

Getting Started with ZIC[®]-HILIC

Sample Solvent and Solvent Strength

Sample solvents should consist of 60-100% organic solvent, or initial eluent composition. Water should be minimized. Weak HILIC solvents such as acetonitrile are favoured. It is recommended to have about 5% water in the auto sampler wash solution.

The relative solvent strength for HILIC is:

Acetone < Acetonitrile < Isopropanol < Ethanol < Methanol < Water

Typical Elution Protocols

Isocratic elution: 80:20 (v/v) acetonitrile / NH₄Ac, (5-20 mM) or other suitable buffer salt.

Gradient elution: 90% to 40% acetonitrile in 20 minutes (~2.5%/min).

Flow-rate and Injection Volume

The optimal flow-rate and expected backpressure can be seen in the figure and table below. The recommended injection volume is also listed in the table.

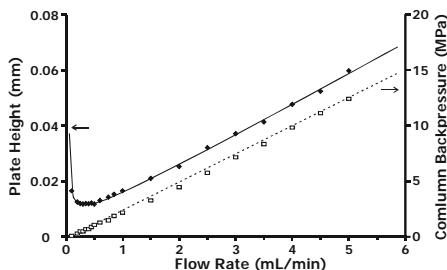


Figure: Column plate height (◆) and backpressure (□) vs. volumetric flow rate. Cytosine injected on a 50 x 4.6 mm ID column at k' 1.3 using an eluent with 80:20 acetonitrile/buffer

Table: Flow-rate, backpressure and injection volume

| Column I.D. (mm) | Injection volume (μL) | Flow-rate (mL/min) | Backpressure | |
|------------------|-----------------------|--------------------|----------------|-----------|
| | | | Expected (MPa) | Max (MPa) |
| 7.5 | 10-150 | 1.3 | 1-5 | 20 |
| 4.6 | 5-50 | 0.5 | 1-5 | 20 |
| 2.1 | 0.5-5 | 0.1 | 1-5 | 20 |

Mobile Phase Considerations

To obtain reproducible results, maintain at least 3% water in the mobile phase, in order to ensure sufficient hydration of the stationary phase particles.

Buffer Recommendations

Suitable buffer systems for HILIC separations are formate and acetate, due to their excellent solubility even in very high concentrations of organic solvent. Avoid phosphate, and other low solubility buffers, to prevent precipitation on the column bed. A buffer concentration in the range 5-20 mM is recommended for most analytes, with an upper limit of 200-300 mM, depending on the solubility in the eluent. TFA and other ion pair reagents should be avoided, as they can interfere with the HILIC separation mechanism, and suppress MS signals.

Suitable pH range: 3-8.

Upper Temperature limit: 70°C.

Column Regeneration and Storage

If the backpressure increases or a shift in selectivity is observed, use the following washing protocol to regenerate the column.

1. 30 column volumes of deionized water
2. 30 column volumes of 0.5 M NaCl
3. 30 column volumes of deionized water

Store columns as shipped:

Acetonitrile / NH₄Ac 5 mM, pH 6.8; 80:20 (v/v)

Support

If you have further questions that cannot be solved using this guide or the supplied *Instructions for Use*, please visit our website or contact our support team.

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