

Aqueous Normal Phase or ANP HPLC What is it - HPLC PRIMER

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If you're working with polar compounds like acids, bases, or peptides and struggling to retain them using traditional HPLC methods, Aqueous Normal Phase (ANP) chromatography might be the solution you're looking for.

What Is ANP Chromatography?

Aqueous Normal Phase (ANP) is a unique HPLC technique that combines features of both Reversed Phase (RP) and Normal Phase (NP) chromatography. It's especially useful for separating polar compounds that don't retain well in RP without pH extremes or paired ions.

- Mobile Phase: Mostly organic (e.g., starts with 98% acetonitrile) with a small amount of water (2%) and an acid or buffer like formic acid, TFA, ammonium acetate, or ammonium formate.
- Stationary Phase: Slightly hydrophobic, silica hydride, found in Cogent TYPE-C™ columns.

How Does ANP Work?

In ANP mode:

1. The organic-rich mobile phase allows the stationary phase to adsorb hydroxide ions from the small amount of water.
2. This creates a negatively charged surface on the column.
3. Polar, ionized compounds (like acids and bases) interact with this surface and are retained.
4. As you increase the water content in the mobile phase, the polarity increases, and polar compounds are more attracted to the mobile phase than the column—so they elute.

Why Use ANP?

ANP offers several advantages for chromatographers:

- Excellent retention of polar compounds
- Fast equilibration between runs
- High reproducibility and run-to-run consistency
- Unique selectivity not possible with RP or HILIC
- Long column lifetime
- No need to purge the system when switching between RP and ANP modes

Flexible Column Use

All Cogent TYPE-C™ Silica columns can be used in:

- ANP mode
- Reversed Phase (RP) mode
- Normal Phase (NP) mode

You can switch between modes simply by adjusting the mobile phase—no need for extensive flushing or reconditioning. Some bonded phases are more optimized for ANP, while others perform better in RP or hybrid conditions.

Quick Summary for Beginners

Feature	ANP HPLC
Best for	Polar compounds (acids, bases, peptides)
Mobile phase	High organic (e.g., 98% to 40% acetonitrile) + low water depending on the analytes
Stationary phase	Silica hydride (slightly hydrophobic)
Retention mechanism	Surface adsorption via hydroxide ions
Key benefits	Fast, reproducible, flexible, MS-compatible



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