How To Select Reference Wavelength On DAD or MWD

Why we need to use the Reference Wavelength in Diode Array Detector**.**

In the Gradient analysis the absorbance value of sample is varying as the composition of the mobile phase is varying, refractive index also changes during the gradient. This change in Sample absorbance is not because of the sample itself but because of change in composition of mobile phase. In Isocratic analysis the baseline will drift and wander induced by room temperature fluctuations along with changes the absorbance value of sample.

The absorbance value from the reference wavelength is subtracted from the values of the sample wavelength continuously. The signal comprises a series of data points over time, with the average absorbance in the sample wavelength band minus the average absorbance of the reference wavelength band. By this procedure the drift and wander induced by non sample effects like mobile phase changes are nullified.
The use of a reference wavelength is highly recommended to further reduce baseline drift and wander induced by room temperature fluctuations or refractive index changes during a gradient. An example of the reduction of baseline drifts is shown in the analysis of PTH-amino acids. Without a reference wavelength, the chromatogram drifts downwards due to refractive index changes induced by the gradient. This is almost completely eliminated by using a reference wavelength. With this technique, PTH-amino acids can be quantified in the low picomole range even in a gradient analysis.
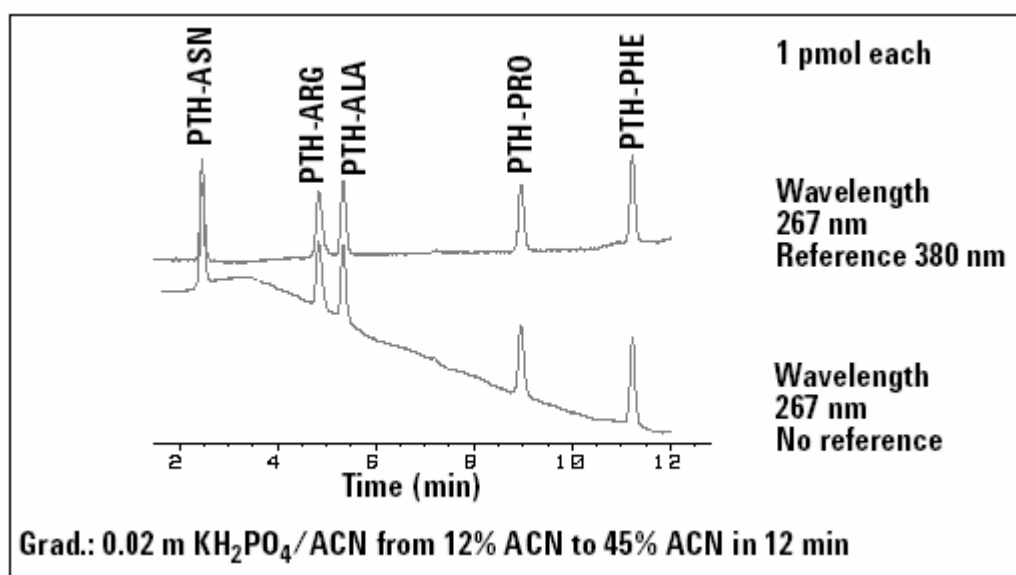
Gradient Analysis of PTH-Amino Acids (1 pmol each), with and without Reference