

Analyte retention decreases with mobile phase water content using the Cogent Bidentate C18 HPLC column has anything happened to the column - FAQ

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When using the Cogent Bidentate C18 column, a decrease in analyte retention with increasing water content in the mobile phase can be attributed to two primary scenarios:

1. Aqueous Normal Phase (ANP) Retention Mechanism

This behavior may be entirely expected and is characteristic of the Cogent TYPE-C™ silica-based columns. These columns can exhibit ANP-like retention, especially for polar analytes, where retention increases with higher organic content (e.g., acetonitrile) and decreases with higher aqueous content. This is not an anomaly but a normal operational mode for these stationary phases under certain conditions.

2. Instrument Configuration or Method Setup Error

If the expected retention mechanism is Reversed Phase (RP) and retention decreases with increasing water content, this may indicate a system or method configuration issue. Consider the following troubleshooting steps:

- **Verify Solvent Identity and Placement:** Ensure that mobile phase A (typically aqueous) and mobile phase B (typically organic) are correctly assigned and placed in the appropriate reservoirs. Mislabeling or switching these can invert the gradient profile.
- **Inspect Solvent Line Routing:** Trace each solvent line from the reservoir through the degasser and into the pump. Confirm that no lines are crossed or misconnected, especially in systems with shared degassers or multi-channel pumps.
- **Check Pump Calibration (Dual Pump Systems):** If using a binary pump system, verify that each pump delivers the correct flow rate. A simple test involves timing how long it takes to fill a 5 mL volumetric flask from each solvent line. Discrepancies in flow delivery can lead to incorrect mobile phase composition and unexpected chromatographic behavior.

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