

New Column Use and Method Suggestions

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New Column Verification and Method Development Best Practices

1. Review the QC Test Chromatogram

Every new Cogent™ HPLC column includes a packing test chromatogram that documents:

- Peak symmetry
- Plate count
- Test analytes
- Complete test conditions

This chromatogram serves as the primary performance benchmark for the column. It should be retained for reference, as it is essential for verifying that the column operates as expected on your specific chromatographic system.

2. Reproduce the Factory Test Conditions

To verify column performance, set your LC system to match the QC test conditions as closely as possible (mobile phase, flow rate, temperature, and analytes).

Run several injections and compare the resulting chromatograms with the QC reference.

This is the fastest and most reliable way to determine whether any abnormalities originate from the column or from your instrument.

3. Evaluate Key Performance Indicators

Compare your data to the QC documentation and assess:

- Peak shape
- Retention time
- Column efficiency (plate count)
- Baseline stability

Significant differences often indicate instrument-related issues, such as extra-column volume, improper fittings, or injection problems.

4. Begin Routine Use

Once the column's performance aligns with the QC reference:

- Flush the column with the starting mobile phase of your method
- Allow adequate equilibration
- Begin running your samples confidently

5. If Problems Occur

If performance begins to drift or peaks degrade:

1. Re-run the column under the original QC test conditions to confirm whether the column still performs as expected.
2. If results differ from the initial QC reference, first check for system-related causes:
 - Tubing or connection problems
 - Injector or detector contamination
 - Mobile phase quality issues

If no instrument-related causes are identified, the observed decline in chromatographic performance is most likely attributable to stationary-phase degradation—a normal outcome given the finite lifetime of HPLC columns as consumables—and the column should be replaced.

6. Additional Guidance for Method Development

During method development, it is routine to investigate a wide range of experimental variables, including buffer composition, ionic strength (salts), acidic or basic additives, organic modifier concentration, and pH conditions. These additives can have short-term or cumulative effects on the stationary phase. Even if the column appears stable during development, certain conditions—such as high salt content, extreme pH, or aggressive additives—can alter the column surface over time, affecting retention, selectivity, peak shape, and reproducibility.

Best Practice for Method Development

Once you finalize your method, always:

Test the final method using a *brand-new* column.

This ensures that:

- Your chosen additives and conditions do not negatively impact a fresh stationary phase
- The method is fully reproducible on new columns
- No irreversible surface changes occurred during method experimentation

This final confirmation is essential for developing methods that are robust, transferable, and suitable for regulated QC environments or multi-site implementation.

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