Improving peak shapes in an HPLC method in Aqueous Normal Phase ANP - Tips & Suggestions

Date: 14-APRIL-2020 Lat Updated: 18-JUNE-2025

Achieving sharp, symmetrical peaks in ANP HPLC methods can be challenging, especially with complex analytes or sensitive compounds. Here are some expert tips to help you optimize your method and get the most out of your Cogent TYPE-C™ silica hydride columns:

---

1. Optimize Sample Solvent Strength Relative to the Mobile Phase

- For best results, ensure that your sample solvent is stronger than the initial mobile phase composition, particularly at the start of a gradient. This promotes efficient analyte focusing at the head of the column and minimizes peak broadening.
- Cogent TYPE-C™ columns are inherently more resistant to peak distortion compared to traditional HILIC phases, thanks to their unique silica hydride surface. This allows for greater flexibility in solvent selection and improved peak shapes, even for polar compounds.

---

2. Address Metal-Sensitive Compounds Proactively

- Compounds such as polyprotic acids, nucleotides, and phosphate-containing species can interact with trace metal ions in the HPLC system, leading to peak tailing or signal suppression.
- To mitigate this:
  - Use metal-free coated stainless steel columns to eliminate unwanted metal interactions.
  - If using phosphoric acid (PA) as a mobile phase additive, dedicate the column to that method. PA can irreversibly modify the column surface, affecting performance in other applications.
  - Consider a ***metal ion passivation protocol*** for your system if persistent issues arise.

---

3. Control Injection Volume and Sample Concentration

- Overloading the column with excessive injection volume or highly concentrated samples can lead to distorted peaks and poor reproducibility.
- Always optimize injection parameters based on column dimensions and analyte properties. For guidance, refer to our detailed resource on the effects of injection volume Click ***HERE***.

Additional Suggestions:

- Gradient Design: Tailor your gradient to the retention characteristics of your analytes. A shallow gradient may improve resolution for closely eluting peaks.
- Column Equilibration: Ensure adequate equilibration between runs, especially when using high aqueous content in the mobile phase.

- pH Considerations: While TYPE-C™ columns are stable across a wide pH range, adjusting pH can significantly impact analyte ionization and retention.

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