

The advertisement features a dark blue background with several large, glowing spheres of varying sizes. Each sphere is composed of a dense grid of small, golden-brown particles, representing the chromatography columns. A central sphere is the largest and most prominent, with a dark brown circular area in its center containing the product name. The text is white and orange, with a registered trademark symbol. The overall aesthetic is scientific and high-tech.

HALO[®]
PENTA-HILIC
UHPLC COLUMNS

HALO®

PENTA-HILIC

- † Highly retentive, proprietary penta-hydroxy-ligand
- † Excellent peak shape for polar compounds with a variety of functional groups:
 - † acids, bases, zwitterions
 - † strong and weak acids, bases
 - † peptides, sugars, nucleoside bases, nucleosides, nucleotides, and derivatives
- † Compatible with all typical HILIC operating conditions
- † Fast equilibration of the column, especially useful for gradient separations
- † High stability under acidic and mildly basic conditions of operation
- † Very high efficiency packed columns (>200,000 plates per meter)
- † Utilizes Halo Fused-Core® particles with 2.7 µm particle diameter and 0.5 µm thick porous layer, for maximum performance at any flow rate
- † Available in a broad range of column dimensions for a variety of analytical applications
- † Moderate back pressure places less stress on instrument parts and permits these UHPLC columns to be used with conventional HPLC equipment.
- † The use of 2 µm porosity column inlet frits reduces the inconvenience caused by pressure increases from plugged frits and makes HALO columns more forgiving and easier to use than columns packed with sub-2 µm particles.

HALO Fused-Core particle technology facilitates ultra-fast, high resolution “UHPLC” separations with either UHPLC or conventional HPLC equipment. Now, HALO Penta-HILIC columns join HALO C18, C8, RP-Amide, ES-CN, Phenyl-Hexyl, PFP, Peptide ES-C18 and the standard silica HILIC phase to offer a wide range of powerful selectivity choices to accomplish the most demanding separations. HALO Penta-HILIC is particularly well suited for the separation of highly polar compounds that are poorly retained under typical reverse phase conditions. In addition, HALO Penta-HILIC columns are highly recommended for LC-MS application due to their compatibility with MS-friendly mobile phases and exhibit extremely low bleed.

TABLE 1: Features of the various chemistries that AMT has developed for HILIC:

Product	Features	Analyte Considerations	Mobile Phase Considerations
HALO HILIC	polar silanols	highest base retention with good peak shape, some anionic character	typical HILIC, higher ionic strength at neutral pH, avoid pH >8, highly stable low pH
Halo Penta-HILIC	polar C-OH	high acid, base, zwitterion retention, with good shape neutral character	typical HILIC, forgiving for ionic strength, avoid pH <2 at high temperature
Halo PFP	polar C-F	unique selectivity, high retention for acids, neutrals, some cationic character	typical HILIC, may require higher Ionic Strength, avoid pH >8, pH<2 at high temperature

The HALO Penta-HILIC column provides superior retention to silica HILIC and amide HILIC phases for acids and zwitterionic analytes (figure 2)

HALO Penta-HILIC uses proprietary bonding chemistry that includes five hydroxyl groups on the bonded ligand. This unique bonded phase provides a highly polar stationary phase on which to perform HILIC separations. The high bonding density and polarity of the bonded phase minimize ion-exchange effects with residual silanols, thereby improving peak shape and lot-to-lot reproducibility.

FIGURE 1: HALO Penta-HILIC penta-hydroxyl group

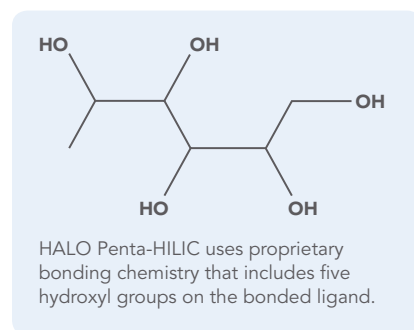
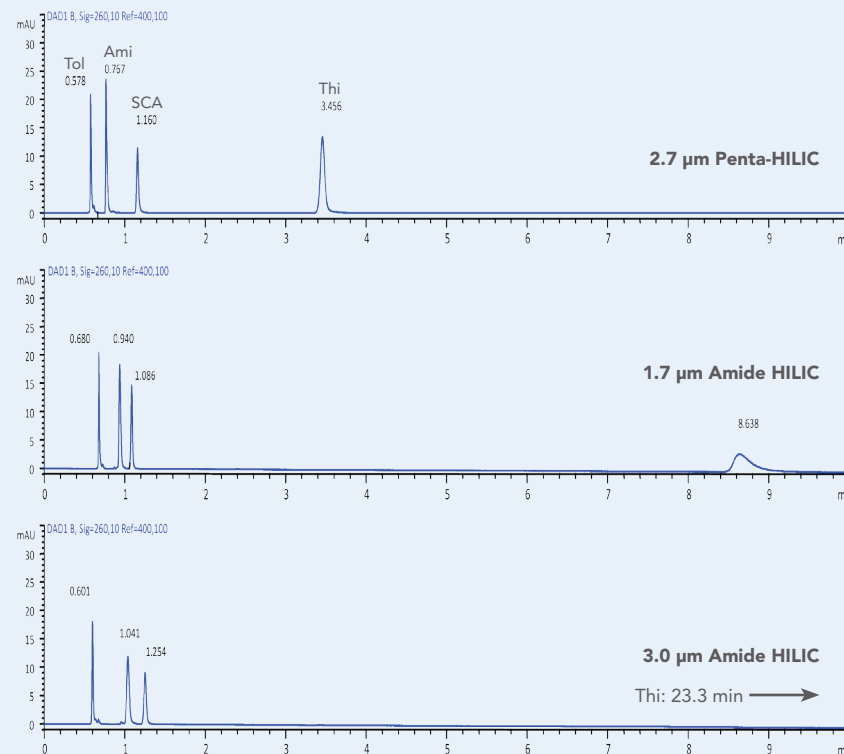


FIGURE 2: Comparative Retention of HILIC Columns

TEST CONDITIONS:

Column: 2.1 mm ID x 150 mm
 Test: 90% AcN/10 mM Ammonium Formate pH 3.0, 0.5 mL/min, 23 °C
 Toluene/Amitriptyline/Salicylic Acid/Thiamine



Mechanism of Separation

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 that forms on the polar surface of the Penta-HILIC particles promotes interaction with polar solutes. Retention in HILIC mode 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 Penta-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 mode, the organic solvent type can be varied to change retention and separation selectivity. Solvent strength (from weakest to strongest) for the Penta-HILIC phase 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 9 usually are not recommended because they may enhance the slow dissolution of the silica support.

Sample conditions

As with reversed phase, the solvent used to inject the sample is an important consideration with Penta-HILIC. As in reversed-phase, 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 for 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.

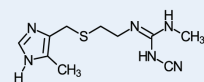
FIGURE 3: Separation of Antiulcer Drugs

TEST CONDITIONS:

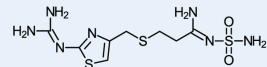
Column: 4.6 x 100 mm, HALO Penta-HILIC
Part Number: 92814-605
Mobile Phase: 10/90: A/B
A= 0.04 M Ammonium formate, pH=3.0 (adj.)
B= Acetonitrile
Flow Rate: 3.0 mL/min.
Pressure: 210 Bar
Temperature: 30° C
Detection: UV 254 nm, VWD
Injection Volume: 2 µL
Sample Solvent: mobile phase
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

PEAK IDENTITIES:

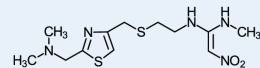
1. Cimetidine
2. Nizatidine
3. Famotidine
4. Ranitidine



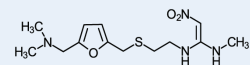
Cimetidine



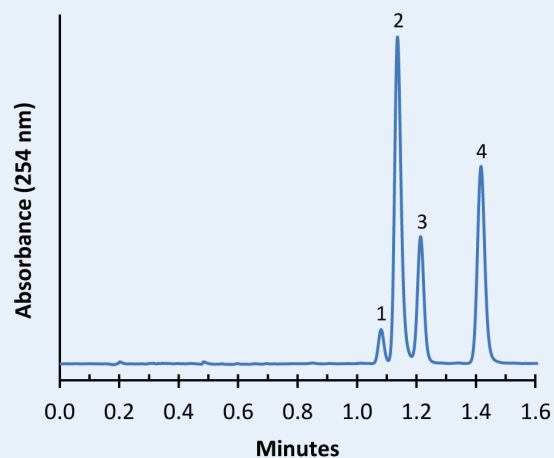
Famotidine



Nizatidine



Ranitidine



These strongly basic antiulcer drugs can be rapidly separated on HALO Penta-HILIC phase using a mobile phase that works well with a mass spectrometer detector.

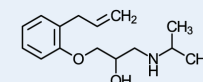
FIGURE 4: Separation of Beta Blocker Drugs

TEST CONDITIONS:

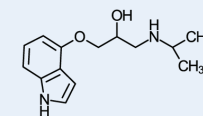
Column: 4.6 x 100 mm, HALO Penta-HILIC
Part Number: 92814-605
Mobile Phase: 10/90: A/B
A= 0.04 M Ammonium formate buffer, pH=3.0
B= Acetonitrile
Flow Rate: 3.0 mL/min.
Pressure: 215 Bar
Temperature: 30° C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: mobile phase
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

PEAK IDENTITIES:

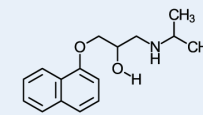
1. Alprenolol
2. Propranolol
3. Pindolol
4. Acebutolol
5. Atenolol



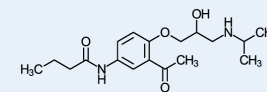
Alprenolol



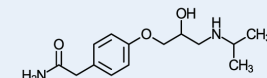
Propranolol



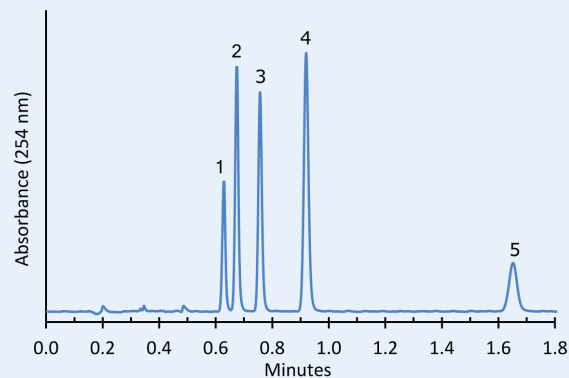
Pindolol



Acebutolol



Atenolol



High resolution separations of these highly basic compounds are easily obtained using HALO Penta-HILIC.

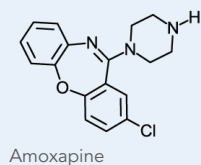
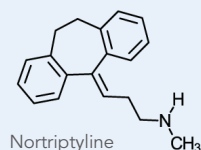
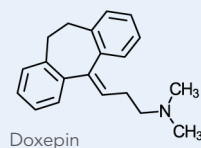
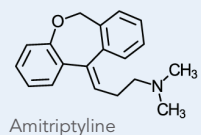
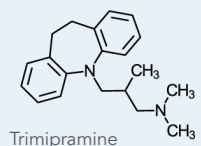
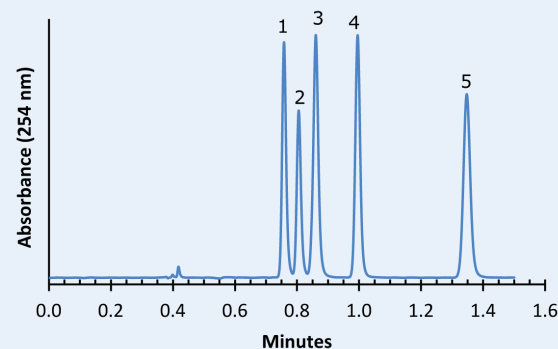
FIGURE 5: Separation of Antidepressants

TEST CONDITIONS:

Column: 4.6 x 100 mm, HALO Penta-HILIC
 Part Number: 92814-605
 Mobile Phase: 7/93: A/B
 A= 0.1 M Ammonium formate, pH=3.5 (adj.)
 B= Acetonitrile
 Flow Rate: 2.5 mL/min.
 Pressure: 165 Bar
 Temperature: 30°C
 Detection: UV 254 nm, WWD
 Injection Volume: 0.5 µL
 Sample Solvent: 10/90: Water/Acetonitrile
 Response Time: 0.02 sec.
 Flow Cell: 2.5 µL semi-micro
 LC System: Shimadzu Prominence UFLC XR
 ECV: ~14 µL

PEAK IDENTITIES:

1. Trimipramine
2. Amitriptyline
3. Doxepin
4. Nortriptyline
5. Amoxapine



Basic drugs such as antidepressants can be separated rapidly under HILIC conditions with good peak shape using HALO Penta-HILIC stationary phase.

HALO Penta-HILIC Specifications

- ♦ Hydroxylated-ligand (five hydroxyl groups)
- ♦ Ultra-pure, "Type B" silica
- ♦ 1.7 µm solid core particle with a 0.5 µm porous silica layer fused to the surface
- ♦ 150 m²/gram surface area
- ♦ 90 Å pore size
- ♦ pH range: 2 to 9
- ♦ Maximum Temperature: 60°C
- ♦ Maximum Pressure: 9,000 psi, 600 bar

HALO Penta-HILIC Ordering

HALO Penta-HILIC Columns

Column Dimensions	Part Number
2.1x20mm	92812205
2.1x30mm	92812305
2.1x50mm	92812405
2.1x75mm	92812505
2.1x100mm	92812605
2.1x150mm	92812705
3.0x20mm	92813205
3.0x30mm	92813305
3.0x50mm	92813405
3.0x75mm	92813505
3.0x100mm	92813605
3.0x150mm	92813705
4.6x20mm	92814205
4.6x30mm	92814305
4.6x50mm	92814405
4.6x75mm	92814505
4.6x100mm	92814605
4.6x150mm	92814705

HALO Penta-HILIC Guard Columns (3/pk)

Column Dimensions	Part Number
2.1mm ID	92812105
3.0mm ID	92813105
4.6mm ID	92814105
Guard Holder	94900001

HILIC references

1. A. J. Alpert, *J. Chromatogr.*, 499 (1990) 177-196.
2. P. Hemström, K. Irgun, *J. Sep. Science*, 29 (2006) 1784-1821.
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.
5. J. Pesek, M. T. Matyska, *LC-GC*, 25 (2007) 480-490.
6. D. V. McCalley, *J. Chromatogr. A*, 1171 (2007) 46-55.