

## Better Methods Than HILIC for Polar Compound Analysis in HPLC and LC-MS - Tips & Suggestions

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### Improving Polar Compound Analysis: ANP vs. HILIC for LC and LC-MS

Over the years, analytical scientists have faced persistent challenges when developing HPLC and LC-MS methods for highly polar compounds. While Hydrophilic Interaction Liquid Chromatography (HILIC) has been a go-to technique, many chromatographers report significant drawbacks—especially when using gradient methods.

#### Common HILIC Challenges

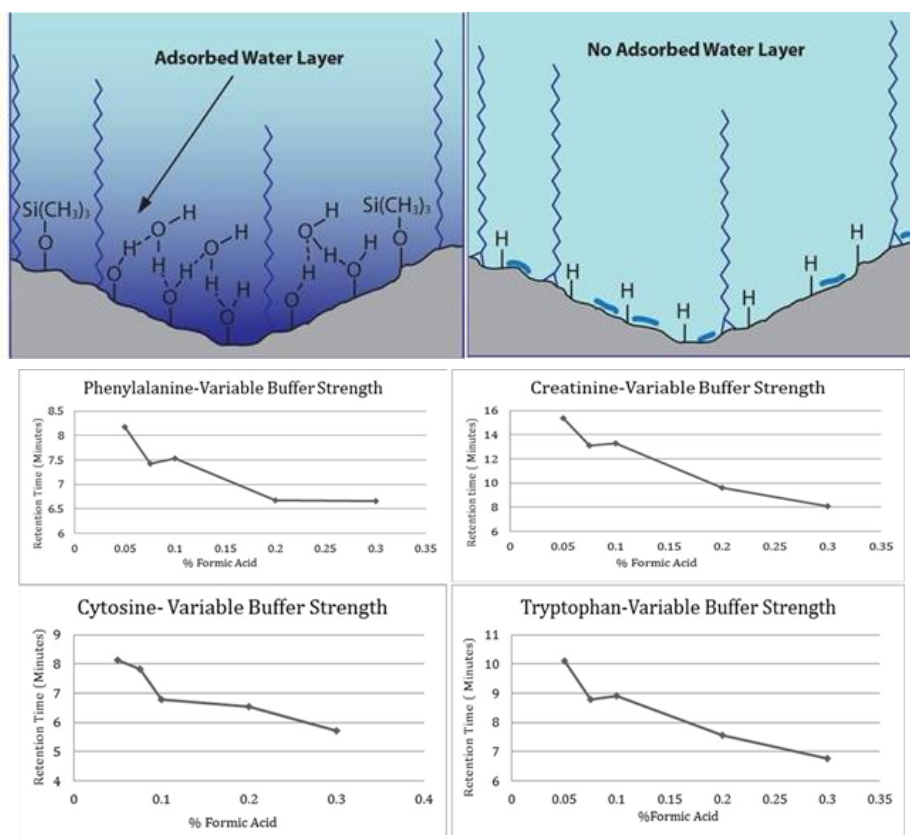
- **Slow Equilibration:** HILIC columns, like conventional silica-based phases, require extended equilibration due to the persistent water layer (hydration shell) on the silica surface. This leads to increased downtime between runs.
- **Reproducibility Issues:** The hydration shell is sensitive to environmental changes (e.g., temperature), often resulting in poor precision and retention time variability.
- **Column Instability:** HILIC columns may degrade unpredictably, particularly during overnight sequences.
- **High Salt Requirements:** Retention often depends on mobile phases with salt concentrations up to 100 mM—problematic for LC-MS systems and preparative workflows due to ion suppression and fouling.

#### Aqueous Normal Phase (ANP): A Smarter Alternative

ANP chromatography, particularly with **Cogent TYPE-C™ silica hydride columns**, offers a robust solution. Like HILIC, ANP retains polar compounds using high-organic mobile phases and inverse gradients. However, the underlying chemistry is fundamentally different:

- **No Hydration Shell:** TYPE-C silica lacks the persistent water layer, enabling faster equilibration (typically 3–5 column volumes).
- **Minimal Silanol Activity:** With <5% residual silanols and direct Si–C ligand bonding, ANP columns avoid the secondary interactions that plague HILIC.
- **Low Salt Operation:** ANP methods typically require ≤15 mM salt, eliminating the need for buffers and reducing LC-MS contamination risk.

**All standard silica depicted on the left and TYPE-C Silica below on the right. No hydration shell.**



**Effect of Salt Concentration on Retention in ANP Methods shown above in the graphs.**

### Key Benefits of ANP over HILIC

- **Rapid Equilibration** → Higher throughput and reduced solvent use.
- **Exceptional Precision** → Reliable retention times across runs and days.
- **Extended Column Lifetime** → Fewer failures and lower replacement costs.
- **LC-MS Compatibility** → Low salt minimizes ion suppression and system fouling.
- **Versatile Selectivity** → Retains both polar and some non-polar analytes.
- **Simplified Method Development** → Broad selectivity range and reproducibility.

### Technical Highlights

1. **Surface Chemistry:** Cogent TYPE-C columns exhibit <1 monolayer of water vs. 3–12 in HILIC, improving surface consistency.
2. **Fast Re-equilibration:** 3–5 column volumes vs. significantly longer for HILIC.
3. **Stable Surface Charge:** Silica hydride surfaces are negatively charged due to surface hydroxide ions.
4. **Dual-Mode Capability:** TYPE-C columns can operate in both ANP and reversed-phase modes.
5. **Low Additive Requirements:** Most polar analytes can be retained with  $\leq 15$  mM salt—no buffers needed.

For further reading, see:

**Kulsing et al., "Insights into the Origin of the Separation Selectivity with Silica Hydride Adsorbents," J. Phys. Chem. B, 2015, 119, 3063–3069.**

**Notes:**

1. *Less than one Monolayer of Water on Cogent TYPE-C columns vs. 3-12 layers on HILIC materials leading to less variability in surface composition and hence greater Precision in analyte retention.*
2. *Rapid Equilibration of the stationary phase after gradients, typically in the range of 3-5 column volumes for Cogent TYPE-C Columns.*
3. *Negatively Charged Surface on Silica Hydride due to free, surface Hydroxide ions.*
4. *Difference in Selectivity and Enhanced Retention in ANP.*
5. *Ability of Cogent TYPE-C Columns to Function in Reversed-Phase HPLC Mode.*
6. *Excellent run to run and column to column Reproducibility with Cogent TYPE-C Columns.*
7. *No need for high Concentrations of Additives in the Mobile Phase; only 15mM or less required for most Hydrophilic Compound Analyses in ANP.*

See also: [Main Differences between HILIC & Cogent TYPE-C Columns...](#)

See also: [Why ANP HPLC Methods are Better to use than HILIC.](#)

See also: [Efficiency of ANP v. HILIC Columns](#)

Reference below for details on this topic published in J. Phys. Chem. B.:

C. Kulsing, Y. Nolvachai, P.J.	Insights into the Origin of the Separation Selectivity with Silica	J. Phys. Chem. B.	2015	119	3063-3069
Marriott, R.I. Boysen, M.T.	Hydride Adsorbents				
Matyska, J.J. Pesek, M.T.W. Hearn					



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