Difficulties with wavelength accuracy readings while performing a PQ of an HPLC – Tips & Suggestions

Evaluation of wavelength accuracy on older, less sensitive detectors can be a little difficult at times.

Getting bubbles in the flow cell is a problem and we recommend that you pull the Wavelength Calibration solution through the flow cell, then disconnect the syringe, and allow the system to rest, and keep the inlet/outlet tubing ends above the flow cell level, to prevent syphoning. Once everything is stabilized, you can begin the process by auto-zeroing in a region of no absorbance (ca. 590 nm is recommended), then begin the readings.

These older detectors are not the most sensitive, and have wide bandwidths. This will make accurate detection of the lower absorbing, narrow peaks (i.e., 241, 278 and 287 nm bands) difficult. Absorbance readings on the order of 0.1-0.2 AU are typical for these types of detectors, and the differences between the readings near the maxima are not that strong. This is because the detector is averaging the signals over a broad range of wavelengths. My guess is older instruments have a practical bandwidth of 5-8 nm. You might be forced to look only for the strong, isolated bands such as 641, 537, 451 and 361 nm. The 241 nm **band** is difficult, as it is sharp, and near a baseline rise at lower wavelengths. An older detector simply may not be capable of resolving the 278 and 287 nm bands, and we use that test as a kind of indicator of the detector resolving power, or lack of.

The 204 **band** of caffeine should be resolvable, even on an older instrument, while the 273 nm **band** should be clearly discernable on any instrument. Perhaps the above suggestions of obtaining a more stable signal will help. Also, you are looking for the maximum in a known wavelength region — don't stray too far away from the expected maximum, or you might get enough signal change to falsely indicate another change in the signal direction.

These limitation lie in the instrument, rather than the solutions. We suggest that you use the results from the strong, available bands, and issue the qualification based on those values. In this case, you might be limited to reporting values for the 273, 451 and 537 nm bands, and noting that the instrument was incapable of resolving the finer **band** structure of Holmium Oxide.

Using the few wavelengths that can be resolved will still produce NIST traceability to the holmium oxide solution.