• Alternate, complementary selectivity to C18 and C8 bonded phases

• Particularly recommended for compounds containing aromatic groups

• Excellent bonded phase stability for durable, long-lived performance plus minimum bleed for LC/MS applications

• Base deactivated for good peak shape when separating basic compounds

• Moderate back pressure allows for UHPLC-like performance with conventional HPLC equipment

• The use of 2 μm porosity column inlet frits reduces the inconvenience caused by pressure increases from plugged fits and makes HALO columns more forgiving and easier to use
HALO Phenyl-Hexyl UHPLC Columns

HALO Fused-Core® particle technology is used to produce UHPLC columns that deliver high speed, high resolution separations with UHPLC equipment, and can even deliver near-UHPLC performance with conventional HPLC equipment. HALO columns generate significantly less back pressure compared to other UHPLC columns, putting less stress on the system and facilitating rugged reliable performance. It is this moderate back pressure of HALO columns that permits them to be used with most conventional HPLC equipment. Furthermore, HALO columns utilize a column inlet frit with a porosity that is significantly larger than other UHPLC columns (2 μm versus 0.5 μm). This larger porosity column inlet frit reduces a problem that plagues UHPLC columns, inlet frit plugging. In fact the inlet frit on HALO columns is no smaller than that typically used on columns packed with 5 μm particles. Imagine that. A UHPLC column with the ease of use and reliability of a column packed with 5 μm particles.

HALO Fused-Core particles are now available with a phenyl-hexyl bonded phase. The phenyl functionality of this bonded phase provides an additional mechanism of separation that can be particularly useful when separating mixtures that contain compounds with aromatic groups. HALO Phenyl-Hexyl columns are an excellent choice to try when a C18 or C8 phase fails to provide an adequate separation.

As with the HALO C18, C8, and RP-Amide phases, ultra-pure reagents, “Type B” silica, dense bonding technology, and exhaustive endcapping generate a base deactivated stationary phase that provides excellent peak shape for polar compounds.

Mechanism of Separation

HALO Phenyl-Hexyl can retain analytes via several different mechanisms, including π–π interactions between the overlap of the delocalized electrons on the analyte and the stationary phase phenyl group, and via partitioning between the mobile phase and the hydrophobic aryl-alkyl phase. See Figure 1 for the structure of the HALO Phenyl-Hexyl bonded phase.

**FIGURE 1:** Structure of bonded phase of HALO Phenyl-Hexyl

```
Silica Stationary Phase Support  Si — O — Si — C₆H₁₂ — Phenyl
```

A phenyl group is bonded to the silica surface via a hexyl chain.
Acetonitrile tends to decrease the π–π interactions between aromatic and polarizable analytes and a phenyl stationary phases, but methanol enhances those same interactions, giving both increased retention and changes in selectivity. This does not mean that acetonitrile should not be used with a phenyl bonded phase or that it might not provide an acceptable separation, but methanol is more likely to deliver the additional selectivity that is desired from a phenyl phase. Figures 2 and 3 provide examples of the type of high speed, high resolution separations possible with HALO Phenyl-Hexyl.

**FIGURE 2:** Fast Separation of Aromatic Compounds

**Peak Identities:**
1. uracil
2. benzyl alcohol
3. cinnamyl alcohol
4. 4'-bromoacetanilide
5. nitrobenzene
6. butyl paraben
7. chlorobenzene
8. ethylbenzene
9. naphthalene
10. hexanophenone
11. biphenyl

**Test Conditions:**
- **Column:** 4.6 x 50 mm HALO Phenyl-Hexyl
- **Mobile Phase:** 75/25 MeOH/H₂O
- **Flowrate:** 1.8 ml/min
- **Temperature:** 35 °C
- **Pressure:** 220 bar
- **Detection:** UV at 254 nm

**Peak Identities:**
1. uracil
2. benzamide
3. benzonitrile
4. propyl paraben
5. benzyl benzoate
6. diethylphthalate
7. toluene
8. 1-chloro-4-nitrobenzene
9. di-n-propyl-phthalate
10. n-propylbenzene
11. n-butylbenzene
12. biphenyl
13. acenaphthene
14. phenanthrene
15. unknown impurities

**Test Conditions:**
- **Column:** 4.6 x 50 mm HALO Phenyl-Hexyl
- **Mobile Phase:** 77/23 MeOH/H₂O
- **Flowrate:** 1.8 ml/min
- **Pressure:** 200 bar
- **Detection:** UV at 254 nm
- **Temperature:** 40 °C

These two chromatograms illustrate how HALO Phenyl-Hexyl columns can provide separation of complex mixtures in a little over a minute.
**HALO UHPLC Columns** | **High resolution, Hyper-fast, Super-rugged**

HALO Fused-Core particles are designed for high speed, high resolution liquid chromatography. They are unique particles consisting of a 0.5 μm porous silica “halo” fused to a 1.7 μm solid silica core (Figure 4). The high density and extremely narrow size distribution of these Fused-Core particles facilitate the packing of columns with unexpectedly high efficiencies - efficiencies more in line with what you would expect from columns packed with sub-2 μm particles. The reason for this unexpectedly high efficiency is apparently the unusually well-ordered packed bed that minimizes the eddy diffusion contribution to band broadening. HALO columns do, however, generate the back pressure that one would expect from columns packed with 2.7 μm size particles. This pressure is low enough to permit HALO UHPLC columns to be used effectively with conventional HPLC equipment to deliver near-UHPLC performance.

**FIGURE 3:** Fast Separation of Anticoagulants

![Graph showing separation of anticoagulants](image)

**Peak Identities:**
1. uracil
2. 4-hydroxycoumarin
3. coumarin
4. 6-chloro-4-hydroxycoumarin
5. warfarin
6. coumatetralyl
7. alpha-chloroxycoumarin

**Test Conditions:**
- **Column:** 4.6 x 50 mm HALO Phenyl-Hexyl
- **Mobile Phase:** 20% ACN/ 20% MeOH/ 60% 0.1% Formic Acid (pH=2.6)
- **Temperature:** 45 °C
- **Flowrate:** 2.0 ml/min
- **Pressure:** 215 bar
- **Detection:** UV at 254 nm

Six anticoagulants are well separated on a HALO Phenyl-Hexyl column in less than 72 seconds.

**FIGURE 4:** Fused-Core Particle Technology

Fused-Core particle technology was developed by Jack Kirkland to produce UHPLC columns that could be effectively used with either UHPLC or conventional HPLC equipment. As the name implies, Fused-Core particles are manufactured by fusing a porous silica layer onto a solid silica particle.
HALO columns deliver over 90% more separating power (theoretical plates) than a column of the same length packed with 3.5 μm particles and almost three times the plates of a column packed with 5 μm particles (Figure 5). And, because of Fused-Core particle technology, HALO columns maintain their resolving power at high mobile phase velocity. This means that shorter columns and higher flow velocities can be used to achieve remarkably fast high resolution separations.

**FIGURE 5: HALO columns deliver more separating power**

![Bar chart showing separating power comparison between 5 μm, 3.5 μm, 3 μm, 2.2 μm, and sub-2 μm particles.]

HALO columns deliver over 90% more separating power (theoretical plates) than columns of the same length packed with 3.5 μm particles and almost three times the separating power of columns packed with 5 μm particles. Note: N/Meter values were calculated at the optimum mobile phase linear velocity for each of these stationary phases.

The combination of extremely narrow particle size distribution and very dense particles allows for the production of columns that are incredibly rugged and reliable, even when used under UHPLC conditions of high pressure and high flow velocity. In addition, the narrow size distribution of the Fused-Core particles permits the use of 2 μm porosity inlet frits on HALO columns. This is the same inlet frit porosity typically found on columns packed with 5 μm particles. The result is columns capable of delivering the speed and resolution of other UHPLC columns packed with sub-2 μm particles, but with the ease of use and durability of columns packed with 5 μm particles.
**HALO** Specifications:

**Stationary Phase Support:**
- Ultra-pure, “Type B” silica
- 1.7 micron solid core particle with a 0.5 micron porous silica layer fused to the surface
- 150 m²/gram surface area
- 90 Å pore size

**Bonded Phase:**
- Phenyl-Hexyl
- Densely bonded phase: 3.4 μmoles/m²
- Maximised endcapping
- Maximum Pressure: 9,000 psi, 600 Bar
- pH Range: 2 to 9

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**HALO** Phenyl-Hexyl Ordering Information:

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<thead>
<tr>
<th>Column Dimensions</th>
<th>Part Number</th>
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<tr>
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<tr>
<td>2.1 X 50 mm</td>
<td>92812-406</td>
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<tr>
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