Controlling Solvent Gradient Effects using Evaporator Gas Flow

All ELS detectors operate with a fixed nebulizer gas flow, which is optimized for the least volatile portion of the mobile phase. However, under gradient chromatography, using a fixed nebulizer gas flow generates the response change shown in Figure 1. The cause of this response curve is due to the increasing number of droplets formed during nebulization, as the eluent increases in organic composition. This increase in droplet number in turn augments the number of analyte particles reaching the optical region, resulting in an increase in the amount of scattered light detected. If the number of particles is kept constant during gradient elution, the ELSD response will remain uniform throughout the run.

The Varian 380-LC series addresses this non-uniformity of response by using the evaporator gas flow to ensure that the number of particles reaching the optics is kept constant throughout the solvent gradient. To achieve this control over the particle stream, the evaporator gas is controlled in real-time during the gradient, using an on-board timetable method. The benefit of this on-board gas control can be seen by comparing Figures 2 and 3. Figure 3 displays the response obtained when 5-fluorocytosine (5-FC) is injected every minute across a 10-minute gradient of 5-95 % ACN, under fixed ELSD gas flow. Under a fixed gas flow setting, the 5-FC response increases 7-fold across the gradient, while the UV signal remains uniform as expected.

In order to control the increasing ELSD response, an on-board timetable method was created using Dimension software, where the evaporator gas flow was increased in a similar trend to the % organic portion of the mobile phase (Figure 3). Using this ELSD gas timetable program, the 5-FC response was kept uniform across the whole gradient run, matching the UV response almost exactly, as shown in Figure 5.

The evaporator gas program indicated in Figure 4 is specific to the HPLC system and LC conditions. Therefore, following an initial calibration of the system with a known compound, the ELSD gas program can subsequently be used to quantify unknown samples under the same LC conditions. This real-time gas control feature of the Varian ELSD offers an alternative approach to mobile phase compensation when quantifying unknowns under gradient elution.

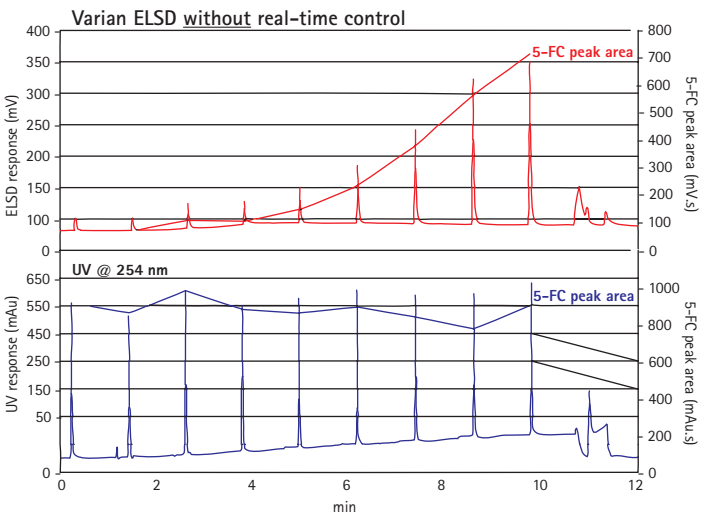

Figure 2. 5-FC response across solvent gradient using UV and ELSD at fixed gas flow.

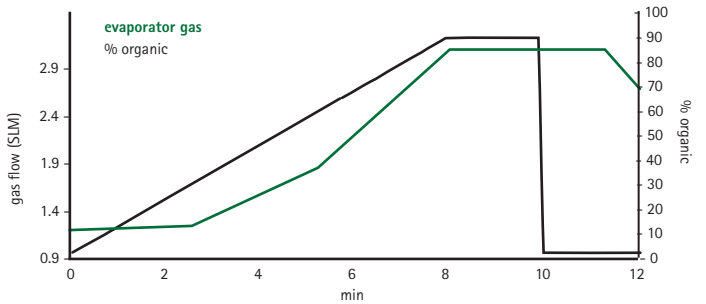

Figure 3. Varian ELSD Evaporator Gas Program, from Dimension software, to provide the gradient response in Figure 2 (solvent program overlaid for comparison).

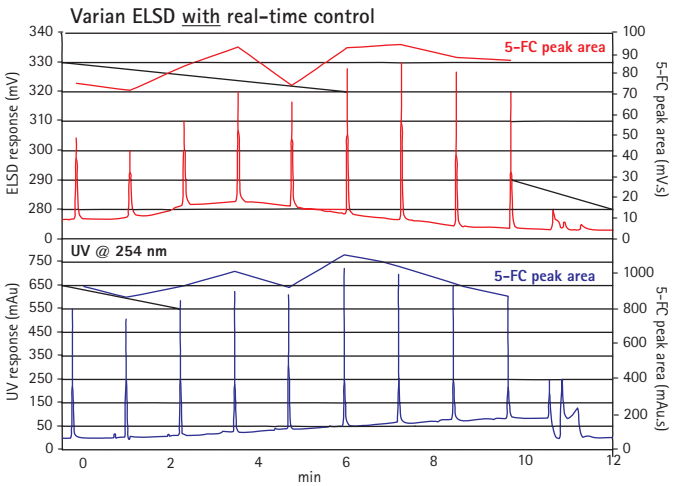

Figure 4. 5-FC gradient response using the ELSD gas program from Figure 3.

Conclusions

In the past, quantification and purity measurements using evaporative light scattering detection showed poor accuracy due to compound volatility and response variation across a solvent gradient. The Varian 380-LC series of evaporative light scattering detectors can be programmed to change parameters during an injection in order to control detector response. Thus, real-time control of the evaporation gas can be used to keep the ELSD response uniform during a solvent gradient.

References

1. B. T. Mathews *et al.* (2004) Chromatographia, 60, 625–633.
2. André de Villiers *et al.* (2007) Journal of Chromatography A, Vol 1161, 183-191.

Varian, Inc.
www.varianinc.com
North America: 800.926.3000, 925.939.2400
Europe The Netherlands: 31.118.67.1000
Asia Pacific Australia: 613.9560.7133
Latin America Brazil: 55.11.3238.0400
Other sales offices and dealers throughout the world—check our Web site

VARIAN

SI-1289 03/08 Printed in UK