Universal quantification using the Agilent 385-ELSD Evaporative Light Scattering Detector

Technical Overview

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Introduction

Pharmaceutical laboratories produce many thousands of new compounds that require identification and quantification of impurity levels. To be considered for drug development, the new compounds must meet a minimal standard of purity. Reliable purity measurements can be achieved using calibration curves generated from well-characterized reference standards, but this approach is not feasible for compound libraries containing unknown impurities, where reference standards are not available.

Gravimetric purification can be performed for every new compound but this is time-consuming and impractical for the large number of unknown compounds generated daily. Pharmaceutical companies thus require a universal process for analytical and small-scale preparative HPLC to enable accurate quantification of crude samples.[1]

Universal calibration, where all unknown impurities can be quantified against a well-characterized single compound, is theoretically the simplest approach to measuring compound purity. This single calibrant technique requires a universal detection process that gives uniform response for chemically different compounds. The evaporative light scattering (ELS) detector is currently the most suitable detector for this task and has been used, with limited success.[2,3]

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One of the main reasons for the limited value of evaporative light scattering as a universal detection technique was attributed to the fact that the detector's response is dependent on mobile phase composition, which is problematic when using gradient elution.[3]

The Agilent 385-ELSD Evaporative Light Scattering Detector is the next generation of ELS detector, specifically designed to improve accuracy of results by overcoming this limitation.

Minimizing solvent enhancement effects

A well-known property of aerosol-based detectors is their response dependency on mobile phase composition. This is highlighted by the red trace in Figure 1, where a single compound of fixed concentration was injected every minute into an ELSD across a reversed phase solvent gradient.

This increase in compound response across the solvent gradient is caused by an increase in the number of droplets formed during nebulization, which is a result of the change in solvent viscosity. The increase in the droplet number translates to an enhancement in detector response due to the increased number of particles reaching the detection stage.

For a typical reversed phase HPLC gradient, an aerosol-based detector's response can change by a factor of ten, depending on the mobile phase program and detector design. This nonuniform response across a solvent gradient has several implications when quantifying with aerosol-based detectors.

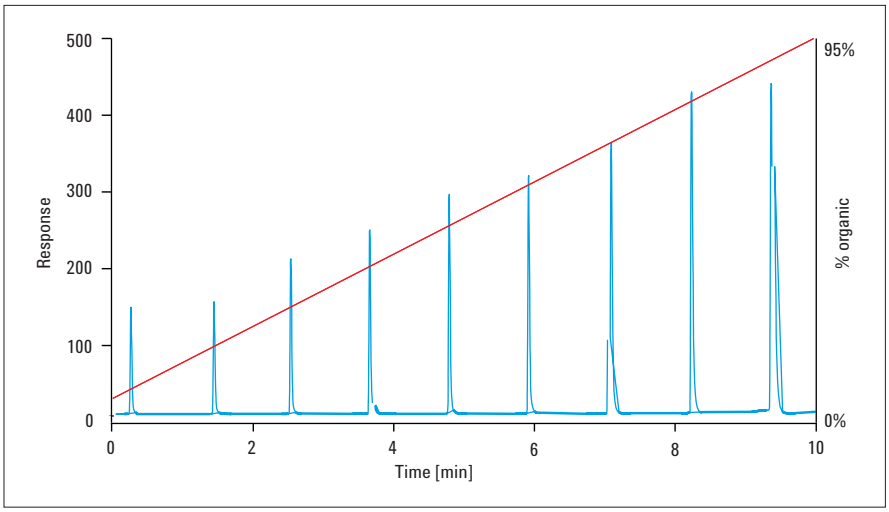

Figure 1
Typical nonuniform response of aerosol-based detectors across a solvent gradient.

1. A slight shift in a compound's retention time between injections will lead to poor reproducibility in area response.
2. Irreproducible gradient programs due to poor pump performance will produce poor accuracy due to variations in detector response.
3. If universal calibration of unknowns is performed, the enhancement effect of the mobile phase has to be considered, otherwise large errors are observed.

There have been several attempts to compensate for this solvent enhancement effect in order to minimize errors when quantifying with aerosol detectors. One method uses a 3-D calibration model to characterize a detector's response with respect to compound concentration and retention time.[4]

A second approach uses a secondary HPLC pumping system to combine the exact inverse solvent gradient to the main gradient program to ensure that the detector receives a constant isocratic mixture of mobile phase.[5]

Both approaches reduce quantification errors but they are both labor intensive, as they require careful configuration to minimize system errors. Also, the need for an additional pumping system increases investment costs.

Correcting for solvent gradient effects using the Agilent 385-ELSD

The Agilent 385-ELSD can control the number of particles entering the detection stage by using evaporation gas technology.

The effect of the evaporation gas in delivering real-time control over the ELSD response is highlighted in Figures 2, 3, and 4. A 5-Fluorocytosine (5-FC) solution was injected every minute across a ten minute gradient of 5–95% ACN, under fixed ELS detection conditions. As with other aerosol-based detectors, under these settings the response of 5-FC increases across the gradient. For comparison, the UV signal remains uniform, as expected for a bulk property detector.

To correct for this response enhancement in Figure 2, a gas flow gradient was programmed into the Agilent 385-ELSD, which compensates for the increase in droplet number and maintains a uniform response. The gas program used to achieve this is shown in Figure 3 and follows closely the trend in organic composition.

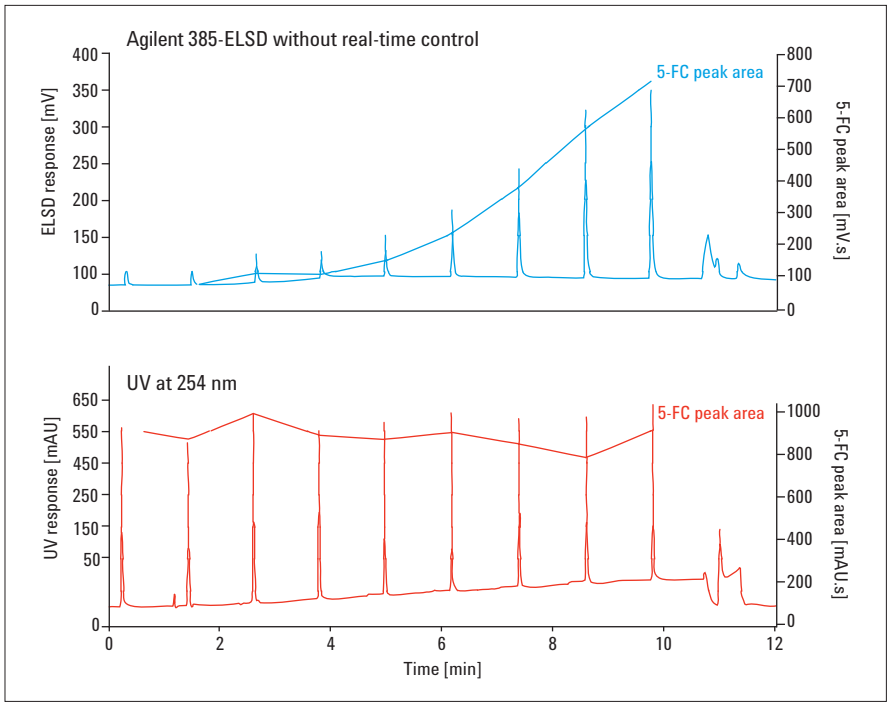

Figure 2
5-FC response across solvent gradient using UV and ELSD at fixed gas flow.

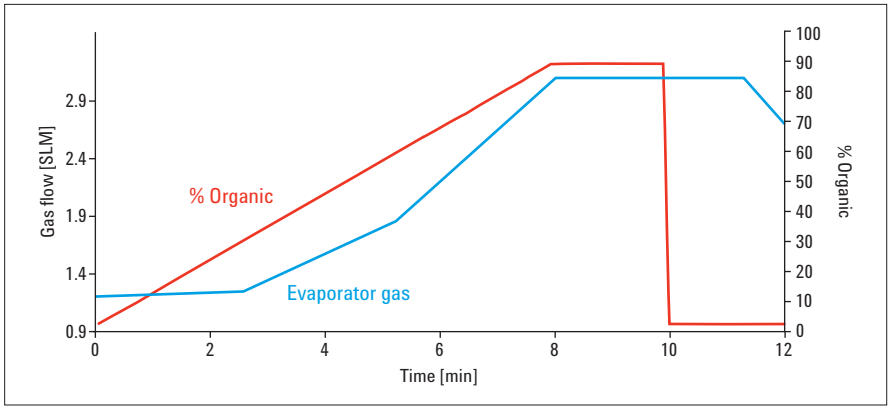

Figure 3
Evaporator gas method programmed into the Agilent 385-ELSD to correct the response in Figure 2.

The benefit of using the gas gradient is clear from Figure 4, where the response of 5-fluorocytosine is kept uniform across the entire gradient run, closely matching the UV response. This gas program can be used repeatedly for a sequence of injections to quantify unknown samples, without the need for a secondary LC pump or 3-D calibration.

The ability of the Agilent 385-ELSD to overcome solvent enhancement effects during gradient elution makes it ideal for quantifying unknown compounds. Combined with the improved sensitivity to semivolatile compounds at sub-ambient temperature, the Agilent ELSD offers a simple and accurate solution to universal quantification.

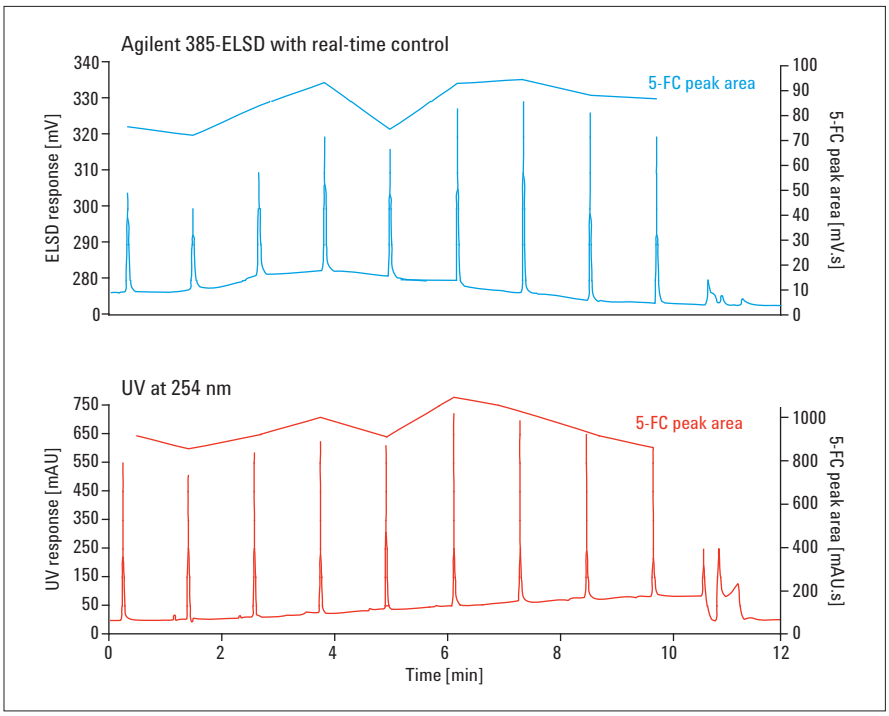

Figure 4
5-FC response across solvent gradient using real-time gas control from Figure 3.

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