Universal Detection of Combinatorial Libraries by HPLC and Low Temperature ELSD

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Introduction
The Varian evaporative light scattering detector is universal and is not dependent on the optical properties of the compound. Consequently, its good discriminating power and sensitivity is well suited to combinatorial compounds that have weak or no UV chromophores.

Low molecular weight degradation products in DMSO are detected, without interference from DMSO, providing a rapid means of highlighting unstable compounds that could lead to false positive results in early stages of drug discovery.

The prolonged storage of organic compounds in solution can lead to significant sample degradation and to a subsequent increase in the number of false positives in high throughput screening assays. Consequently, pharmaceutical companies, who typically store their libraries in frozen DMSO at 4 °C, are faced with decomposition of a high proportion of their compounds.

Studies such as the COMDECOM project have been established to determine the extent of this problem with the aim of developing a predictive model that will highlight compounds susceptible to decomposition. While this model will improve the storage of compounds in the future, for pharmaceutical companies there is an urgent need to analyze the composition of their libraries that are currently in storage, to minimize any further decomposition of their samples.

The use of liquid chromatography, UV detection and mass spectrometry are the typical detection methods used to analyze combinatorial libraries. However, these techniques can miss potential drug compounds if the compound has no UV chromophore or is difficult to ionize. The presence of DMSO is also problematic.

Instrumentation
Column: C18 5 µm, 150 x 4.6 mm
Detection: Varian ELSD (neb=25 °C, evapor=25 °C, gas=1.6 SLM)

Materials and Reagents
Eluent A: 0.1 % HFBA in water
Eluent B: 0.1 % HFBA in ACN

Sample Preparation
A typical scaffold (2 mg compound A/mL in 100 % DMSO) was kept in DMSO at room temperature for three days, prior to analysis, to accelerate sample decomposition.

Conditions
Flow Rate: 1.0 mL/min
Injection Volume: 20 µL
Gradient: 5-30 % B in 10 min; 30-80 % B in 5 min

Results and Discussion
The benefits of using the Varian ELSD to determine the decomposition of samples stored in DMSO are shown in Figure 1 (degradation pathway for A is shown inset). Compound A and its degradation products would go undetected by UV due to their lack of a UV chromophore, whereas the Varian ELSD was able to detect all three components in the degradation process, providing vital information about the purity of the sample. Figure 1 might be typical of a compound in short-term storage, where the number of breakdown products is small.

However, for longer storage periods, or for highly unstable compounds, the composition could look similar to Figure 2. Compound E has also been stored in DMSO for three days. However, its unstable nature leads to a greater number of impurities than compound A.
Conclusion

By operating the Varian ELSD at 25 °C, the ELSD was able to detect the low molecular weight degradation products, as well as a host of other impurities. Early eluting impurities that would normally be masked by the DMSO peak are also detected with the Varian ELSD. The Varian ELSD surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications.

The Varian ELSD’s unique gas control 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 Varian ELSD provides superior performance compared to competitors’ detectors for the analysis of semi-volatile compounds.

References