MICROSOLV

# Phosphorylated Compounds Analyzed with Cogent Diamond Hydride Columns - Tips and Suggestions

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Analyzing phosphorylated compounds by HPLC can be challenging due to their high polarity and tendency to interact with metal surfaces. However, the Cogent Diamond Hydride™ column, used in Aqueous Normal Phase (ANP) mode, offers a powerful solution for retaining and separating a wide range of phosphate-containing analytes.

## ✅ Compatible Compound Types

The column has demonstrated excellent retention and separation for:

- Phosphorylated sugars
- Herbicides
- Nucleotides
- Isoprenoid phosphates and other phosphate-functionalized molecules

## 🔬 LC-MS Detection Tips

When using LC-MS, monitor for $[M - H]^-$ ions in negative ion mode extracted ion chromatograms (EICs) for optimal detection of phosphorylated species.

## 🧪 Recommended Mobile Phase Conditions

Use the following mobile phase system for ANP:

- Solvent A: DI water + 10 mM ammonium acetate (or ammonium formate)
- Solvent B: 95:5 Acetonitrile:DI water + 10 mM ammonium acetate

Start with a high %B and apply a gradient to a moderate or low %B to adjust retention. Fine-tune the gradient based on your compound's behavior.

## 🛠️ Troubleshooting Peak Shape Issues

To improve peak shape and reproducibility, consider these strategies:

1. Adjust pH: Add a small amount of dilute ammonia to raise the mobile phase pH to ~7.0.
2. Avoid Sodium Contamination: Use Teflon bottles instead of glass to prevent sodium leaching.
3. Metal Ion Interference: Add 5–10 µM EDTA to the mobile phase to chelate trace metals that can distort peaks of anionic compounds.

4. Column Hardware: Use metal-free, coated HPLC columns to avoid unwanted interactions with stainless steel surfaces.

## 📦 Ordering Information

Clicke ***HERE*** for ordering the Cogent Diamond Hydride™ column or to learn more about its specifications and available formats, please visit our [product page] or contact our technical support team.


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