

Factors that affect sensitivity in HPLC - Tech Information

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Factors That Affect Sensitivity in HPLC

What is Sensitivity in HPLC?

In High Performance Liquid Chromatography (HPLC), *sensitivity* refers to how well the system can detect small amounts of substances (called analytes) in a sample. A more sensitive system can "see" even tiny traces of a compound.

Understanding Signal-to-Noise Ratio (S/N)

One of the biggest factors that affects sensitivity is something called the **signal-to-noise ratio**. This is a measure of how strong your analyte signal is compared to the background "noise" from the system.

Think of it like this:

Imagine trying to see stars in the sky. During the day, the sunlight (noise) makes it hard to see the stars (signal). But at night, especially in a dark, remote area, the stars become much clearer—even though the stars themselves haven't changed. The difference is the background light (noise) is lower.

In HPLC, the same idea applies. The analyte signal might be the same, but reducing the background noise makes it easier to detect.

Types of Detectors and Their Role

Different detectors have different sensitivities. Some common types include:

- UV detectors (measure light absorption)
- Mass spectrometers (MS) (measure mass of molecules)
- Refractive index detectors
- Charged aerosol detectors (CAD)
- Evaporative light scattering detectors (ELSD)

Each has its strengths depending on what you're analyzing.

How to Improve Sensitivity (Without Changing the Detector)? You can improve sensitivity in two main ways:

✓ Increase the Signal

- Use the best wavelength for your compound (especially in UV detection).
- Optimize ionization settings in MS.
- Pre-concentrate your sample before injection.

✓ Reduce the Noise

- Use high-purity HPLC-grade solvents to avoid extra absorbance from impurities.
- Keep your system clean and well-maintained.

- Use proper mobile phase preparation and filtration.

What About the HPLC Column?

The column doesn't directly control sensitivity, but it can help. A high-efficiency column produces **sharper peaks**, which means the signal is more concentrated and easier to detect. However:

- Sensitivity \neq Selectivity (selectivity is about separating compounds, not detecting them).
- It's not accurate to say a column is "more sensitive"—it just helps improve the signal shape.

Helpful Tips for Beginners

- Always check your baseline noise before running samples.
- Run a blank to see if your solvents or system are contributing to background noise.
- Keep a log of detector settings and performance to track changes over time.



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