Chromatography
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NextGen Evaporative Light Scattering Detector and High-Boiling Solvents in Normal Phase Chromatography

Keywords: ELSD, Combi*Flash*, Thermo-Split.

Abstract

Although most normal phase chromatography uses volatile solvents such as hexane, petroleum ether, ethyl acetate, dichloromethane, or methanol, there is a need for solvents with high boiling points such as toluene or water. Heptane is also used because of a perceived reduced toxicity, while toluene is useful to purify aromatic compounds. Water is used for purifying very polar compounds. The importance of adjusting the ELSD temperature settings to increase the sensitivity of ELSD expanding to high-boiling point solvent systems is shown.

Background

Although hexane is commonly used for normal phase chromatography, it is commonly replaced by heptane because heptane is considered less toxic. However, heptane has a higher boiling point than hexane, requiring changes to the default ELSD parameters to normal phase columns. Some other solvents used for normal phase chromatography also have high boiling points. Water is used for Hydrophilic Interaction Liquid Chromatography (HILIC) for very polar compounds, while toluene offers a unique selectivity used to purify compounds containing aromatic groups.

Solvent	Boiling point (°C)
Hexane	68
Heptane	98
Water	100
Toluene	111

Table 1. Boiling points of several commonly used solvents.

The internal ELSD in Combi*Flash*® systems has a Thermo-Split™ chamber, allowing independent adjustment of the spray chamber and drift tube temperatures to improve sensitivity and limit the effect of droplets on compound detection as shown in Figure 1.

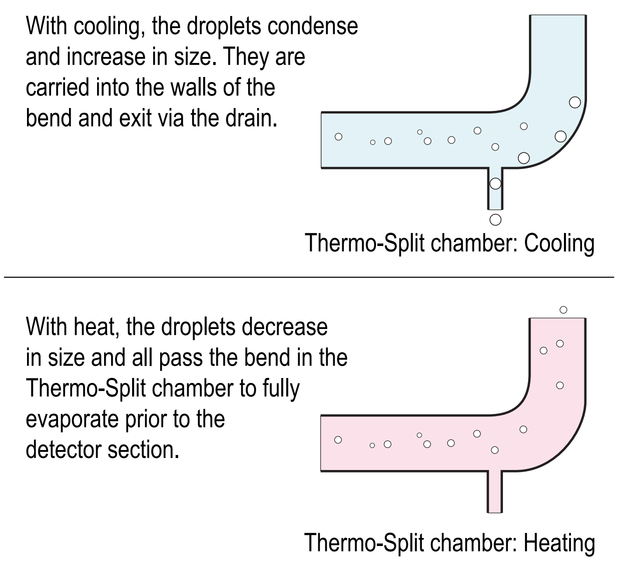

Figure 1. The Thermo-Split chamber allows adjustment of the droplet size sent to the drift tube.

For high boiling solvents, the spray chamber is cooled to induce condensation of the mobile phase droplets, increasing the size of the droplet. The droplets need to make a sharp turn before entering the drift tube. Those droplets too large and massive to evaporate before reaching the detector collide with side of the Thermo-Split chamber and are removed through a drain. The remaining solvent entering the drift tube is easily evaporated, resulting in less baseline noise due to unevaporated solvent passing through the detector cell, increasing the signal to noise ratio of the detection. The Thermo-Split also allows detection of semi-volatile compounds by preventing solvent droplets from entering the drift tube and detector cell. Less solvent entering the drift tube allows the drift tube temperature to be lowered to allow for detection of semi-volatile compounds.

⚠ WARNING

Make sure the drain line from the ELSD is placed in a waste container.

The ELSD settings for high-boiling solvents are shown in Table 2 and are used for all runs in the results and discussion sections below. The settings are similar to those used for reverse phase.

ELSD control	Setting value
Spray Chamber	20 °C
Drift Tube	60 °C
Gain	1
Sensitivity	High

Table 2. ELSD settings for high-boiling solvents

Results and Discussion

Heptane

Teledyne ISCO Universal Test Mix was used for these experiments. The hexane/ethyl acetate run used the Combi*Flash* default ELSD settings for a silica column, while the heptane run used the settings in Table 2.

The heptane chromatogram shows good sensitivity and baseline stability.

Water

The runs in Figure 3 are a HILIC scouting run and a calculated focused gradient from the scouting gradient. Fructose was purified from glucose using a Redi*Sep*® Gold Amine column using an acetonitrile/water gradient.

As this solvent system contains water, the settings in Table 2 were used, with excellent results.

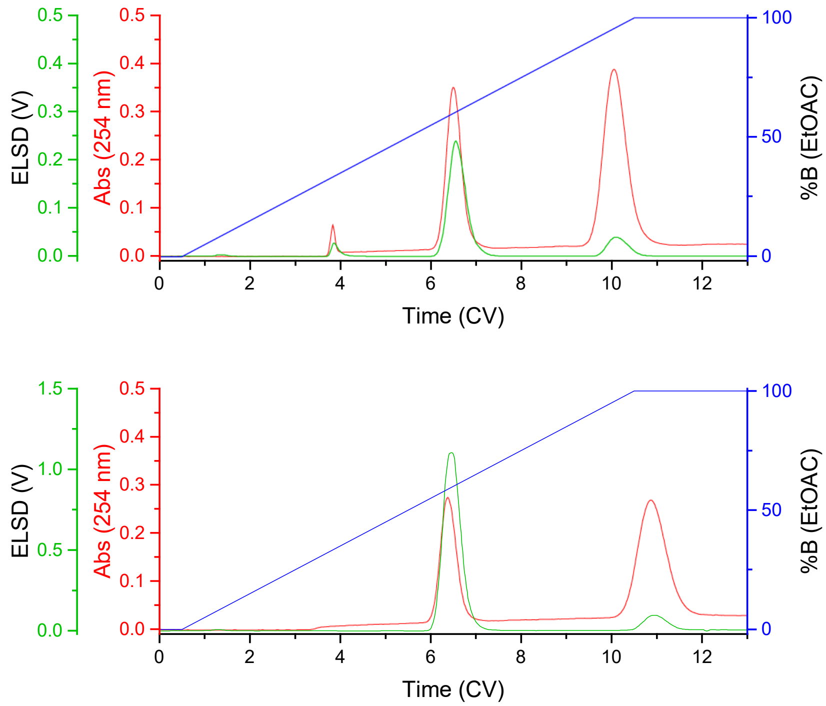

Figure 2. Universal Test mix run in hexane/ethyl acetate (top) and heptane/ethyl acetate (bottom) on silica.

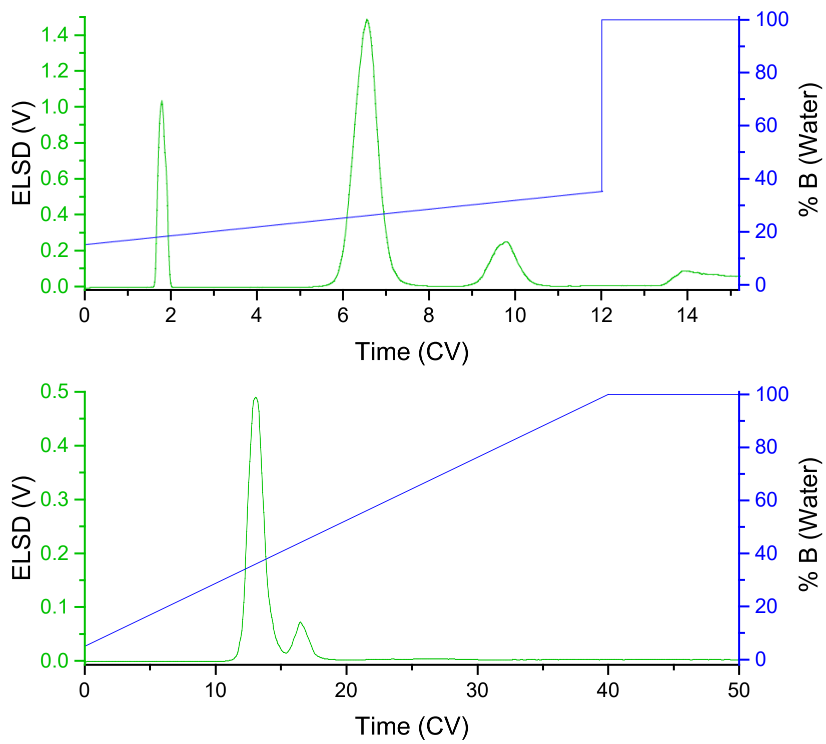

Figure 3. Fructose with sucrose impurity scouting run (top) using an amine functionalized column, A= acetonitrile, B=water. The focused gradient is below; the peak eluting at ~2 minutes is the DMSO used to dissolve the sample.

Heptane and toluene–SARA analysis

SARA is an important crude oil analysis to determine the amount of Saturates, Asphaltenes, Resins, and Aromatics in crude oil. This run contains both heptane and toluene as part of the gradient. The initial gradient segment was heptane only, after which a step gradient employing toluene was used. The run was finished with a tetrahydrofuran gradient segment.

The settings in Table 2 gave excellent results for the heptane and toluene and for the tetrahydrofuran portions of the run.

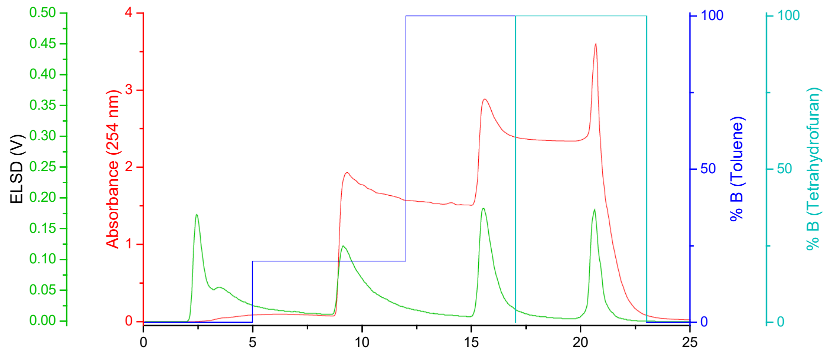

Figure 4. SARA analysis of an asphalt extract. The blue trace is a heptane/toluene step gradient.

Conclusion

ELSD temperature control is important when using high boiling point solvents systems. Using the reverse phase settings work well for solvents with high boiling points on Teledyne ISCO systems. They allow good sensitivity while allowing the ability to detect semi-volatile samples. Use of these solvents expands the range of compounds that can be purified with flash chromatography.