

Separating Hydrophilic and Hydrophobic Compounds in the Same HILIC Method - Tech Information

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Introduction

Developing a chromatographic method for samples containing both hydrophilic and hydrophobic compounds can be challenging. Traditional column selection often forces chromatographers to choose between:

- Reversed phase chromatography for non-polar compounds
- HILIC chromatography for polar compounds

As a result, compounds at one end of the polarity spectrum may be inadequately retained while compounds at the opposite end may be retained excessively.

Cogent™ TYPE-C™ silica hydride columns provide a unique advantage because they can operate effectively under both HILIC and reversed phase conditions, allowing method developers to explore a broader range of selectivity options during method optimization.

Samples Containing Mixed Polarity Compounds

Many real-world samples contain compounds with significantly different chemical properties.

Examples include:

- Pharmaceutical formulations
- Biological samples
- Metabolomics extracts
- Food and beverage products
- Environmental samples
- Chemical reaction mixtures

These samples may contain:

- Highly polar compounds
- Moderately polar compounds
- Hydrophobic compounds

Selecting the optimal chromatographic approach often requires evaluating more than one retention mode.

Start Method Development with Two Screening Gradients

When working with unknown mixtures or samples containing a broad polarity range, a practical approach is to evaluate both HILIC and reversed phase conditions early in method development.

Reversed Phase Screening Gradient

A useful reversed phase screening method may begin with:

- High aqueous content
- Lower organic content

and progress toward increased organic solvent concentration during the run.

This approach is often effective for:

- Hydrophobic compounds
- Moderately retained analytes
- Traditional reversed phase applications

HILIC Screening Gradient

A complementary HILIC screening method can be evaluated using:

- High organic content at the start of the run
- Gradual increase in aqueous content during the gradient

This approach is often effective for:

- Polar compounds
- Highly hydrophilic analytes
- Metabolites
- Ionic species
- LC-MS applications

Compare the Two Chromatograms

After running both screening methods, compare:

- Retention
- Resolution
- Peak shape
- Selectivity
- Analysis time

This comparison usually reveals which retention mechanism is most appropriate for the sample.

In many cases, one approach clearly provides:

- Better retention
- Better peak spacing
- Improved resolution
- More efficient separations

The preferred separation mode can then be optimized further.

Refining the Method

Once the preferred chromatographic direction has been identified, additional optimization can be performed using adjustments to:

- Gradient slope
- Initial mobile phase composition
- Final mobile phase composition
- Flow rate
- Temperature
- Mobile phase additives

These changes can be used to improve:

- Resolution
- Retention
- Sensitivity
- Throughput

Developing Isocratic Methods

An additional benefit of the dual-screening approach is that it often provides information useful for developing isocratic methods.

The gradient results can help determine:

- Appropriate solvent strength
- Approximate retention windows
- Ideal starting conditions

This information can significantly reduce method-development time.

Benefits of the TYPE-C™ Platform

Because TYPE-C™ columns support both HILIC and reversed phase applications, method developers can evaluate multiple chromatographic strategies without switching to a completely different column technology.

Benefits include:

- Expanded selectivity options
- Simplified method development
- Improved flexibility
- Better retention of challenging analytes
- Broad application range
- Efficient screening of unknown samples

This versatility is particularly valuable when compound polarity is uncertain or when samples contain a wide range of chemical classes.

Practical Method Development Strategy

Begin with Reversed Phase Conditions

- Evaluate retention of hydrophobic and moderately polar analytes.

Evaluate HILIC Conditions

- Assess retention of highly polar compounds.

Compare Results

- Determine which mode provides the most useful selectivity and resolution.

Optimize the Preferred Method

- Fine-tune conditions to achieve the desired chromatographic performance.

Consider Isocratic Operation

- Use gradient screening results to establish efficient isocratic methods when appropriate.

Key Takeaways

- Samples containing both hydrophilic and hydrophobic compounds can be challenging to separate using a single chromatographic strategy.
- Cogent™ TYPE-C™ columns support both HILIC and reversed phase operation.
- Initial screening using both reversed phase and HILIC gradients can quickly identify the most effective retention mode.
- Gradient screening results can guide subsequent optimization and isocratic method development.
- The flexibility of the TYPE-C™ platform provides expanded selectivity options for complex mixtures.
- Evaluating both retention modes often results in faster and more efficient method development.

