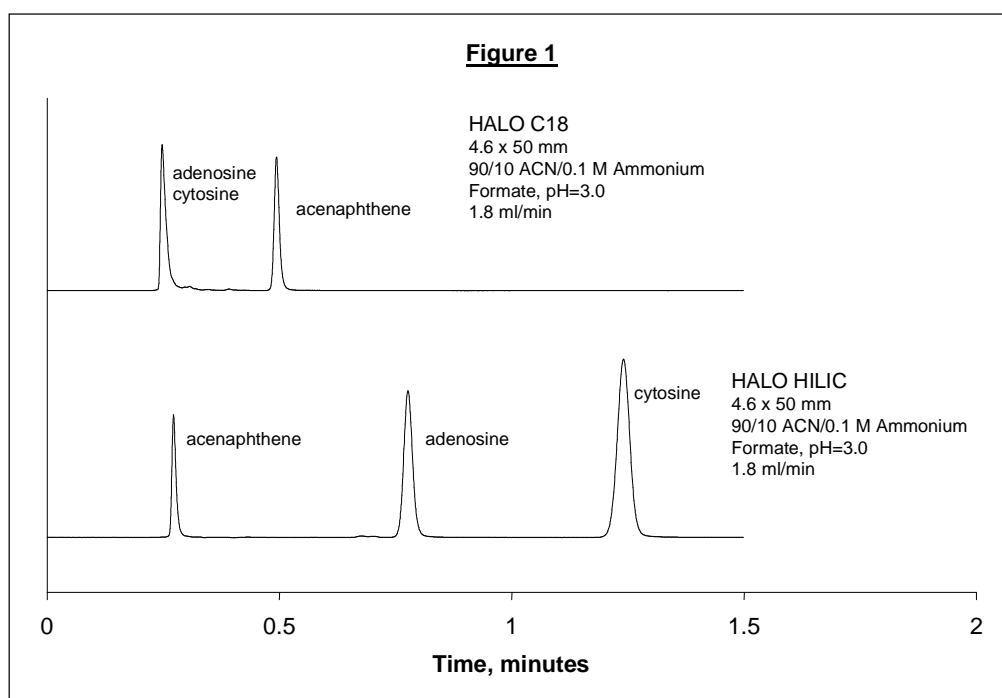


## HALO for Hydrophilic Interaction Liquid Chromatography (HILIC)

This brief summarizes information that might be useful for those interested in using HALO for separating highly polar (water soluble) compounds that are not well suited for reversed-phase chromatography (RPC). As shown in Figure 1, HILIC is a useful and complimentary method to RPC and is especially attractive in situations where compound retention is poor in RPC and very high levels of water are required in the mobile phase for adequate retention.



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 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 RPC. The strongest mobile phase has a high concentration of water and the weakest has a high concentration of organic solvent. Therefore, for gradient separations, the initial mobile phase has a high concentration of organic solvent and the gradient is formed by increasing the aqueous concentration. Greatest retention for basic and acidic analytes is found when using more than about 70% organic (e.g., acetonitrile) in acidic mobile phases. High organic concentrations are used in the mobile phases. Therefore, HILIC is especially favorable for separations using mass spectrometry (MS) detection.

Because of the highly polar mobile phases used for HILIC, both acidic and basic compounds often produce highly symmetrical peak shapes, often superior to that found for RPC. In addition, sample loading effects often are also more favorable for HILIC. When optimized, Halo columns show efficiency that is competitive with results in RPC operation. Column operating temperatures above 60°C generally are not recommended with HILIC.

### **Mobile phase solvents**

Acetonitrile is typically used as the weak organic solvent in the mobile phase. With this solvent, 95% is typically the upper limit and 60 - 65% the lower limit for adequate retention. At least 5% of the mobile phase should be the highly polar solvent such as water or methanol. Water should be the polar solvent if a buffer is included because of solubility limitations. The organic solvent type can be varied to change retention and separation selectivity, much as in RPC. Solvent strength (from weakest to strongest) for HILIC generally is tetrahydrofuran < acetone < acetonitrile < isopropanol < ethanol < methanol < water, where water is the strongest elution solvent.

For using gradients to scout a possible separation, 90 - 95% acetonitrile is suggested as the initial solvent, with a gradient to about 50 - 60%. The resulting elution characteristics can be used to estimate the proper strength mobile phase for isocratic elution in much the same way as for RPC. To further increase retention in HILIC, replacing some of the water in the mobile phase with another polar solvent such as methanol or isopropanol sometimes is 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 are 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 a part of 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 using MS detection. (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 also have been used at concentrations of 5 - 20 mM, but are generally less effective for separating stronger basic and acidic compounds. Buffers or additives above pH 6 usually are not recommended because of slow dissolution of the silica support.

## Sample conditions

As with RPC, the solvent used to inject the sample is an important feature of HILIC. This sample solvent should be as close as possible to the mobile phase strength and type used for separating the sample, just as in RPC. The dissolution solvent can contain a higher organic than the separating solvent, but if it contains a higher concentration 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 sometimes will be useful for sample dissolution, if the sample cannot be presented to the column in the operating mobile phase.

Samples dissolved in very strong solvents such as dimethylformamide or dimethylsulfoxide will usually result in very poor peak shapes and are not recommended. Such samples generally will have to be diluted with a weaker solvent such as acetonitrile before satisfactory peak shapes can be obtained.

## Useful references

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<b>Part Numbers</b>	<b>Description</b>
92812-401	2.1 x 50 mm HALO HILIC
92812-601	2.1 x 100 mm HALO HILIC
92812-701	2.1 x 150 mm HALO HILIC
92814-401	4.6 x 50 mm HALO HILIC
92814-601	4.6 x 100 mm HALO HILIC
92814-701	4.6 x 150 mm HALO HILIC

Other dimensions available by special order

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