

Response Improvement in Your HPLC UV Detector - Tips and Suggestions

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In accordance with Beer's law, there are only three things you can do to obtain increased analyte response in UV detection:

1. The first is increasing the analyte concentration.
2. The second is increasing the path length of the UV flow cell.

To do this, you could use an instrument with or allows a longer path length.

3. The third is to increase the value of the molar extinction coefficient.

You can do this by operating at a wavelength corresponding to the Lambda max of the analyte.

In an already developed and validated method, most of these parameters have already been decided upon, and cannot be changed substantially. However, to the extent there is some flexibility in the method parameters, as for example, with permissible changes to USP and EP column and operating condition selection, it may be possible to increase peak sensitivity.

For concentration detectors (UV, RI, Conductivity), the detector is sensing concentration in the flow cell. For a given injection volume and solute concentration, the maximum concentration for a peak is determined by its width, i.e., column efficiency. So within permissible method limits, a smaller particle column will usually produce a higher efficiency (plate count), and thus a higher peak concentration. Another way is to decrease the column diameter and/or length, keeping the injection volume the same. Conversely, changes to the flow rate will have no significant effect on the peak sensitivity, assuming no other changes to the system.

You normally do not want to go over approximately 1500 mAU because deviations from the ideal linear behavior predicted by Beer's law can result above this region. The Chemical Solutions HPLC Qualification kits can be used to measure absorbance linearity up to any desired absorbance value, to demonstrate the upper absorbance limit of your HPLC instrument.

Click [HERE](#) for the Chemical Solutions HPLC Qualification Kit Ordering Information.