

Why Use ELSD if My Compound Absorbs UV?

Abstract

Evaporative Light Scattering Detection (ELSD) is a useful technique for weakly absorbing compounds. Although these compounds can be detected with a UV detector, their response is very weak resulting in low compound recovery. If the absorbance of the compound overlaps that of the solvent, it may be difficult to fractionate the compound due to a decreased signal after subtracting the solvent baseline.

Experimental

Tocopherols were dissolved in ethyl acetate and mixed 10% (w/w) with Celite. The mixture was evaporated to a free flowing powder. The sample (load listed in each section) was placed in a 5g empty RediSep® solid load cartridge (PN 69-3873-235) and run with the solvents and gradients as described in each section. Normal phase ELSD parameters were used on a CombiFlash® Rf-200i (PN 68-5230-011).

Compound Weakly Absorbs UV Light

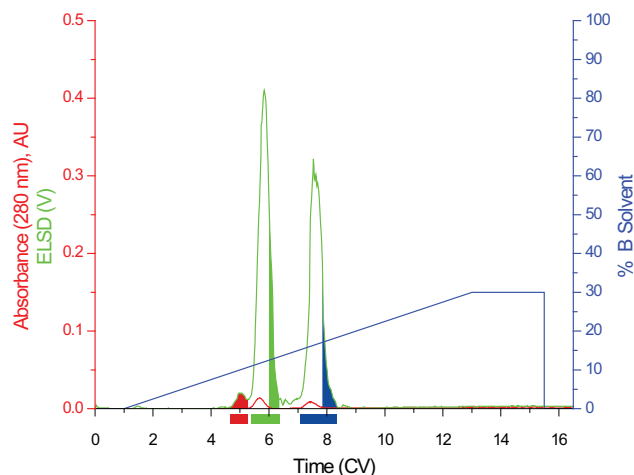


Figure 1: Purification of tocopherols on a diol column with a hexane/ethyl acetate gradient. Shaded area denotes area of improved recovery using ELSD compared to UV absorbance.

In *Figure 1*, a mixture of tocopherols is purified using a 30g RediSep® Rf Gold Diol column (PN 69-2203-516) with a hexane/ethyl acetate gradient. Tocopherols

exhibit a weak absorbance at 280 nm. Using ELSD allows enhanced detection of the compounds and improved recovery, denoted by the shaded areas. The shaded areas denote material not detected with the UV detector, but collected with the ELSD. 0.2g Celite/tocopherol mixture used (20 mg tocopherols).

Compound UV Absorbance is Obscured by Elution Solvent

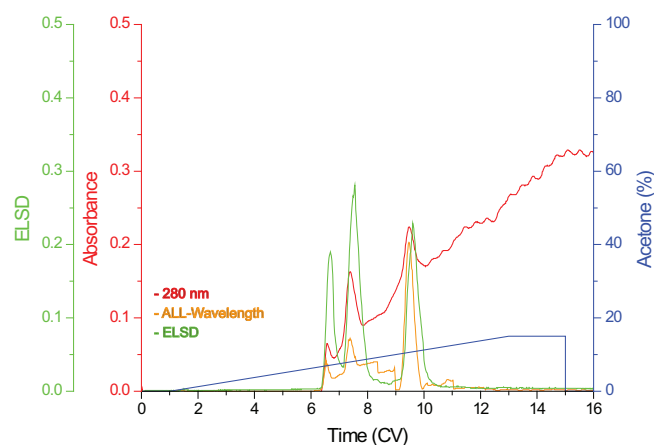


Figure 2: Tocopherols purified on a diol column using a hexane/acetone gradient. Detection using ELSD, All-Wavelength Collection, and absorbance at 280 nm.

The sample in *Figure 2* was run with an acetone gradient, which absorbs at 280 nm. Detection at 280 nm was obscured by the solvent absorbance. The sample load was 0.5g Celite/tocopherol mixture (50 mg tocopherols). The ELSD detection was unaffected by the solvent. All Wavelength Collection weakly detected the first two peaks. The collection at 280 nm was partially obstructed by the solvent absorbance.

Conclusion

ELSD detection can improve compound purification even when the sample exhibits UV absorbance. When the sample exhibits weak absorbance, the ELSD can improve yield by detection of the sample at lower concentrations. ELSD can also improve detection when the compound absorbance is obscured by interference from the solvent.

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