How to improve LOD or detection limits in HPLC - Tips & Suggestions

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The Limit of Detection (LOD) in HPLC LOD: defined as the lowest concentration or amount of an analyte in a sample that can be reliably detected but not necessarily quantified . In other words, it's the smallest amount that can be distinguished from background noise or a blank sample .

1. Increase the Signal

- Optimize Detection Wavelength: For UV detection, ensure you're operating at the analyte's λmax. If analyzing multiple compounds, select a compromise wavelength or use multi-wavelength detection if your system supports it.
- Enhance Peak Efficiency: While the column doesn't directly affect detection, improving chromatographic efficiency results in sharper, taller peaks, enhancing signal intensity.
- Mobile Phase Additives:
  - For amines, use 0.1% formic acid to reduce tailing.
  - If not using LC-MS, 0.1% TFA can also improve peak shape.
- Gradient Elution: A sharp gradient often produces narrower peaks compared to isocratic runs.
- Column Selection: The ***Diamond Hydride™*** column with Aqueous Normal Phase (ANP) is highly effective for hydrophilic analytes, often outperforming reversed-phase (RP) in peak shape and signal intensity.
- Pre-ionization in LC-MS: ANP-compatible mobile phases can pre-ionize analytes, further boosting MS signal response.

2. Reduce the Noise

- Mobile Phase Volatility: In LC-MS, ANP methods use more volatile solvents than RP, which helps lower baseline noise.
- UV Transparency of Solvents:
  - Acetonitrile is preferred due to its low UV absorbance above ~190 nm.
  - Avoid acetone, which absorbs strongly in the UV range and increases noise.
- **Additive Considerations**: Ensure that mobile phase additives do not contribute to UV absorbance in your detection range.

By carefully optimizing both signal generation and noise suppression, you can significantly improve the sensitivity and reliability of your HPLC method.

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MicroSolv Technology Corporation
9158 Industrial Blvd. NE, Leland, NC 28451
Tel: (732) 380-8900
Fax: (910) 769-9435
Email: customers@mtc-usa.com
Website: www.mtc-usa.com