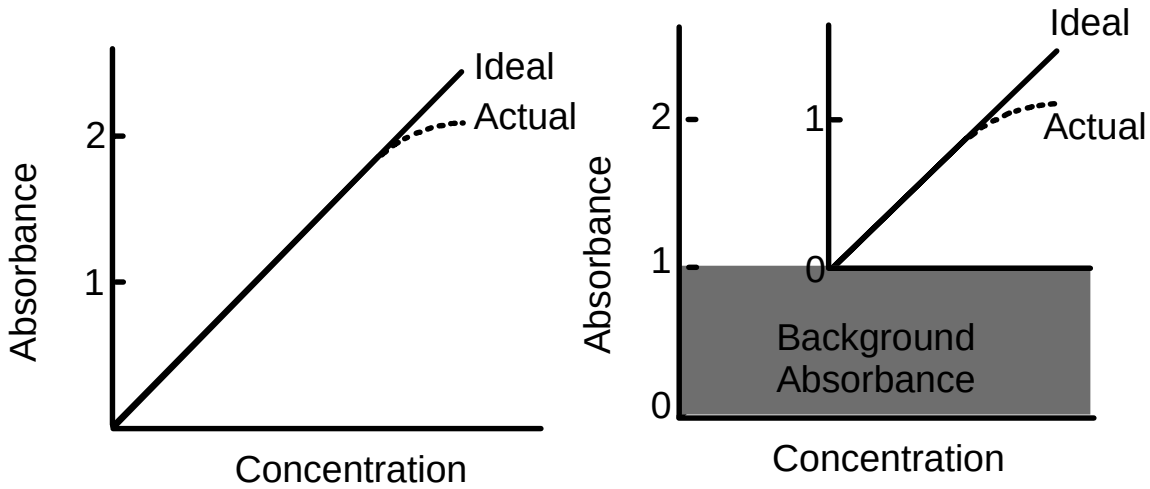
Performance PerSPECtives

UV/Vis Detector Linearity: Mobile Phase Considerations

Your choice of mobile phase or wavelength can decrease the linear range of your detector.



For quantitation, a linear relationship between the detector response, AU (absorbance units) in this case, and the concentration of the analyte is required. Ideally, this relationship remains linear over the working range of the detector. For today's HPLC detectors, a 2 AU range of linearity should be expected.

This relationship is known as Beer's or Beer-Lambert Law. The most ideal situation is when the light is monochromatic, a single wavelength. With tunable HPLC UV/Vis detectors this is not the case. Most variable wavelength HPLC UV/Vis detectors have a band of light, e.g. 254 nm ± 2 nm, determined by the slit width. This is one of the causes of limited linearity in UV/Vis tunable HPLC detectors.

Background absorbance also influences the working range of the detector.

Waters

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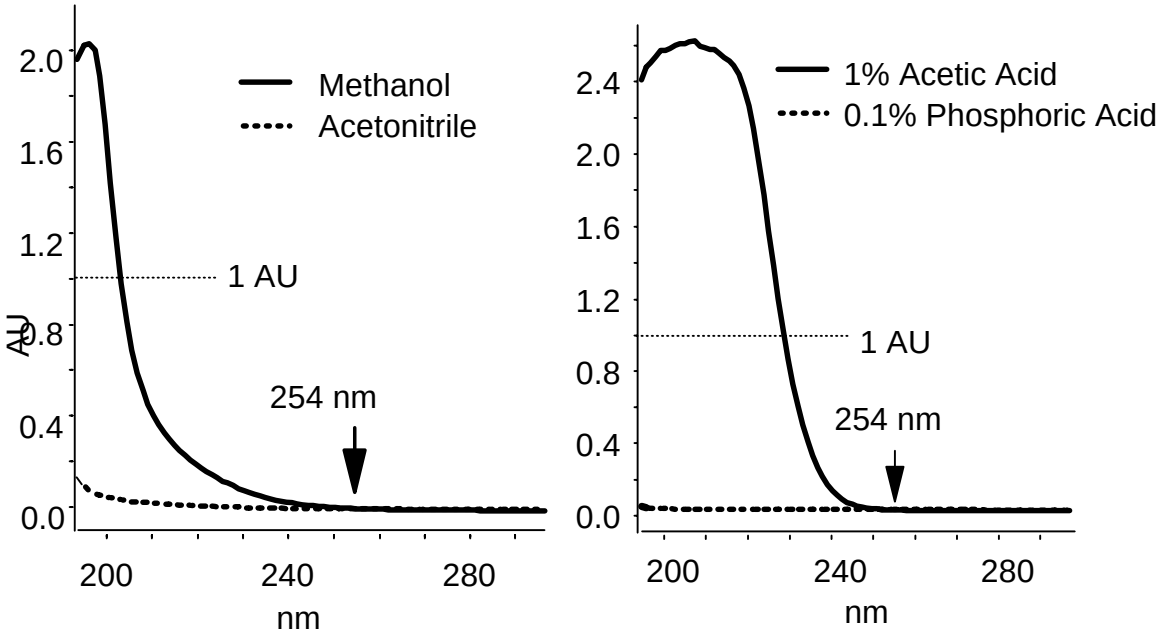
★ Background Absorbance

Many solvents and mobile phase additives have UV absorbance that contribute to the background absorbance. The amount of background absorbance is not observed because the detector autozeroes at the beginning of the chromatographic run. It can be substantial at some wavelengths. In the left panel, methanol is compared to acetonitrile. In the right panel, 1% acetic acid is compared to 0.1% phosphoric acid. For the best linearity, select a detector with good linearity characteristics and use a transparent mobile phase for the wavelength(s) you are monitoring. For photodiode array detection, all wavelengths must be considered. Background absorbance can limit the working linear range of your HPLC detector and therefore, your method.



★ Waters UV/Vis Detectors

Waters UV/Vis detectors, the tunable single wavelength (Waters 486), the programmable multiwavelength (Waters 490) and the photodiode array detector (Waters 996) all have been designed to have 2 AU of linearity when there is no background absorbance.

References

1. Li, J.B., LC-GC 10: (11), 856-864, 1992
2. Seaver, C., Sadel, P., LC-GC: 12 (10), 742-746, 1994

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