

Solvent Strength Order in Aqueous Normal Phase ANP HPLC - Tech Information

Date 5-APRIL-2013 Last Update: 12-FEBRUARY-2026

Understanding Solvent Strength in Aqueous Normal Phase (ANP) Chromatography

Aqueous Normal Phase (ANP) chromatography is a powerful mode enabled by silica-hydride-based Cogent TYPE-C™ columns.

Unlike reversed phase, where organic content weakens elution strength, ANP has a **unique solvent strength order** that directly influences retention, resolution, and matrix cleanliness. Knowing this order can dramatically improve method development, especially for polar compounds, biological matrices, and LC-MS workflows.

The Solvent Strength Order in ANP

In ANP, solvent strength does **not** align with reversed-phase expectations. Instead, the elution power follows this order (strongest to weakest):

DI Water > Methanol > Isopropanol (IPA) > Acetone > Acetonitrile

Why Water Is the Strongest Solvent

Mechanistic note on ANP (vs. HILIC). In Aqueous Normal Phase (ANP) on silica hydride (e.g., Diamond Hydride/TYPE-C), retention does not rely on partitioning into a thick, water-rich layer.

Instead, studies show the silica hydride surface exhibits adsorbed hydroxide ions (OH⁻) in the presence of aqueous mobile phase, creating a strongly negative interfacial charge; retention of polar analytes then arises primarily from electrostatic attraction (for cations), ion-displacement/repulsion (for anions), and adsorptive effects for neutral polar compounds, and typically strengthens under organic-rich conditions.

By contrast, HILIC depends on a water-rich layer on polar phases (e.g., amide, diol, bare silica, zwitterionic), with retention driven largely by partitioning into that layer.

Practical Impact

- High-aqueous conditions **decrease retention**, especially for highly polar compounds.
 - Using weaker solvents (like acetonitrile) **increases retention**, helping separate early-eluting metabolites, sugars, amino acids, and small organic acids.
 - Understanding solvent order helps avoid over-elution, broad peaks, or matrix interference.
-

Why Methanol and IPA Are Often Included in the A Solvent

While water is strongest, ANP method development typically avoids using only water in the aqueous phase. Instead, methanol or IPA is added to improve column hygiene. These alcohols help:

- Remove adsorbed matrix components from biological extracts.
- Reduce gradual buildup that alters retention.
- Decrease carryover-related baseline noise in subsequent injections.

This is especially beneficial for LC–MS work, where cleanliness, reproducibility, and short equilibration cycles matter.

Acetone vs. Acetonitrile: Not Interchangeable

Although both are considered weaker ANP solvents, acetone and acetonitrile behave differently with certain analytes.

- For some amino acids, retention behavior is similar between the two solvents.
- For others, retention can differ significantly, impacting selectivity and elution order.

This variability means substitutions should be carefully tested during method optimization rather than assumed equivalent.

Acetone as an ACN Substitute in LC–MS

Acetone can be an excellent ACN replacement when:

- Lower UV background is needed, or
- The MS method tolerates acetone's volatility and ionization characteristics.

Acetone's UV transparency can improve detection windows in certain applications.

Using Solvent Strength to Improve ANP Method Robustness

Because ANP retention is sensitive to the water content in the mobile phase, solvent choices provide powerful tools to tune separation quality:

Use cases of stronger solvent (water):

- Reducing retention of polar compounds that otherwise stick too strongly
- Shortening run time for high-throughput assays
- Cleaning the column at end-of-run

Use cases of weaker solvents (ACN, acetone):

- Maximizing retention for extremely polar analytes
 - Improving resolution of early-eluting matrix components
 - Enhancing selectivity in metabolomics and small-molecule workflows
-

Practical Notes for Method Developers

- Don't develop ANP methods using only water and methanol—this can limit method stability and matrix cleanup efficiency.

- Include a moderate percentage of methanol or IPA in the A solvent for better removal of adsorbed compounds.
- When substituting acetone for ACN, verify selectivity and retention with your target analytes.
- Always consider LC–MS compatibility—acetone and IPA can be excellent choices when additives or high salt loads are avoided.

NOTE: For methanol or isopropanol, these are typically used as part of the A solvent to help remove adsorbed sample components and keep the column clean from run to run. This can be very helpful for samples like biological extracts. You would not generally use just water and methanol in ANP. Acetone is a good acetonitrile substitute in certain situations, such as in LC-MS where the UV absorbance of this solvent is not a problem.

[TYPE-C HPLC Column Information](#)



Printed from the Chrom Resource Center

Copyright 2025, All Rights Apply

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