Changing Retention Times and a Spurious Peak with a Silica-C column - Troubleshooting

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Troubleshooting Changing Retention Times and Unexpected Peaks in Blanks When Using Silica-C Columns

Unexpected peaks appearing in blank injections—and shifting retention times—are common troubleshooting challenges in HPLC, especially when using Cogent Silica-C™ (TYPE-C™ silica hydride) columns. These symptoms typically indicate matrix-related contamination or insufficient column cleaning, particularly in gradient methods where the final organic strength may not be high enough to remove strongly retained sample components.

This guide explains the likely cause of these issues and the steps you can take to eliminate them and restore reliable chromatographic performance.

Most Likely Cause: Matrix Effects

When a peak appears in the blank injection after sample runs, the most probable explanation is matrix retention—residual compounds from previous injections slowly eluting in later runs.

These matrix components can:

- Strongly adsorb to Silica-C surfaces
- Elute unpredictably in subsequent injections
- Cause retention time drift or inconsistent peak shapes

This is especially common in methods involving biological matrices, complex formulation excipients, or analytes prone to secondary interactions.

Step 1: Wash the Column with a Strong Solvent

To remove retained contaminants, wash the Silica-C column using a stronger version of the B-solvent—one with a higher organic concentration than your gradient's final %B.

Washing Procedure

1. Run a high-organic wash (e.g., stronger than gradient endpoint).
2. After washing, reinject your blank sample to verify whether the peak disappears.

If the unwanted peak is due to matrix carryover, its intensity should be significantly reduced or completely eliminated after this rinse.

Step 2: Verify That the Peak Is Not the Solvent Front

Before concluding that the peak is contamination, check whether it aligns with:

- The dead volume (t_0)
- The solvent front

If it does, this may indicate a mobile-phase mismatch, injection solvent effect, or normal system behavior rather than contamination.

Step 3: Increase Organic Strength at the End of the Gradient

If the blank peak persists after washing, you may need to increase the final % organic at the end of your gradient.

Recommended solvents:

- Ethanol (EtOH)
- Isopropanol (IPA)

These solvents are more effective in removing strongly retained, hydrophobic, or semi-polar contaminants.

Why this works:

Higher organic strength increases elution power and reduces the likelihood of matrix compounds carrying over into later injections.

Step 4: Add a Routine Washing Step to Your Sequence

If the contamination returns after several injections, the system is accumulating matrix components over time.

In this case, incorporate a scheduled wash cycle, such as after every 5–10 sample injections, using:

- High-organic solvent wash (e.g., 80–100% organic)
- Or higher-strength EtOH/IPA rinse

This prevents matrix buildup and improves long-term stability of retention times.

Why Silica-C Columns Show This Behavior

Cogent Silica-C™ columns are based on TYPE-C™ silica hydride, which provides:

- Strong ANP and RP retention
- Low water layer interaction
- High sensitivity to retained matrix components if not adequately washed

Because the stationary phase is efficient at retaining polar or semi-polar compounds, any insufficiently eluted matrix residue may remain on the surface until washed away with a stronger solvent.

Conclusion

Changing retention times and unexpected peaks in blank injections when using Silica-C columns are typically caused by matrix carryover. The solution involves:

- Performing a strong-organic wash
- Verifying the solvent front is not being mistaken for contamination
- Increasing the end-gradient organic strength (EtOH or IPA recommended)
- Adding periodic washing steps to maintain long-term performance

These steps restore reproducibility and prevent recurring contamination-related artifacts.

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