New! Hydrophilic Interaction Chromatography

An Alternative to RPC for Polar Molecules

Evaluate HILIC for yourself

A novel, non-ion exchange method to increase retention

If you are trying to increase retention of hydrophilic molecules with RPC, there is a versatile, effective alternative to consider: hydrophilic interaction chromatography (HILIC). A rival technique to RPC for separating polar peptides, HILIC is easy to use and works best where RPC works worst – with polar solutes which aren't retained well on RPC. HILIC has been used successfully with:

- Phosphopeptides
- Crude extracts
- Peptide digests
- Carbohydrates
- Histones
- Polar lipids

as well as preparative applications where changing the order of elution affects isolation yields and detergent removal.[1]

How HILIC works

HILIC separates compounds by eluting a hydrophobic or mostly organic mobile phase across a neutral hydrophilic stationary phase, causing solutes to elute in order of increasing hydrophilicity – the inverse of RPC. The technique permits direct LC-MS analysis. For glycopeptides, ammonium formate and reverse organic conditions may be used. Highly charged molecules require salt for ion suppression, and a slight perchlorate or sulfate gradient (in a high organic solvent concentration) effects desorption.

Choice of two columns

- PolyHYDROXYETHYL Aspartamide™ column performs either hydrophilic or small-molecule size exclusion separations[2] under non-HILIC conditions.
- PolySULFOETHYL Aspartamide™ SCX column performs either hydrophilic interaction superimposed upon electrostatic effects, or a cation exchange mixed-mode separation[3] where resolution is enhanced for peptides with the same net positive charge under non-HILIC conditions.

Call for details

For additional technical information, part numbers or prices, contact The Nest Group today at (800) 347-NEST (6378).

If you prefer, for faster response, photocopy and fax the attached card, use our E-Mail address: nestgrp@world.std.com, or visit our website at http://world.std.com/~nestgrp.

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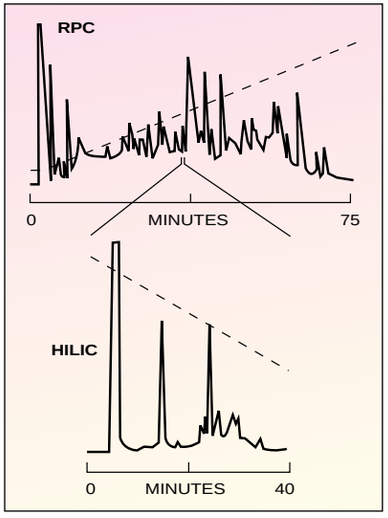
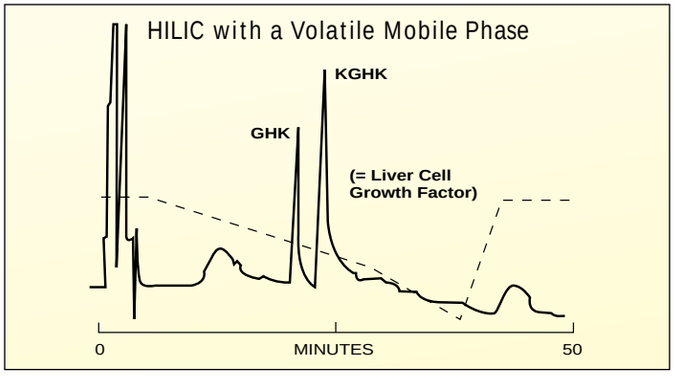


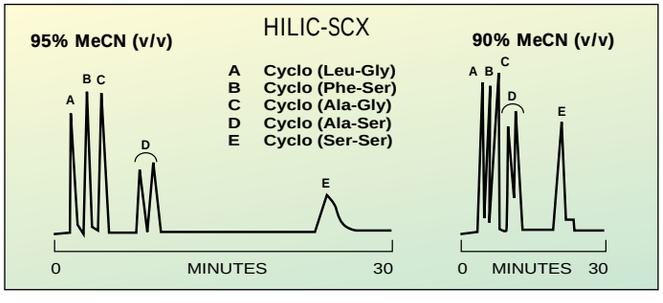
Glycopeptide Isolation by RPC and HILIC

HILIC Conditions
Eluent A: 15mM ammonium formate
80% MeCN
Eluent B: 15mM ammonium formate
30% MeCN

Sequential runs by RPC and HILIC allow analysis and determination of glycosylation sites. Under HILIC conditions at pH 6.5 glycopeptides elute later than peptides.



Tryptic digest of 20-mer peptide from SPARC protein using PolyHYDROXYETHYL Aspartamide column. Mobile Phase: 15mM ammonium formate, pH 2.7. Courtesy Tim Lane (U. of Washington)



Cyclopeptides separated by HILIC on a PolySULFOETHYL A 4.6 x 200 mm, 5 µm column. Mobile Phase: 15mM TEA-PO_4 pH 2.8 with MeCN as noted. Alternatively, run a gradient to 0.3M $NaClO_4$ rather than decrease the organic.

I'm interested in:

❑ HILIC/SEC ❑ HILIC/SCX ❑ Vydac 201HS (C18)

For:

❑ Detergent Removal
❑ Glycopeptides
❑ Histones
❑ Phosphorylated Molecules
❑ Small Hydrophilic Solutes
❑ Carbohydrates

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1 Jeno, P., Scherer, P.E., Manning-Krieg, U., and Horst, M, "Desalting Electroeluted Proteins with Hydrophilic Interaction Chromatography." Analytical Biochemistry, 215, 292-298 (1993).

2 Alpert, A.J., "Hydrophilic Interaction Chromatography (HILIC): A New Method for Separation of Peptides, Nucleic Acids and Other Polar Solutes." J. Chromatogr. 499, 177-196 (1990).

3 Zhu, B., Mant, C. and Hodges, R., "Mixed-Mode Hydrophilic and Ionic Interaction Chromatography Rivals Reverse-Phase Chromatography for the Separation of Peptides." J. Chromatogr. 594, 75-86 (1992).