

## Analyze phosphorylated compounds with the Cogent Diamond Hydride column – Tips & Suggestions

### Phosphorylated compounds can be difficult to analyze by HPLC.

However, a wide variety of compounds with the phosphate functional group have been retained with the Cogent Diamond Hydride™ column in Aqueous Normal Phase ANP methods. Examples include phosphorylated sugars, herbicides, and nucleotides. The strategy can also be applied to other compounds of this type such as isoprenoid phosphates.

*If using LC-MS, look for the  $[M - H]^-$  ions in the EICs (negative mode).*

For the mobile phase, try Solvent A: DI water + 10 mM ammonium acetate (*or formate*) and Solvent B: 95/5 Acetonitrile / DI water + 10 mM ammonium acetate. Then use a standard ANP gradient going from a high %B to a moderate / low %B. Depending on the retention, adjust as needed to get higher or lower retention. To address any peak shape issues, consider the following three strategies:

- Use a dilute ammonia solution to increase mobile phase pH to 7.0
- Use Teflon bottles instead of glass for the mobile phase to prevent leaching of sodium
- Use a very small amount of EDTA (5-10 microM) in the mobile phase to sequester trace metals, which can cause peak shape problems for anionic compounds such as phosphates
- Use our metal free, coated HPLC columns instead of an untreated stainless steel column

[Cogent Diamond Hydride HPLC column ordering information](#)



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