Issues with a steep UV trace slope in gradient HPLC methods - Tips & Suggestions

A common problem encountered in gradient elution chromatography can be the angle or slope of the UV trace.

The reason for this phenomenon can be understood by considering how each of the solvents (A) and (B) have their own UV absorption profile. Because of these differences, the UV readout (trace) will change continuously over the course of the gradient, producing the undesirable slope that is observed. Some of the baseline drift is due to differences in the refractive indices between the two solvents. If the slope is too steep, it can obscure eluting peaks, reducing sensitivity. Therefore, the slope should ideally be as shallow as possible.

Consider the following analogy: Suppose that instead of UV absorbance we are talking about visible absorbance (i.e. colored mobile phase solvents). Say one mobile phase solvent is dark blue and the other is light blue. As they are mixed together, the resulting solvent will be somewhere between the two shades of blue. Because this ratio of mixing continuously changes over the course of the gradient, the shade of blue that the detector sees will continuously vary. Shown as a function of time, this trace is a sloped line.

Some gradients have sharper slopes than others because one solvent is a much "darker blue" than the other. What this would mean in terms of the UV absorbance case is that as the mobile phase content of the gradient changes, the absorption of the UV energy at a particular wavelength will increase due to a more absorptive solvent. This results in a drifting baseline.

If both solvents are similar shades of light blue, the change will be less pronounced over the gradient and the slope will be shallower.

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