

HILIC

Method Development Guidelines

 **WARNING**
Read Before Use



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HILIC

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Tips for Use

This is not Reversed Phase

In HILIC the strong solvent is the more polar solvent (water). To elute polar compounds the polarity of the mobile phase is INCREASED by increasing the % water.

Check Before You Inject

Your sample injection should have the same composition as your mobile phase.

Not All HILIC Columns are Identical

Selectivity will differ between HILIC phases. Different HILIC columns should be treated as complementary selectivities.

Before you Begin

Equilibration Considerations

Luna[®] HILIC and Kinetex[™] HILIC columns are shipped in HILIC mobile phase (90 % acetonitrile/10 % 100 mM ammonium formate, pH 3.2) and can be used immediately after equilibration with your HILIC mobile phase. For best results we highly recommend that you **equilibrate your HILIC column with at least 20 column volumes** of your mobile phase prior to analyzing samples. In addition, maintaining at least 5 % water in your mobile phase will greatly reduce the column equilibration time and thus ensure stable retention times. If gradient elution is used, then care should be taken to ensure complete column equilibration between injections – failure to do so will result in retention time drift and poor reproducibility. **For best reproducibility**, we recommend that you inject a blank sample at the beginning of each sample set. Additionally when using an autosampler, be sure to incorporate a needle wash step with acetonitrile.

HILIC Optimization Techniques

Adjusting Initial % Organic Modifier

Increasing or decreasing the organic modifier in 5 % increments can have a substantial impact on retention. Do not use 100 % organic conditions.

Adjusting Ionic Strength

For maximum reproducibility and ruggedness maintain an effective buffer concentration of at least 5 mM (5 mM in the mixed mobile phase). Adjusting buffer concentration, and the ionic strength, can alter selectivity, retention, and efficiency.

Adjusting Mobile Phase pH

Kinetex[™] HILIC is pH stable from 1.5 – 7.5 and Luna[®] HILIC is pH stable from 1.5 - 8, allowing experimentation with different pH values. Depending on the pK_a values of the analytes, pH manipulation can greatly affect analyte ionization and thus dramatically change retention and selectivity.

For complete details on how to adjust operating parameters and maximize performance, please contact your Phenomenex Technical Consultant or email support@phenomenex.com.

Luna is a registered trademark and Kinetex is a trademark of Phenomenex, Inc.



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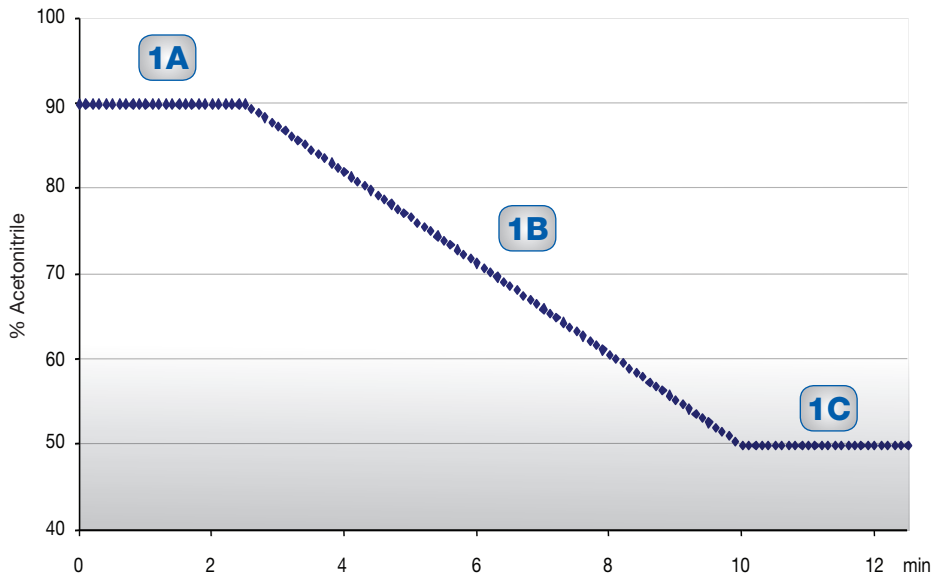
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General HILIC Method Development Guidelines

1. Use gradient conditions to determine compound applicability to HILIC

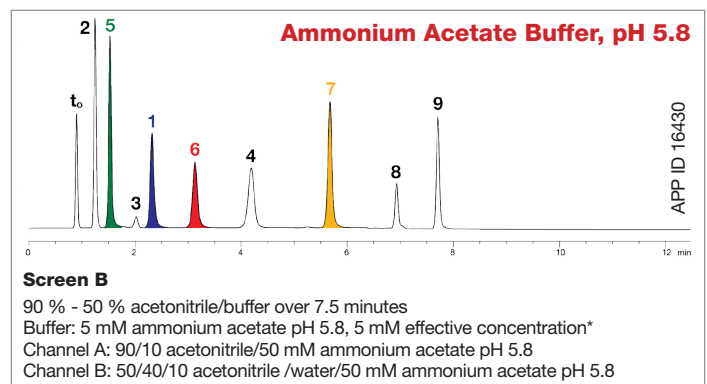
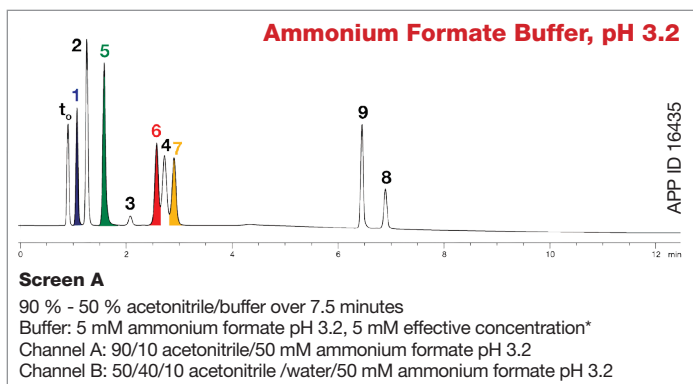
The generic gradient shown below is the recommended starting point for method development.



- 1A** Hold 90 % acetonitrile isocratically for 2.5 min – this period is used to determine if a compound is weakly retained in HILIC mode.
- 1B** The gradient portion of the elution profile spans 7.5 min. This period is the ideal elution region since gradient slope, starting percent organic, and ending percent organic can all be adjusted to maximize selectivity.
- 1C** Hold 50 % acetonitrile isocratically for the last 2.5 min - this period is used to determine if a compound is strongly retained in HILIC mode.

2. Screen your compounds at two different pH levels

Mobile phase pH has a far greater impact on retention and selectivity in HILIC than in reversed phase separations. The conditions below were chosen to maximize selectivity over a broad range of analytes with varying pK_a values.



*Maintaining Effective Buffer Concentration

Effective concentration of buffer is the buffer concentration in the mixed mobile phase. In the Screen A example above 5 mM ammonium formate in 90 v/v % acetonitrile is prepared by adding 100 mL 50 mM ammonium formate pH 3.2 aqueous buffer to 900 mL acetonitrile and mixing. No adjustment for volume contraction is necessary. Having 5 mM buffer in each mobile phase reservoir ensures the mobile phase ionic strength is maintained constant during the gradient giving a more robust method. Constant buffer concentration can be accomplished using a quaternary pumping system without pre-mixing mobile phases by setting the buffer channel to a constant value, e.g. 10 % 50 mM buffer.

3. Use Ammonium as the cation in buffers

The lack of an appropriate cation may lead to irreproducible HILIC results. Phenomenex recommends the use of ammonium as an effective counter ion, as shown in the examples below.

