Selectivity Modification in an ANP Method Using Different Mobile Phase pH - Tips & Suggestions

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Optimizing Selectivity in Aqueous Normal Phase (ANP) HPLC

One of the most effective and straightforward ways to modify selectivity in ANP is by adjusting the mobile phase pH.

Why pH Matters

The ionization state of both the analytes and the stationary phase plays a critical role in ANP retention. Changing the pH can significantly impact chromatographic behavior.

Key Considerations

- Use mobile phase additives that are compatible with your stationary phase and system.
    - Avoid phosphates in LC-MS applications.
- Common additives include:
    - 0.1% formic acid or acetic acid
    - 10 mM ammonium acetate or ammonium formate
- Refer to the Cogent™ column specifications for allowable pH ranges, especially for Diamond Hydride™ columns.

Solvent Choice

The organic component in ANP methods also influences retention. For example:

- Acetone vs. Acetonitrile can produce markedly different retention profiles.
- Combining both solvents may help optimize selectivity:
- A peak pair that co-elutes with acetone may separate with acetonitrile—and vice versa.
- A mixed solvent approach can sometimes achieve complete resolution of all peaks.

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