High-Molecular-Weight Compounds Fail to Retain on the Cogent Diamond Hydride Column — and the Correct Column to Use - Tips and Suggestions

Date: 29-AUGUST-2013 Last Updated: 7-FEBRUARY-2026

When analyzing high–molecular-weight analytes (~10,000 Da or greater), you may observe no retention when using Cogent Diamond Hydride™ (100 Å pore size) columns.

This behavior is expected and is a direct consequence of pore accessibility and surface area utilization in silica-based chromatographic media.

1. Why Large Molecules Do Not Retain on Diamond Hydride™ (100 Å)

The vast majority of a silica particle's interactive surface area resides inside its pores, not on the external particle surface. Retention mechanisms—whether ANP, RP-like, or mixed-mode—require the analyte to enter these pores to interact with the stationary phase.
For very large molecules such as:

- Proteins
- Large peptides
- Polymers
- Other macromolecular species

…the 100 Å pores are too small for the analyte to enter. If the analyte cannot reach the internal surface where retention occurs, it will:

- Pass through the column rapidly
- Elute at or near the **solvent front**
- Show little to no retention despite method changes

This is exactly the behavior observed with high–molecular-weight compounds on the Diamond Hydride™ column

2. Correct Column Choice: Cogent Bidentate C8 300 Å (Wide-Pore)

To retain and separate larger biomolecules, you must use a wide-pore stationary phase, such as the Cogent Bidentate C8 300Å™ HPLC column.

The 300 Å pore size allows macromolecular analytes to:

- Physically enter the pore network
- Access the full functionalized surface
- Exhibit meaningful retention and separation

The Cogent Bidentate C8 300 Å phase is therefore the recommended choice for:

- Proteins
- Peptides
- Polysaccharides
- Polymers
- Large hydrophobic or amphipathic biomolecules

3. Recommended Next Steps for Analysts

A. Choose the Correct Stationary Phase

- For MW > ~10,000 Da: Use Cogent Bidentate C8 300 Å
- For mid-size polar biomolecules (<10 kDa): Diamond Hydride™ may still be appropriate, depending on shape, charge, and folding

B. Modify Mobile Phase Conditions Accordingly

- Matching 300 Å pore phases with proper organic/aqueous balances is crucial
- Typical starting points include:
    - 0.1% formic acid or 10 mM ammonium formate (LC-MS compatible)
    - 60–90% organic (for ANP-compatible macromolecules)
    - Or RP-oriented gradients for hydrophobic peptides/proteins

C. Consider Sample Preparation

Because large biomolecules have strong nonspecific interactions, ensure:

- Adequate filtration (0.2 µm recommended)
- Avoidance of salts that precipitate in high-organic conditions
- Use of appropriate solubility aids (e.g., low % MeOH, ACN, or pH adjustment)

---

4. Summary

High-MW molecules cannot retain on Cogent Diamond Hydride™ (100 Å) because they cannot enter the pores where chromatographic interactions occur. Instead, they elute at the solvent front. The correct solution is to switch to a wide-pore 300 Å column, specifically Cogent Bidentate C8 300™, which is engineered to provide retention for proteins, peptides, polymers, and other high-MW species.

Click HERE for Cogent Bidentate C8 300 HPLC column ordering information

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