



HALO[®] HILIC
PRODUCT BULLETIN



hydrophilic interaction liquid chromatography

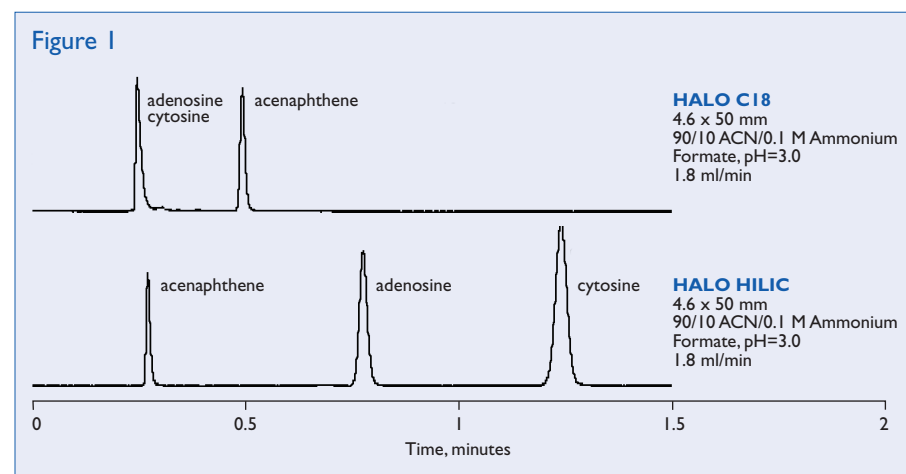


HALO HILIC

- Separate highly polar compounds that are poorly retained on reversed-phase columns
- Complementary selectivity to reversed-phase
- Increase LC/MS sensitivity

HILIC, or hydrophilic interaction liquid chromatography, is especially suitable for separating polar compounds. HALO HILIC columns can be particularly useful for separating acidic and basic compounds that are either not retained or poorly retained on reversed-phase columns. In addition, the complementary selectivity of HILIC may provide a better separation than that achieved by reversed-phase. Because typical mobile phases used with HILIC are composed of a large fraction of volatile organic solvent, such as acetonitrile, HILIC separations can offer significant increases in sensitivity when interfaced to mass spectrometry.

Figure 1 illustrates the complementary selectivity of reversed-phase and hydrophilic interaction liquid chromatography (HILIC).



Retention Mechanism

Retention in HILIC is not well understood but appears to be a combination of hydrophilic interaction, ion-exchange and some reversed-phase retention. The aqueous layer which forms on the polar surface of HILIC particles promotes interaction with polar solutes. Retention in HILIC as a function of the mobile phase is just opposite from that in reversed-phase. The strongest mobile phase has a high percentage of water and the weakest has a high percentage of organic solvent. For gradient separations, the initial mobile phase has a high percentage of organic solvent and the gradient is formed by increasing percentage of the aqueous component. The greatest retention for basic and acidic analytes is found when using more than about 70% organic (e.g., acetonitrile) in acidic mobile phases. Since high organic concentrations are used in the

mobile phases, HILIC is especially favorable for separations using mass spectrometry (MS) detection.

Due to the highly polar mobile phases used in HILIC, both acidic and basic compounds often exhibit highly symmetrical peak shapes, often superior to those obtained in reversed-phase. In addition, sample loading effects often are more favorable for HILIC. When optimized, HALO HILIC columns show efficiency that is competitive with results obtained with reversed-phase. Although increased column operating temperatures can improve efficiency and peak shape in reversed-phase chromatography, temperatures above 60 °C generally are not recommended with HILIC.

Mobile phase solvents

Acetonitrile is commonly used as the weak mobile phase component in HILIC separations. With this solvent, 95% is typically the upper limit and 60% the lower limit for adequate retention. At least 5% of the mobile phase should be a highly polar solvent such as water or methanol. If buffers are used, water is preferred for improved buffer solubility. As in reversed-phase, the organic solvent type can be varied to change retention and separation selectivity. Solvent strength (from weakest to strongest) for HILIC generally is tetrahydrofuran < acetone < acetonitrile < isopropanol < ethanol < methanol < water.

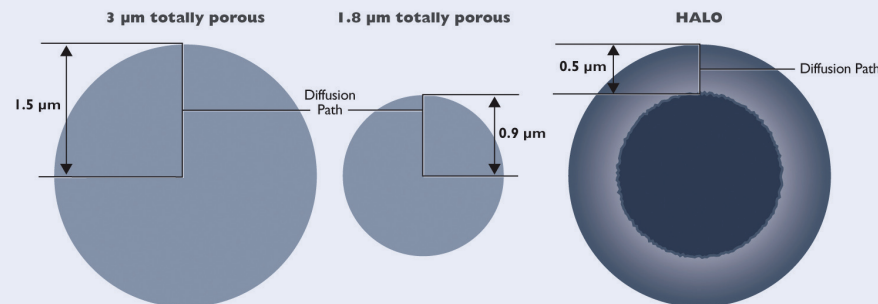
When using a gradient to scout for optimum mobile phase conditions, 90 - 95% acetonitrile is suggested as the initial solvent composition and 50 - 60% acetonitrile as the ending composition. The resulting elution characteristics can be used to estimate the appropriate mobile phase composition for isocratic elution in much the same way as for reversed-phase. To further increase retention in HILIC, replacing some of the water in the mobile phase with another polar solvent such as methanol or isopropanol is sometimes effective.

Mobile phase buffers

For optimum column efficiency and reproducibility, buffers in the range of 10 - 20 mM concentration or additives in the 0.5% range can be used in the mobile phase. Phosphate buffers are not recommended because of their poor solubility in high organic mobile phases and incompatibility with MS detection. Additives such as formic acid,

trifluoroacetic acid and phosphoric acid at concentrations up to about 1% can be used in the mobile phase. Volatile ammonium formate/formic acid buffers up to a final concentration of about 20 mM and pH 3 are especially effective for separating both basic and acidic compounds when interfacing the liquid chromatograph to a mass spectrometer. Acetonitrile/formate mobile phases seem to be a good starting point for many separations of both basic and acidic compounds. Ammonium acetate at pH ~5 has also been used at concentrations of 5 - 20 mM, but is generally less effective for separating stronger basic and acidic compounds. Buffers or additives above pH 6 usually are not recommended because they may enhance the slow dissolution of the silica support.

Figure 2 - The shorter diffusion path of HALO reduces axial dispersion



The shorter diffusion path of HALO particles reduces axial dispersion of solutes and minimizes peak broadening. Because of the shorter diffusion path, the performance advantages of HALO become even more apparent when separating larger solute molecules and operating at faster mobile phase flow rates.

Sample conditions

As with reversed-phase, the solvent used to inject the sample is an important consideration with HILIC. The sample solvent should, as closely as possible, resemble the strength and type of the mobile phase. The sample solvent can contain a higher amount of organic than the mobile phase, but if it contains a higher amount of polar solvent (e.g., water), peak shape will be compromised, especially with early-eluting compounds. A mixture of 75:25 (v/v) acetonitrile/methanol is sometimes useful as the sample solvent, if for some reason it is not possible to inject the sample dissolved in the mobile phase.

Very strong solvents, such as dimethylformamide or dimethylsulfoxide, will usually result in poor peak shapes and are not recommended. These solvents will generally have to be diluted with a weaker solvent, such as acetonitrile, before satisfactory peak shape can be obtained.

HALO HILIC Specifications

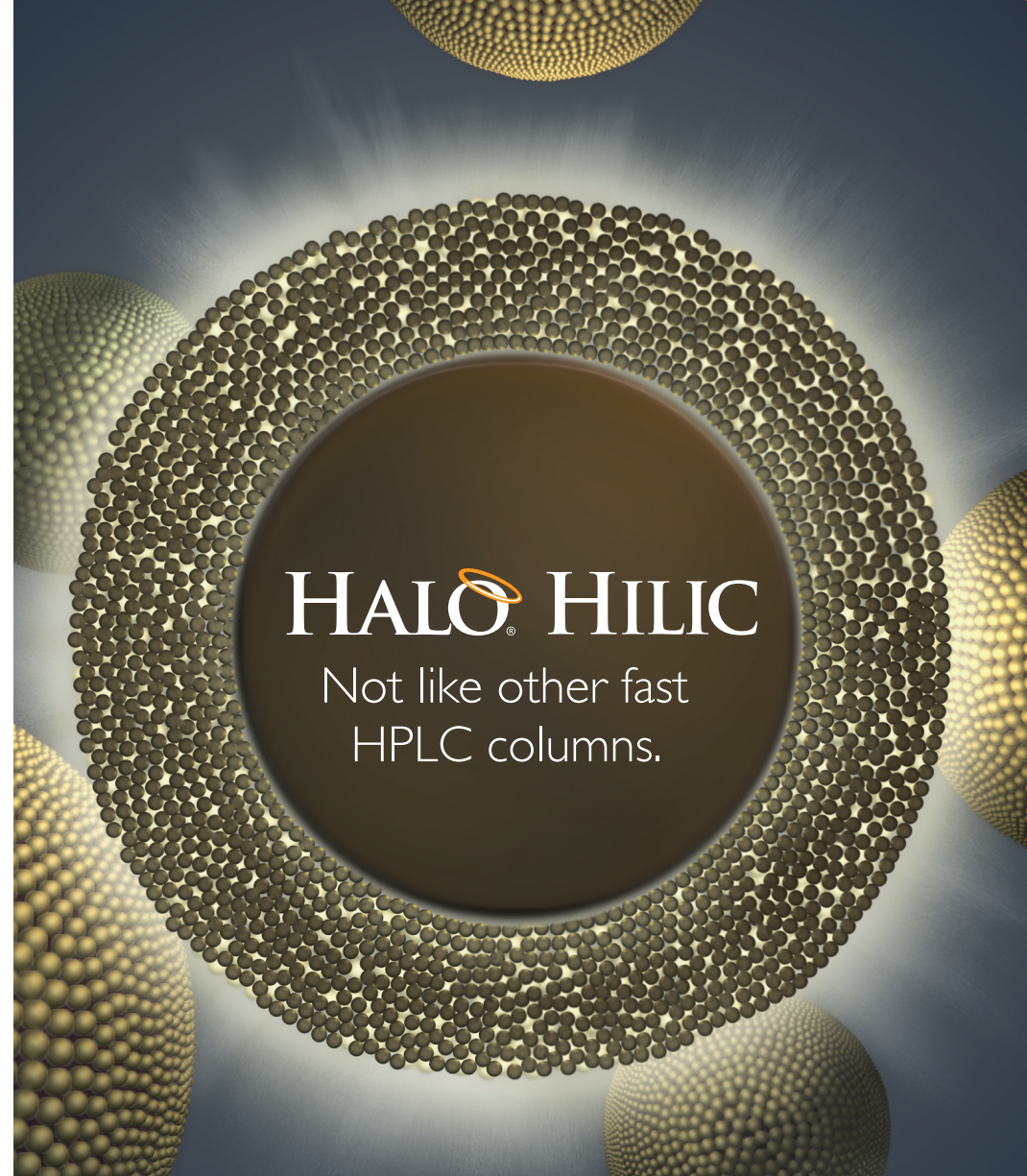
- Ultra-pure, “Type B” silica
- 1.7 μm solid core particle with a 0.5 μm porous silica layer fused to the surface
- 150 m^2/gram surface area
- 90 \AA pore size
- pH range: 2 to 9
- Maximum Pressure: 9,000 psi, 600 bar

HALO HILIC Ordering Information

Column Dimensions	Part Number
2.1x50 mm	92812401
2.1x100 mm	92812601
2.1x150 mm	92812701
4.6x50 mm	92814401
4.6x100 mm	92814601
4.6x150 mm	92814701

Useful References

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3. B. A. Bidlingmeyer, J. K. Del Rios, J. Korpi, *Anal. Chem.*, 54 (1982) 442-447.
4. E. S. Grumbach, D. M. Wagrowski-Diehl, J. R. Mazzeo, B. Alden, P. C. Iraneta, *LCGC*, 22 (2004) 1010-1023.
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