Mobile phase compensation method for the Agilent 1200 Series Evaporative Light Scattering Detector to significantly improve quantification of unknowns

Technical Note

Introduction

The Agilent 1200 Series Evaporative Light Scattering Detector (ELSD) detects and quantifies any compound that is less volatile than the mobile phase with and without available standards. It provides an essentially universal measurement under isocratic and gradient conditions and is independent of a compound´s absorbance, fluorescence, or electroactivity. Here, we show how mobile phase compensation using Agilent 1200 Series binary pumps significantly improves ELSD quantifications of unknowns by reducing the area relative standard deviation (RSD) for both signals, for example, UV from 11 to 0.83 (factor of 13.2) and ELSD from 34 to 1.7 (factor of 20).

Mobile phase compensation method

In gradient runs, quantifying unknowns using available standards can lead to errors due to differences in elution time between standard and unknown compounds. They can lead to different ELSD responses as the response depends on analytes' organic content. The mobile phase compensation method is a method that compensates for changing organic content in gradient elution. In this method, a separate pump is attached post column that runs an opposite gradient to neutralize the effect of changing organic content. In this way, an isocratic flow reaches the detector.

Factors influencing the ELSD response

In the first stage of Agilent 1200 Series ELSD detection, the eluent is nebulized and small droplets are selected to minimize background noise. This is followed by an evaporation stage where solvents in the aerosol evaporate at low temperatures to avoid loss of compounds and form particles. Finally, in the detection stage, solute particles are focused, using gas-supported focusing (GSF) for enhanced signal-to-noise ratios and the intensity of scattered light from the particles is detected. The intensity of detector response depends on many factors at each stage of detection. The three stages of ELSD detection and the various factors that contribute to overall response are shown in Table 1.[1]

If chromatographic and ELSD parameters are kept constant, the factors influencing the response in nebulization, evaporation and detection stages narrows down significantly. The two major factors influencing the response will be the physiochemical properties of the compound and the organic content during elution in gradient runs.

For unknown compound quantification, it is important that the response of ELSD depends only on the physiochemical property of the analyte and not on the organic content during its elution. The mobile phase compensation method balances a changing percentage of organic phase in a typical elution gradient.[1]

Mobile phase compensation set up

A typical mobile phase compensation instrumental setup involves two pumps which are arranged as shown in Figure 1. The second pump is attached post column using a "T" connection. The pumps are designed to start at different times such that each produce gradient which is exactly the same but opposite when it reaches the "T" connector. The two opposite gradient solvents mix at the "T" connector to produce constant isocratic buffer.

Nebulization	Evaporation	Detection
1. Mobile phase properties 2. Percentage of organic phase during elution 3. Gas pressure 4. Flow rate 5. Diameter of nozzle	1. Temperature 2. Volatility/molecular weight/structure of the compound 3. Size of the aerosol 4. Mobile phase additives	1. Size of the particle (concentration of analyte) 2. Shape of the particle 3. Surface properties (refractive index of the compound) 4. Density of particles (peak shape) 5. Wavelength of the incident light. 6. Angle of scattered light

Table 1
The three stages of ELSD detector operation and factors that influence overall detector response.

Matching the two pumps

Matching the two pumps involves adjusting the start time of the pumps so that they produce exactly opposite gradients at the "T" connector. The pumps are elastic (for the Agilent 1200 Series Binary Pump SL only) and solvent calibrated using LC Diagnostic software

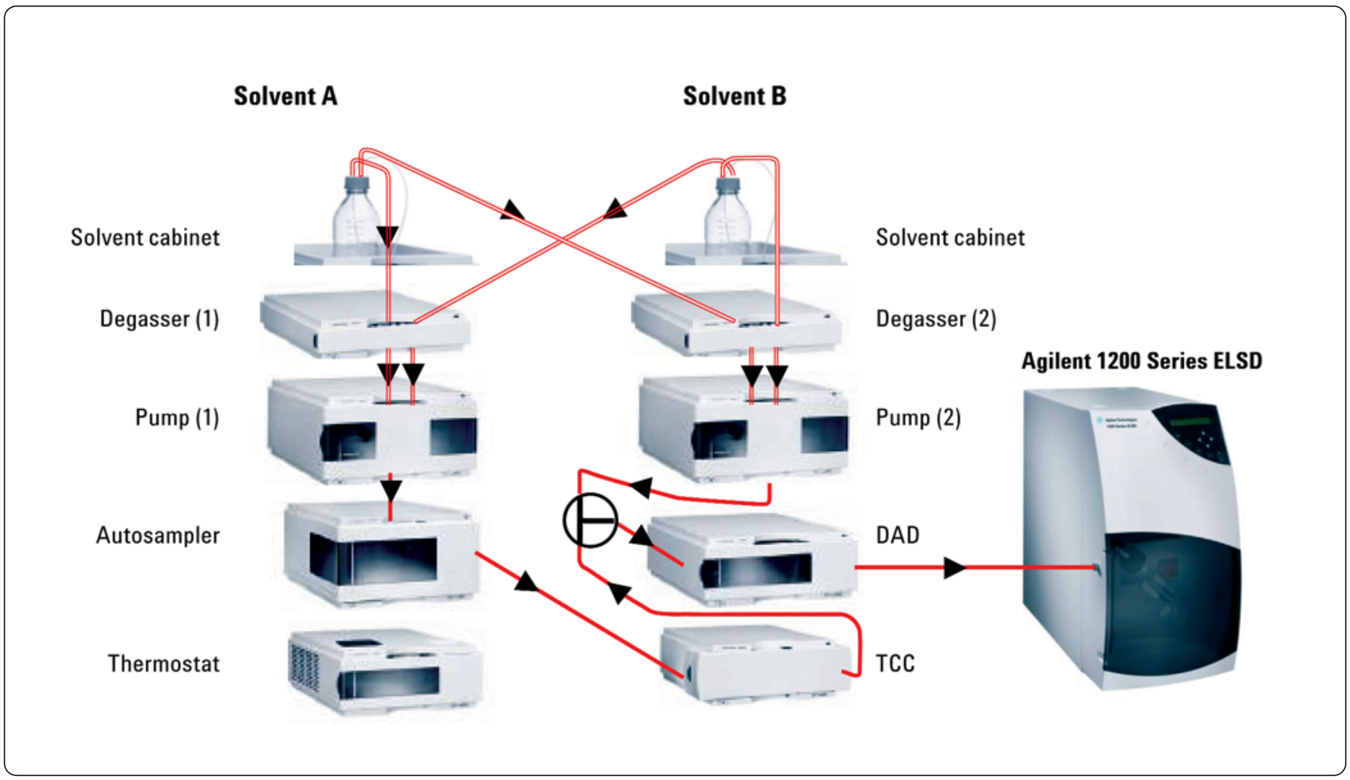

Figure 1
Configuration of two binary pumps for a mobile phase compensation method connected to a UV-DAD and ELSD detector using a "T" connector. Pump (2) produces a gradient exactly opposite to that from pump (1); where both pumps can use same solvent bottles.

(new version: Agilent Lab Advisor software) or the same compressibility factors selected for both the channels in both pumps. As shown in Figure 2, the elution condition in pump (2) was extended for 0.6 min so that it operated exactly opposite to pump (1). When both the pumps are run simultaneously, there is a straight baseline.

In Figure 2, the pumps are matched without the column. Matching the pumps with a column is accomplished by injecting a non-retaining compound such as Uracil or 5-Fluorocytosine to determine the delay volume (delay time × flow rate) and pump (2) was adjusted accordingly.

Repeated injections of sulfamethoxazole

A 50 µg/mL amount of sulfamethoxazole is repeatedly injected using an injector program with both pumps running the same or opposite gradient. Gradient is run from 10% acetonitrile to 90% acetonitrile. As shown in Figure 3, both UV and ELSD traces show a mobile phase dependent detection of sulfamethoxazole which is counterbalanced by running the second pump in the opposite direction (90% to 10% acetonitrile with delay time). Significantly lower RSD value is obtained for area measurement after gradient compensation (Table 2).

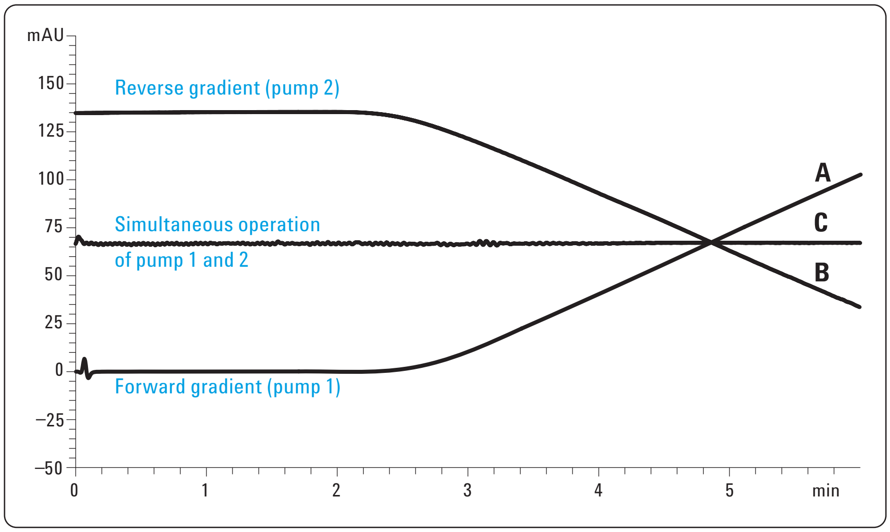

Figure 2
Overlay of gradient using pump (1) - A, reverse gradient using pump (2) - B and when both are run simultaneously - C operating at 0.5 mL.min flow rate. Gradient runs are performed using water in mobile phase A and acetonitrile having 0.1% acetone in mobile phase B. The gradient for pump A is 10% B for 0.5 min and gradient to 90% in 5 min and isocratic at 90% till 6.1 min; while pump B is 90% B for 1.1 min and gradient to 10% B in 5.6 min and isocratic at 10%. UV absorbance of acetone is measured at 279 nm. In C, the baseline is wavy initially, this is because of viscosity differences in liquids mixing at the "T" connector. This disappears as the run progresses.

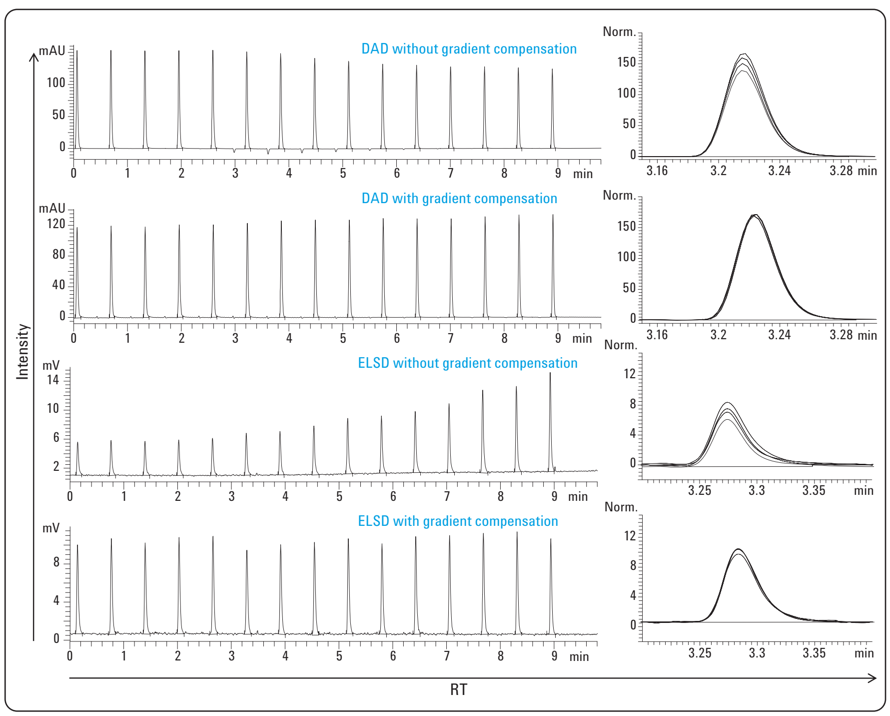

Figure 3
Repeated injection of a single compound, sulfamethoxazole analyzed by DAD and ELSD with and without gradient compensation. The zoom in spectra on the right show the overlay of four peaks from the repeated injection. With gradient compensation enabled, the spectra show less variation in area.

Conclusions

This Technical Note demonstrates the ease of performing gradient compensation methods using Agilent 1200 Series Binary LC pumps. Gradient compensation methods are useful for both UV and ELSD detectors in the area of unknown compound quantification. When the response is dependent upon the mobile phase composition and the exact standards are not available, gradient compensation methods provide a versatile tool for quantifying unknowns by normalizing the response (reduction in RSD from 34 to 1.7).

References

1.
Megoulas, N. C., Koupparis, M. A., "Twenty Years of Evaporative Light Scattering Detection," *Critical Reviews in Anal Chem,* 35:301-316, **2005**.

2.
Villiers, A. D, G´orecki, T., Lynen, F., Szucsb, R., Sandra, P., "Improving the Universal Response of Evaporative Detection by Mobile Phase Compensation," *J. Chromatography A*, 1161:183-191, **2007**.

	Average area ± std dev	RSD of area
UV absorbance without gradient compensation	263 ± 29	11
UV absorbance with gradient compensation	236 ± 1.9	0.83
ELSD without gradient compensation	16 ± 5.5	34
ELSD with gradient compensation	21 ± 0.37	1.7

Table 2
Average area and RSD values (n=4) obtained for UV and ELSD peaks in repeated injections of sulfamethoxazole.

Product	Part number
"T" connector	5022-2144
Red tubing; id: 0.127 mm	5042-6461
ELSD	G4218A
Agilent Lab Advisor software B.01.01	G4800AA
Binary pump	G1312B

www.agilent.com/chem/elsd

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