

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

7-APRIL-2012 Last Updated: 5-JULY-2025

When performing wavelength accuracy checks during a Performance Qualification (PQ) of an HPLC system, the following tips can help mitigate common issues—especially with older detectors:

### 1. Preventing Bubbles in the Flow Cell

Bubbles can significantly disrupt wavelength accuracy readings. To avoid this:

- Draw the Wavelength Calibration Solution through the flow cell using a syringe.
- Disconnect the syringe and allow the system to rest.
- Keep both the inlet and outlet tubing ends elevated above the flow cell to prevent siphoning.
- Once the system stabilizes, auto-zero the detector in a region with minimal absorbance (around 590 nm is recommended), then proceed with measurements.

### 2. Understanding Detector Limitations

Older detectors often have:

- Lower sensitivity
- Wider bandwidths (typically 5–8 nm)

These characteristics make it difficult to resolve narrow, low-absorbance peaks such as those at 241, 278, and 287 nm. Absorbance values in the range of 0.1–0.2 AU are typical, and peak maxima may appear flattened due to signal averaging across a broad wavelength range.

### 3. Recommended Wavelengths for Reliable Results

Given these limitations, focus on stronger, more isolated bands:

- Holmium Oxide: 361, 451, 537, and 641 nm
- Caffeine: 204 nm (resolvable on most instruments), 273 nm (should be clearly visible)

Avoid relying on the 241 nm band, as it is sharp and close to a baseline rise, making it difficult to resolve. The 278 and 287 nm bands are useful as indicators of detector resolution but may not be distinguishable on older systems.

### 4. Best Practices for Peak Identification

- Stay close to the expected peak maxima during scans.
- Avoid scanning too broadly, as this may introduce false signal direction changes.

## 5. Qualification Strategy

These challenges are instrument-related, not due to the calibration solutions. If your detector cannot resolve finer band structures:

- Base your qualification on the clearly resolved peaks (e.g., 273, 451, 537 nm).
- Document that the instrument is not capable of resolving narrower bands like 278 or 287 nm.
- This approach still maintains NIST traceability through the holmium oxide solution.

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