
HALO®
RP-AMIDE

HALO® RP-AMIDE

- Alternate, complementary selectivity to C18 and C8 bonded phases
- Particularly recommended for samples containing acidic and basic compounds
- Compatible with highly aqueous mobile phases to facilitate the retention and separation of polar compounds
- Enhanced 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® Fused-Core® particle technology facilitates ultra-fast, high resolution separations similar to UHPLC, but without the need for UHPLC equipment. The moderate back pressure of HALO columns permits them to be used with most conventional HPLC equipment. Now HALO columns are available packed with a polar-embedded phase that offers a powerful alternate selectivity to HALO C18 and C8. HALO RP-Amide columns provide enhanced selectivity for samples containing acidic and basic compounds and are an excellent choice when a C18 or C8 phase fails to provide an adequate separation. The HALO RP-Amide columns are also particularly well suited for the separation of highly water soluble compounds that require high aqueous mobile phases, since the polar amide group ensures that the stationary phase is fully “wettable”, even when using 100% aqueous mobile phases.

HALO RP-Amide should not be confused with other amide embedded phases that exhibit weak hydrolytic stability. Proprietary bonding chemistry is used in the production of the HALO RP-Amide phase to achieve excellent stability and long column life. The extremely low bleed characteristics of the HALO RP-Amide phase make it particularly well suited for LC/MS applications.

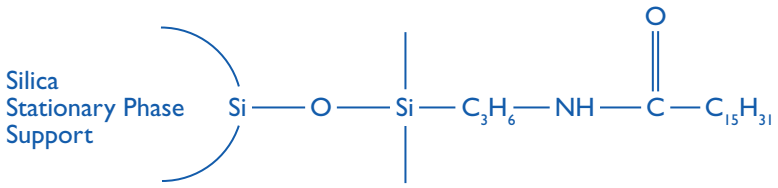
As with the HALO C18 and C8 phases, ultra-pure reagents, “Type B” silica, dense bonding technology, and exhaustive endcapping generate a base-deactivated stationary phase that provides excellent peak shape for polar compounds.

Mechanism of Separation

Separations on HALO RP-Amide columns are influenced by both hydrophobic interaction with the alkyl chain and hydrogen bonding with the embedded amide group. (See Figure 1 for structure of the RP-Amide bonded phase.) Analytes with hydrogen bond donor characteristics can be expected to be more retained on the HALO RP-Amide phase. An example of this can be seen in Figure 2 where 2-chlorophenol, 3-ethylphenol, and butyl paraben, are more strongly retained on the HALO RP-Amide than the HALO C18. In general, acids will be retained more, bases will be retained slightly less, and neutral analytes will have approximately the same retention on the HALO RP-Amide as they will on the HALO C18.

This "different" selectivity provided by the HALO RP-Amide makes it a very useful alternative phase to a C18 phase. Compounds that are poorly separated on a C18 phase may be well separated on the HALO RP-Amide. Figure 3 is a good example. Here, 2-nitroaniline, 4-bromoacetanilide, and 2,2'-biphenol co-elute on a C18 phase, but are baseline separated on the HALO RP-Amide.

Figure 1: Structure of bonded phase of HALO RP-Amide



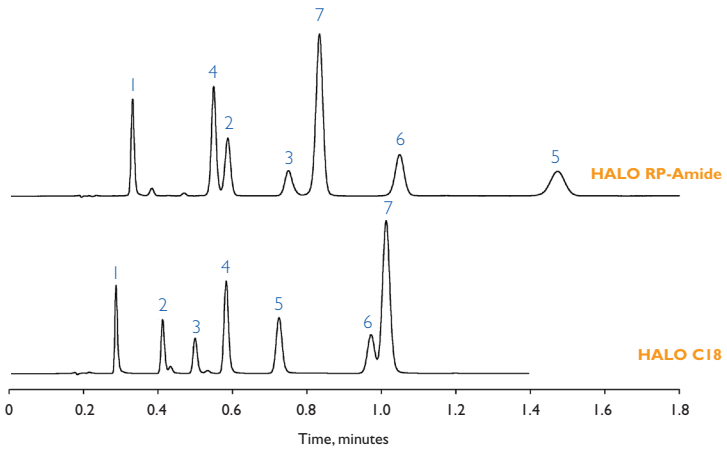
A stable amide group embedded in an 18-carbon chain is the bonded phase used for the HALO RP-Amide phase.

Figure 2 : HALO RP-Amide offers an alternate selectivity to C18

Conditions:

Columns: 4.6 x 50 mm
Mobile Phase: 50/50 ACN/ 20 mM potassium phosphate buffer (pH = 7.0)
Flowrate: 2.0 ml/min
Pressure: ~175 bar
Sample: 1 µL of solution containing:

1. benzyl alcohol
2. 2-chlorophenol
3. 3-ethylphenol
4. benzylbenzoate
5. butyl paraben
6. 4-chloro-3-nitroanisole
7. N,N-dimethylaniline

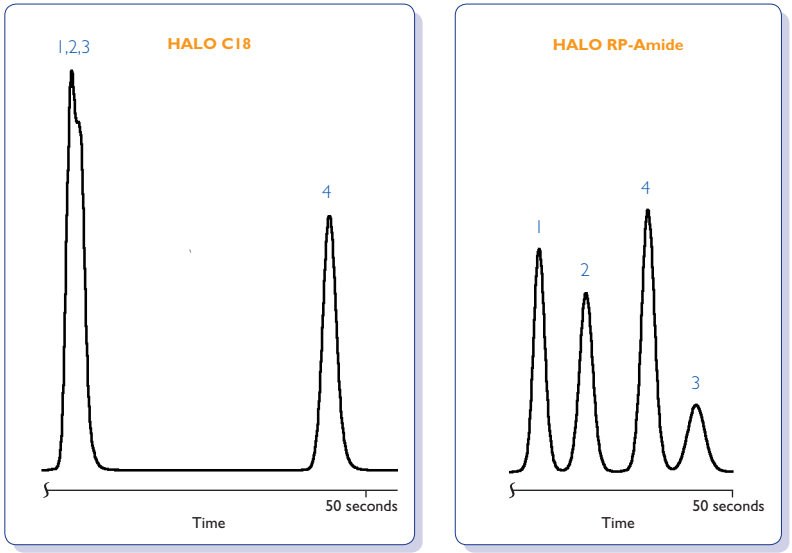


Hydrogen bond donors, like 2-chlorophenol, 3-ethylphenol, and butyl paraben are more strongly retained on the HALO RP-Amide phase than on the HALO C18 phase. Basic compounds, like N,N-dimethylaniline, are slightly less retained on the HALO RP-Amide phase. This difference in selectivity makes HALO RP-Amide an extremely useful alternate selectivity to the HALO C18 phase.

Figure 3 : The alternate selectivity of HALO RP-Amide often provides a better separation

Conditions:

Columns: 4.6 x 50 mm
Mobile Phase: 35/65 ACN/20 mM phosphate buffer, pH = 7.0
Flowrate: 3.0 ml/min
Pressure: 310 bar
Temperature: Ambient at 26 °C
Peak Identities:
1. 2-Nitroaniline **2.** 4-Bromoacetanilide **3.** 2, 2'-Biphenol
4. Benzylbenzoate



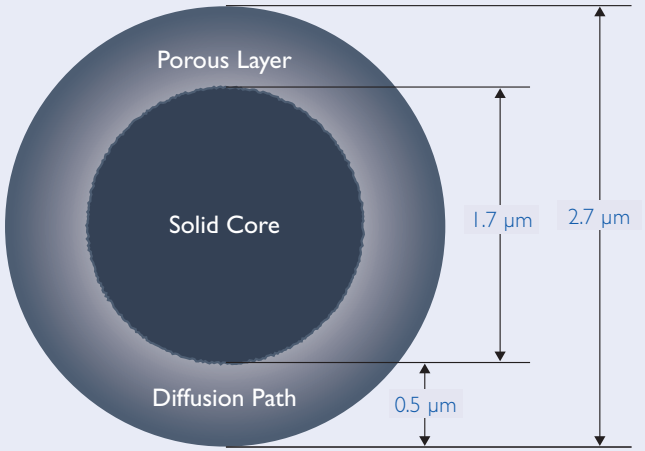
Three compounds in this sample mix do not separate on a C18 phase. However, under the identical mobile phase conditions, they are baseline separated on the HALO RP-Amide.

HALO Columns

High-resolution. Hyper-fast. Super-rugged.

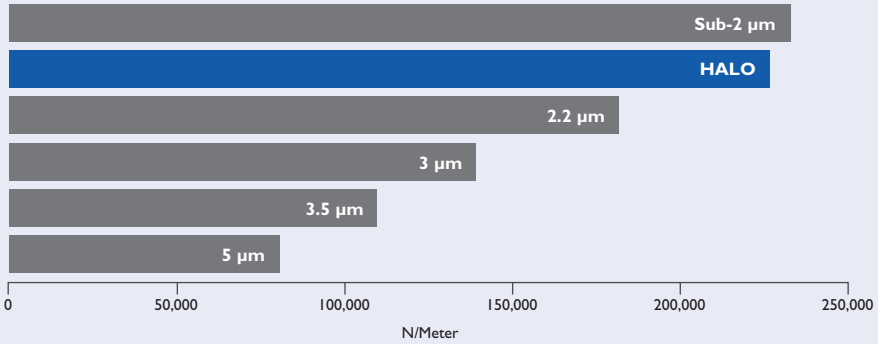
HALO Fused-Core particles are designed for high speed, high resolution liquid chromatography. They are unique particles consisting of a 0.5 µm porous silica "halo" fused to a 1.7 µm solid silica core (Figure 4). The high density and extremely narrow size distribution of these Fused-Core particles facilitate the packing of columns with unexpectedly high efficiencies - efficiencies more in line with what you would expect from columns packed with sub-2 um particles, but with half the operating pressure. 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 columns to be used effectively with conventional HPLC equipment, avoiding the need to purchase expensive UHPLC equipment.

Figure 4 : Fused-Core Particle Technology



Fused-Core particle technology was developed by Jack Kirkland to produce HPLC columns that deliver UHPLC-like performance with conventional HPLC equipment. As the name implies, Fused-Core particles are manufactured by fusing a porous silica layer onto a solid silica particle.

Figure 5 : HALO columns deliver more separating power



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.

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 6).

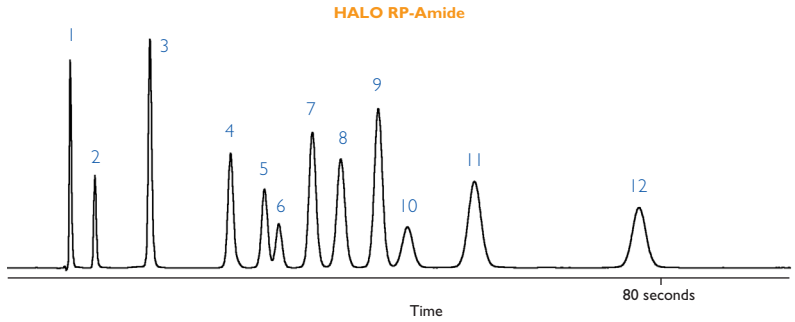
The combination of extremely narrow particle size distribution and very dense particles allows for the production of columns that are incredibly rugged and reliable, even when used under conditions of high pressure and high flow velocity. In addition, the narrow size distribution of the Fused-Core particles 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 speed and resolution similar to columns packed with sub-2 µm particles, but with the ease of use and durability of columns packed with 5 µm particles.

Figure 6 : Ultra-fast separation of a 12 component mixture on a HALO RP-Amide column

Conditions:

Columns: 4.6 x 50 mm
Mobile Phase: 35/65 ACN/20 mM phosphate buffer, pH = 7.0
Flowrate: 3.0 ml/min
Pressure: 310 bar
Temperature: Ambient at 26 °C
Peak Identities:

1. Uracil
2. Benzamide
3. Aniline
4. Cinnamyl Alcohol
5. Dimethylphthalate
6. Phenylacetonitrile
7. 2-Nitroaniline
8. 4-Bromoacetanilide
9. Benzylbenzoate
10. 2,2'-Biphenol
11. 4,4'-Biphenol
12. N,N-dimethylaniline



The selectivity offered by the alkyl amide phase combined with the high efficiency and high speed of the HALO Fused-Core particles facilitates the separation of these 12 compounds in under 80 seconds.

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 m^2/gram surface area
- 90 Å pore size

Bonded Phase:
- Alkyl amide
- Densely bonded phase, 3 micromoles/m^2
- Maximized endcapping

Maximum Pressure: 9,000 psi, 600 Bar

pH Range: 2 to 9

HALO RP-Amide Ordering Information

Column Dimensions	Part Number
2.1 x 30 mm	92812-307
2.1 x 50 mm	92812-407
2.1 x 75 mm	92812-507
2.1 x 100 mm	92812-607
2.1 x 150 mm	92812-707
3.0 x 30 mm	92813-307
3.0 x 50 mm	92813-407
3.0 x 75 mm	92813-507
3.0 x 100 mm	92813-607
3.0 x 150 mm	92813-707
4.6 x 30 mm	92814-307
4.6 x 50 mm	92814-407
4.6 x 75 mm	92814-507
4.6 x 100 mm	92814-607
4.6 x 150 mm	92814-707

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