

Injection Technique for Aqueous Samples in Water using in ANP - Tips and Suggestions

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In Aqueous Normal Phase (ANP) on Cogent TYPE-C™ silica (e.g., *Diamond Hydride*™), the usual best practice is to inject samples prepared in a high-organic diluent to maximize focusing and minimize band distortion.

However, in many real-world applications you can inject entirely aqueous diluents (100% water) and still obtain sharp, symmetrical peaks—as demonstrated in an application analyzing folic acid (standards and juice samples) on a Cogent Diamond Hydride™ column with 100% water as the sample diluent.

When 100% Water Works (and Why)

- For certain highly polar, ionizable analytes (e.g., folic acid), the hydrated silica-hydride interface plus ANP's high-organic mobile phase start can still provide strong focusing, even if the injection plug is fully aqueous. In practice, excellent peak shape has been observed under these conditions.
- This approach is particularly helpful when matrix compatibility or sample preparation constraints require water-only diluents (e.g., food/beverage extracts), and you wish to avoid non-volatile additives or excessive sample manipulation.

Key point: Although a high-ACN diluent is still the *default* recommendation for ANP, 100% water can be a viable, validated option for specific targets and matrices, with minimal compromise to peak integrity.

Practical Setup & Method Tips

1. Mobile-phase strategy (ANP):
 - Start at high %B (ACN) with standard volatile additives (e.g., 0.1% formic acid or 10 mM ammonium acetate) to ensure MS compatibility and robust retention of polar analytes.
2. Injection volume & focusing:
 - If switching from high-organic to 100% water diluent, consider slightly smaller injection volumes or a brief high-%B pre-segment to preserve focusing at the column head. (This preserves the focusing advantage typically delivered by an organic-rich plug.)
3. Gradient design:
 - Maintain a high-organic start (typical ANP), then decrease %B to elute the most strongly retained polar species. This approach has produced sharp, symmetrical folic acid peaks with 100% aqueous sample diluent.

4. Matrix robustness:

- For food/juice/bio-like matrices, add a short clean-out hold (e.g., 50:50 water/MeOH or water/IPA) after the gradient or between runs to prevent matrix build-up; this practice complements the aqueous-diluent approach and protects column longevity.

When to Prefer High-Organic Diluents

- Very early eluters (extremely hydrophilic analytes) that risk partial breakthrough with water-only plugs.
- Large injection volumes or high-salt samples that can disrupt focusing or increase band broadening.
- Highly throughput-sensitive assays where the tightest possible focusing is required across diverse analyte classes.

In these cases, revert to ACN-rich diluents or reduce injection volume for optimal focusing.

Summary

- Default ANP guidance favors high-organic sample diluents, but 100% water can absolutely work on Cogent TYPE-C™ columns for specific analytes and matrices. This has been shown for folic acid (standards and juice) on **Cogent Diamond Hydride™** with sharp, symmetrical peaks even from fully aqueous injections.
- Use ANP-consistent gradients (high %B start), adjust injection volume, and include clean-out holds as needed for robust performance over complex matrices.

[Click HERE for Cogent Diamond Hydride HPLC Column Ordering Information.](#)



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