Serum Samples Use Suggestion for Cogent Diamond Hydride Columns in LC-MS Methods - Tips and Suggestions

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Mobile Phase Recommendations for Using Cogent Diamond Hydride™ Columns with Serum Samples in ANP-LC-MS

Analyzing biological matrices such as serum or plasma presents unique challenges in LC-MS. These samples contain proteins, lipids, and other matrix components that can quickly contaminate or foul an HPLC column. When using Cogent Diamond Hydride™ columns under Aqueous Normal Phase (ANP) conditions, it is especially important to choose mobile phases that both maintain ANP retention mechanisms and help keep the column surface clean.

This guide provides optimized mobile-phase recommendations and cleaning strategies specifically for serum-based LC-MS methods.

Why Serum Samples Require Special Mobile Phase Handling

Serum and similar biological matrices can leave behind:

- Adsorbed proteins
- Lipids and phospholipids
- Salt residues and buffers
- Hydrophobic contaminants

These impurities accumulate on the column surface, gradually reducing retention, weakening ANP selectivity, and degrading peak shapes. To counter these effects, the mobile phase must support continuous column cleaning while maintaining ANP performance.

Recommended Mobile Phases for ANP on Diamond Hydride™ Columns

Solvent A (Critical for Column Cleanliness)

Use continuously, not just during conditioning.
Recommended composition:

- 50:50 DI water : methanol *(or 50:50 DI water : 2-propanol (IPA))*
- Additives:
    - 0.1% formic acid for positive-mode MS, *or*
    - 10 mM ammonium acetate or ammonium formate for negative-mode MS

Why this works:

- Methanol or IPA actively solubilizes biological residues, reducing buildup.
- Maintaining the organic content helps stabilize ANP retention behavior.

Solvent B (Standard ANP Mobile Phase)

Use your normal ANP B solvent composition, for example:

- 98% acetonitrile / 2% DI water
- 1–5 mM acid or base (e.g., formic acid, ammonium acetate, ammonium formate)

This maintains the strong organic environment required for ANP retention.

Cleaning Tip for Biological Matrices

Because serum, plasma, and urine are high-fouling matrices, an explicit cleaning step may be necessary.

If Solvent A cannot contain methanol or IPA (e.g., method restrictions):

- Add a washing step every ~10 injections
- Use the methanol/IPA-containing Solvent A formulation listed above

This intermittent washing minimizes buildup and preserves column performance without interrupting your normal gradient.

Why These Recommendations Work

- Methanol / IPA actively removes proteins and lipophilic residues that stick to silica-hydride surfaces.
- Diamond Hydride™ in ANP mode benefits from stabilized surface hydration, achieved by maintaining appropriate water/organic ratios.
- Proper additives ensure strong MS sensitivity and stable ionization for positive or negative mode.

Together, these strategies significantly improve reproducibility and column lifetime for serum LC-MS methods.

Conclusion

Using serum samples in ANP-LC-MS requires mobile phases that both preserve ANP retention and continually clean the column. A 50:50 water/methanol (or water/IPA) Solvent A paired with a standard ANP Solvent B delivers reliable retention, excellent peak shape, and longer column life for Cogent Diamond Hydride™ columns. Incorporating periodic washing steps further protects performance when high-fouling biological matrices are analyzed.

Related article: General solvent conditioning for TYPE-C Silica columns

COGENT HPLC Columns™

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