Using the Agilent 1290 Infinity ELSD for High Throughput Screening of Samples Stored in DMSO

Application Note

Small Molecule Pharmaceuticals & Generics

Author

Graham Cleaver

Agilent Technologies, Inc.

Abstract

This Application Note describes how the Agilent 1290 Infinity Evaporative Light Scattering Detector can be used in high throughput screening of drug candidates to analyze samples from combinatorial libraries that are stored in dimethylsulfoxide as solvent.

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Introduction

Combinatorial chemistry is an important tool in drug discovery. The technique enables researchers to create small molecule libraries that can include hundreds or thousands of compounds directed at one drug target. The vast majority of combinatorial libraries are stored in dimethylsulfoxide (DMSO) prior to screening, because of its high chemical stability, high solvating power, and low toxicity. High throughput screening (HTS) methods are employed to screen these libraries for potential drug candidates. It is vital that the screening process can detect all the compounds present in the library. Analysts working in high throughput screening rely on UV and mass spectrometry to ensure all components are detected. However, compounds without a UV chromophore or those that do not ionize easily will be missed by both these detectors. Evaporative light scattering detection (ELSD), as exemplified by the Agilent 1290 Infinity Evaporative Light Scattering Detector, is a major improvement, as it responds to all compounds less volatile than the mobile phase. The 1290 Infinity ELSD handles the high flow rates and rapid gradient separations that are typically employed in HTS methods. The unique design of the 1290 Infinity ELSD gives DMSO transparency at low temperatures, enabling the detection of fast eluting semivolatile compounds.

Experimental

Instrumentation

An Agilent 1290 Infinity ELSD was used for all the experiments.

Chromatographic conditions	
Column	Agilent ZORBAX SB C8, 4.6 × 50 mm, 5 µm
Mobile phase	A: Water, B: Acetonitrile
Gradient	5% B at 0 minutes; 100% B at 5 minutes
Flow rate	1 mL/min
Injection volume	20 µL

Results and Discussion

Traditionally, with ELSD, the removal of DMSO in HTS, to allow detection of fast eluting compounds, can be facilitated by increasing the evaporator temperature as shown in Figure 1. Depending on manufacturer, when this occurs, the temperature can vary from approximately 40 °C to 90 °C. However, Figure 1 shows that as the detector temperature increases, the response of the semivolatile compounds, such as acetanilide (peak 5), is reduced.

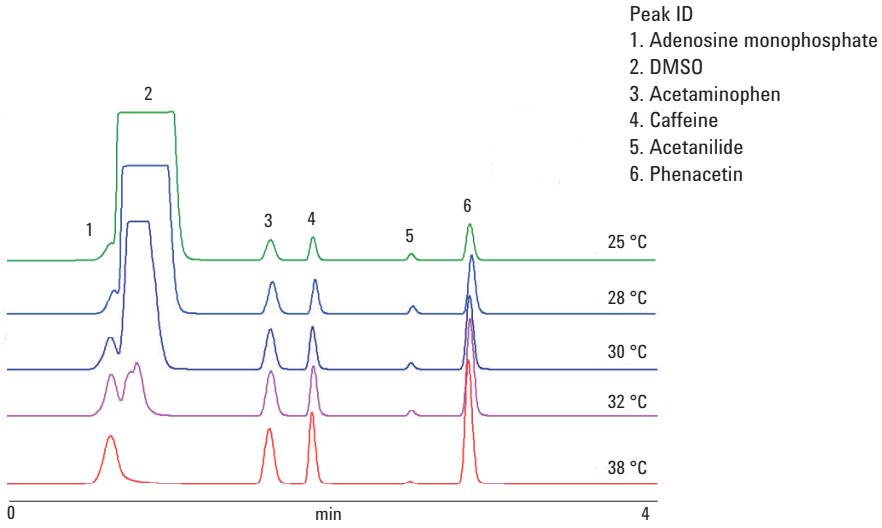

Figure 1. Removal of DMSO by increasing evaporator temperature (neb = 25 °C, gas = 1.6 SLM).

Such an approach is not required with the 1290 Infinity ELSD, which features unique gas control. In the 1290 Infinity ELSD, the addition of a carefully controlled stream of gas to the evaporation step enables complete removal of the eluent to take place without increasing the temperature. The benefits of this unique design are highlighted in Figure 2, where DMSO is completely removed at 30 °C, by increasing the evaporator gas flow, without any loss of the volatile acetanilide.

Further improvements can be gained by reducing the amount of sample entering the 1290 Infinity ELSD. This is done by reducing the injection volume, or splitting the eluent flow as shown in Figure 3. By halving the amount of eluent entering the detector, the temperatures and gas flow parameters can be reduced. This further improves the signal-to-noise (S/N) ratio and limits the loss of thermally labile compounds.

Conclusion

The use of the Agilent 1290 Infinity ELSD in combinatorial chemistry allows even faster gradients to be performed to increase sample throughput, while providing ambient analysis to improve the detection of volatile compounds. The 1290 Infinity ELSD surpasses other ELSDs for low temperature HPLC applications with semivolatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The unique gas control of the 1290 Infinity ELSD 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 1290 Infinity ELSD provides superior performance compared to detectors from other vendors for the analysis of semivolatile compounds.

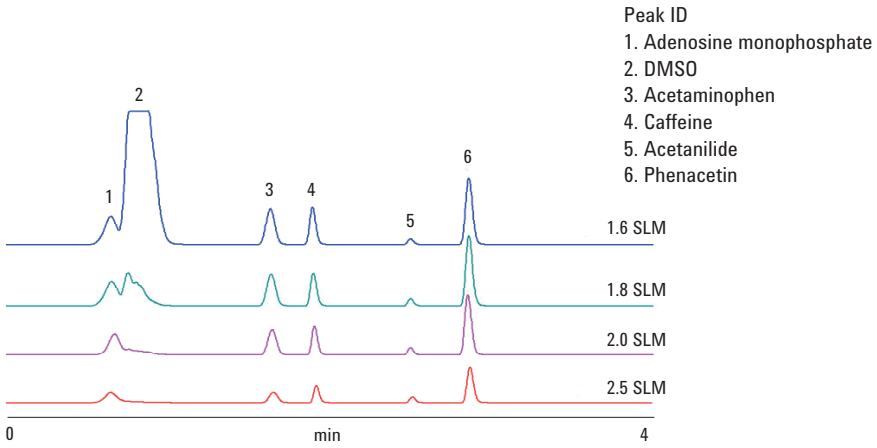

Figure 2. No loss in acetanilide when DMSO is removed by the Agilent 1290 Infinity ELSD (neb = 25 °C, evap = 30 °C).

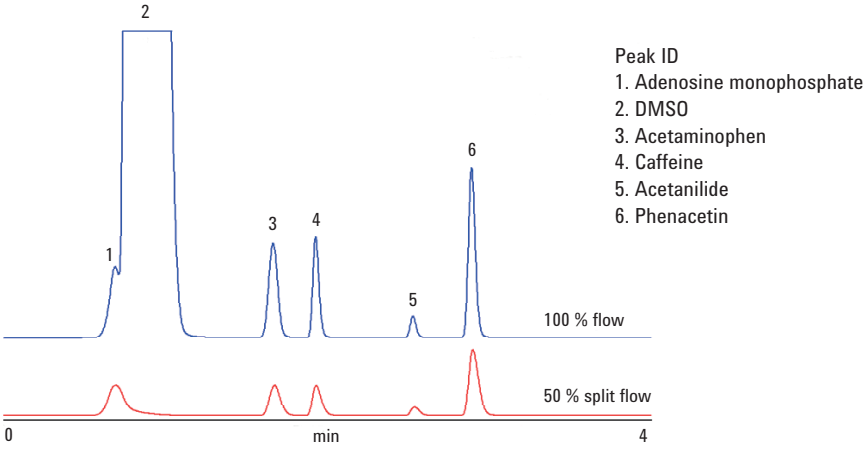

Figure 3. Reduction in S/N ratio and no loss of heat sensitive compounds with the Agilent 1290 Infinity ELSD by reducing the injection volume (neb = 25 °C, evap = 28 °C).

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