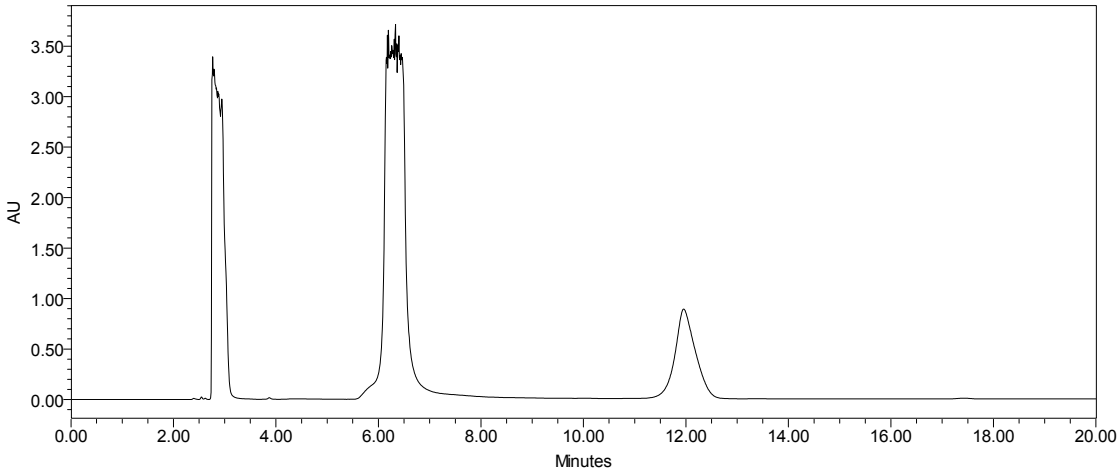
Peak Apex Flattening and Disturbances

Peak shape issues at the apex (top) of a peak are typically related to detector saturation. This symptom can be observed as flattening and/or noise at the peak apex.



Preparative Scales

This is commonly observed in preparative separations, where such a symptom is often not a problem when the chromatogram is used only as a time marker for fraction collecting, and not for quantification purposes.

Analytical Problems

For quantitative purposes, this will be a problem as the peak area/height will fall above the linear calibration range of the detector. Injecting a lower concentration within the calibration range will be necessary. If subsequent impurities present in low amounts cannot be quantified at their required levels with a lower injection concentration, a “high-load:low-load” injection scheme could be used. With a “high-load:low-load” injection scheme, the same sample is injected at the high concentration for the impurity quantification and at the low concentration for assay level quantification.

Beer-Lambert Law

The Beer-Lambert Law relates the absorption of light to the properties of material through which the light is traversing. For UV/Vis detection in chromatography, it is often simplified to $A=\varepsilon cl$, where A is the absorbance (inversely proportional to the transmittance that is measured), ε is the extinction co-efficient (for a particular molecule eluted in a given set of conditions), c is the concentration of the molecule(s), and l is the path length through which the light is traveling. The extinction co-efficient and path lengths are therefore considered constant, leaving the absorbance directly proportional to the concentration.

Troubleshooting

If this is observed under method conditions previously established without the problem, likely contributing causes could be an incorrect dilution factor in the sample preparation, a different detector with a lower range being used, or an otherwise higher than expected sample concentration in the detection path. As seen in the Beer-Lambert equation, changing the detection path length with a different sized flow cell could also change the absolute responses.

Key words: HPLC, Beer-Lambert Law, detector, UV/Vis, saturation, peak shape, troubleshooting

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