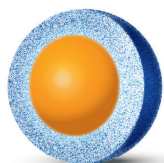


HALO[®]

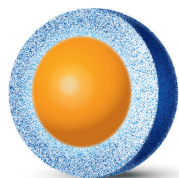
Discover the Advantages of HALO[®] and HALO[®] BioClass Fused-Core[®] Columns

HALO®

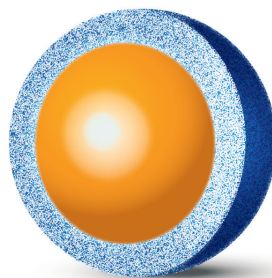
SMALL MOLECULE



2 micron particle

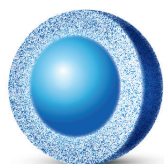


2.7 micron particle

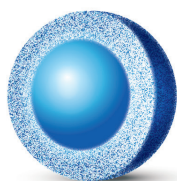


5 micron particle

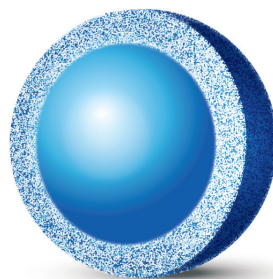
BIOCLASS



2 micron particle

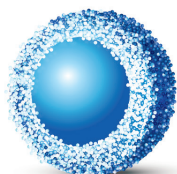


2.7 micron particle

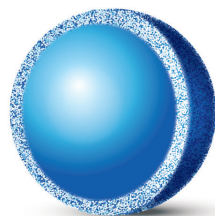


5 micron particle

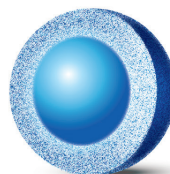
PEPTIDE



2.7 micron particle



3.4 micron particle



2.7 micron particle

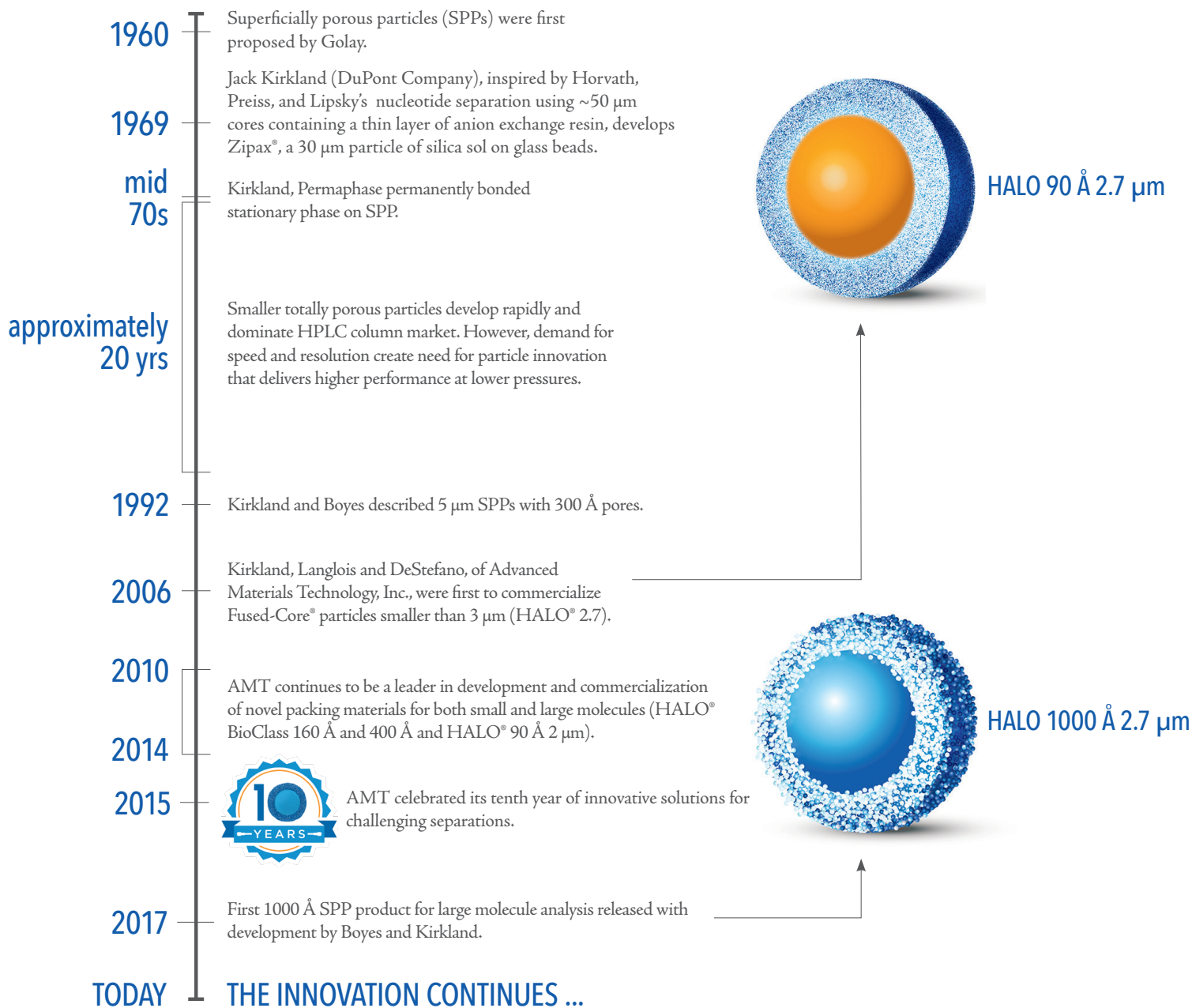
PROTEIN

GLYCAN

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MILESTONES IN THE DEVELOPMENT OF FUSED-CORE PARTICLES



SUMMARY:

- Dr. Joseph (Jack) Kirkland was involved in the development of HPLC packings, including porous and Fused-Core (SPP), throughout his distinguished career.
- Columns packed with these 2.7 µm particles created a revolution in HPLC technology.
 - Performance is comparable to the performance of sub-2 µm non-core particles, but with half the back pressure.
 - Analysts can obtain very high efficiencies and faster separations using their existing HPLC instruments, which may be limited to 400–600 bar.

SUPERIOR PERFORMANCE OF HALO FUSED-CORE COLUMNS:

HALO FUSED-CORE COLUMNS

HALO 2 μm columns will deliver reliable high speed and high resolution separations at pressures lower than non-core sub-2 μm columns.

HALO 2.7 μm columns can meet or exceed the performance of most non-core sub-2 μm columns at pressures one-third to one-half the back pressure under the same conditions.

HALO 5 μm columns match the performance of totally porous 3 μm columns at roughly half the back pressure under the same conditions.

Early Explanations for Superior Performance

- ♦ *Faster Mass Transfer* due to a thin porous bonded-phase layer exterior to particle's solid silica core
- ♦ *More Uniform and Stable Column* beds due to very narrow particle size distribution ($\sim 4\text{--}6\%$ RSD vs. $\sim 20\%$ RSD for non-core particles)

Figure A. FIB - SEM image of first commercial HALO particle with 2.7 μm total size consisting of a 1.7 μm solid silica core and a 0.5 μm shell.

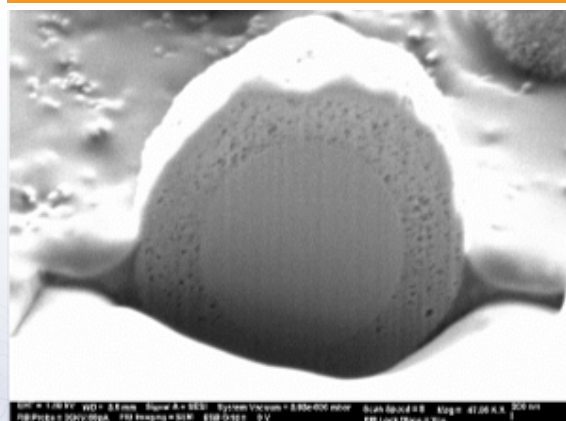


Figure B. SEM image of a focused-ion beam cleaved HALO 1000 Å 2.7 μm silica particle. This "cut-away" view shows the solid core and shell with large pores allowing unrestricted access of macromolecules to the bonded phase.

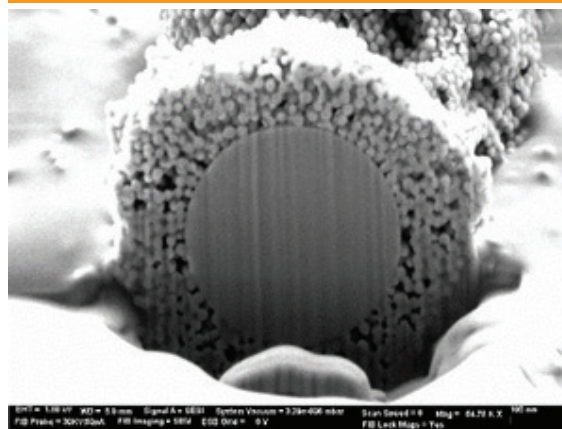
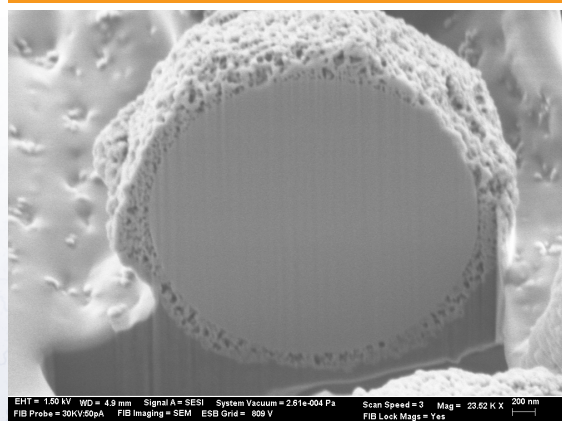


Figure C. SEM image of a focused-ion-beam cleaved HALO Protein 3.4 μm silica particle. This "cut-away" view shows the solid core with its very thin 0.2 μm outer porous layer.



Understanding SPP Performance (Figure D)

The superior performance of Fused-Core SPP columns is now believed to be due to:

- ♦ Reduction in eddy diffusion
 - 40% smaller van Deemter “A term” due to more uniform analyte flow paths through the column bed
- ♦ Much lower longitudinal broadening, flat van Deemter plot and higher optimum linear velocity (flow rate)
 - Due to the presence of the particle’s solid core (25–30% smaller van Deemter “B term”)
- ♦ Much smaller reduced plate heights and high efficiencies for SPP columns due to smaller van Deemter A and B terms for SPP particles

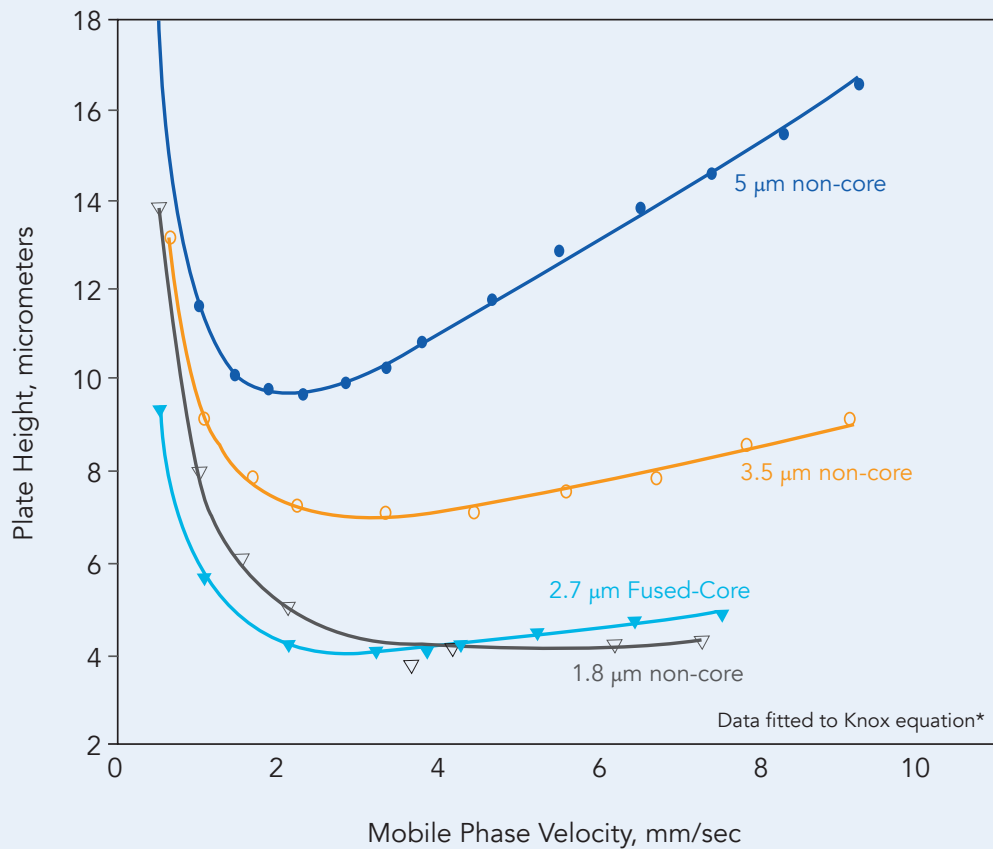
Figure D. van Deemter Plot of Plate Height vs. Linear Velocity (flow rate)

Effect of Particle Size and Type

Column Dimensions: 4.6 x 50 mm, Non-core C18, 5 µm; Non-core C18, 3.5 µm;

Non-core C18, 1.8 µm; HALO C18, 2.7 µm

Solute: naphthalene; mobile phase: 60% ACN/40% water, 24 °C



$$H = A + \frac{B}{\mu} + C\mu$$

van Deemter Equation

H = height equivalent to theoretical plate

A = eddy diffusion term

B = longitudinal diffusion term

C = resistance to mass transfer term

μ = mobile phase linear velocity (L/t_r)

*G.J. Kennedy, J.H. Knox, *J. Chromatogr. Sci* 10 (1972) 549.

KEY ADVANTAGES OF HALO FUSED-CORE COLUMNS

HALO FUSED-CORE PERFORMANCE

High Speed Separations (Figures F and G)

- ✦ Smaller reduced plate heights lead to high efficiencies; narrower and taller peaks, for improved resolution and lower detection limits (LODs and LOQs)
- ✦ Flat van Deemter plot and higher linear velocity optimum (Figure D, page 3) allow higher flow rates with minimal column efficiency loss

High Resolution Separations (Figures E and H)

- ✦ High efficiency with longer geometries (100, 150, 250 mm) provides greater resolving power for challenging applications
- ✦ Lower back pressure permits columns to be used in series for the most demanding UHPLC and HPLC separations

Excellent Ruggedness and Reproducibility

- ✦ Less plugging, longer usable column lifetime and greater uptime due to larger porosity frits (vs. sub-2 μm totally porous (non-core) columns)
 - 2 μm frits for HALO 2.7 μm and 5 μm columns
 - 1 μm frits for HALO 2 μm columns vs. 0.2–0.5 μm frits for sub-2 μm non-core columns

- ✦ Excellent column-to-column and lot-to-lot reproducibility thanks to tight manufacturing controls
- ✦ Robust pores in multiple sizes for a tailored application solution (90 \AA , 160 \AA , 400 \AA and 1000 \AA)

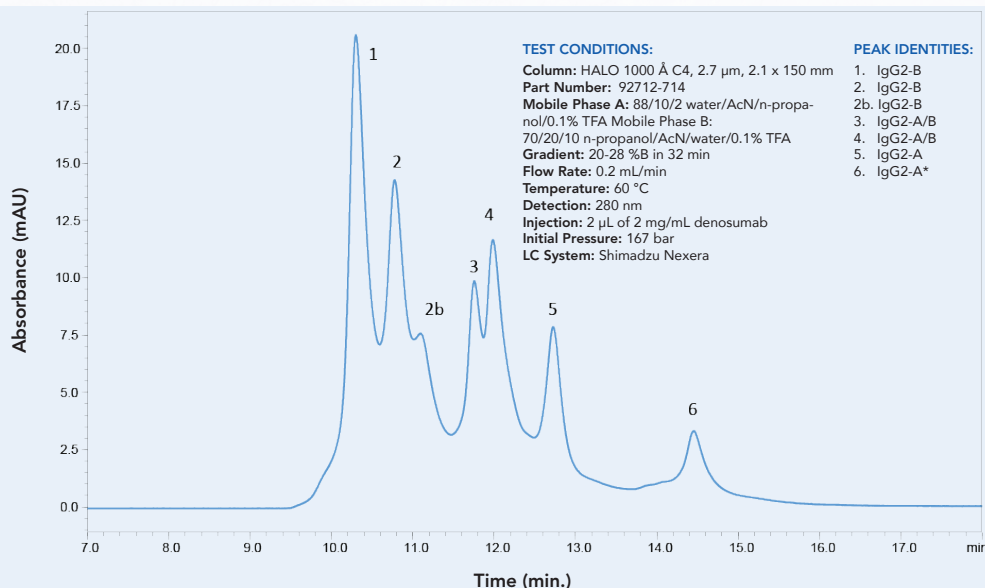
HALO BIOCLASS

Solutions for Proteins, Peptides and Glycans

- ✦ Application specific columns for bioseparations that outperform non-core columns
- ✦ Up to 1/2 the back pressure
- ✦ Offer better peak shape and peak capacity
- ✦ Breakthrough 1000 \AA pore particles for large molecule enablement

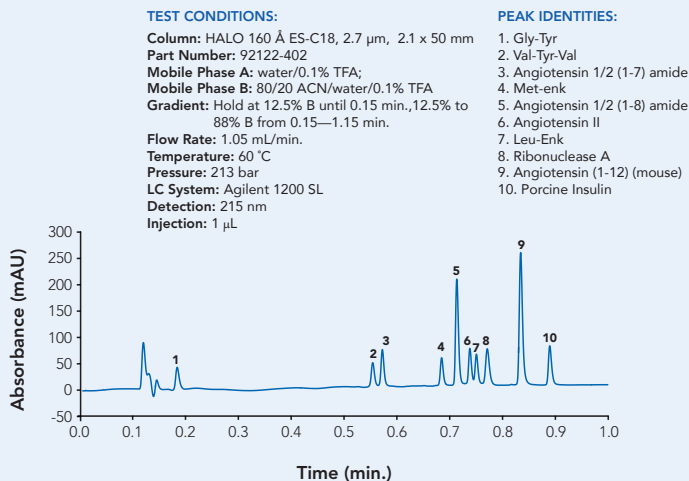
Figure E. High Resolution of IgG2 with HALO 1000 \AA C4

Very high resolution separations are achieved with HALO 1000 \AA C4 for a complex IgG2 such as denosumab. The assignments are based on non-reduced Lys-C digestion mapping.



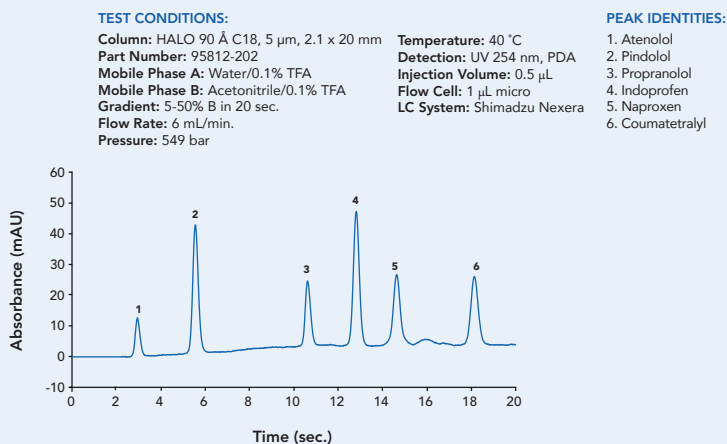
ULTRAFAST PEPTIDE SEPARATION

Figure F. Separation of a 10 peptide mixture is accomplished in less than one minute using a HALO Peptide ES-C18 column on a delay-volume minimized and optimized Agilent 1200 system.



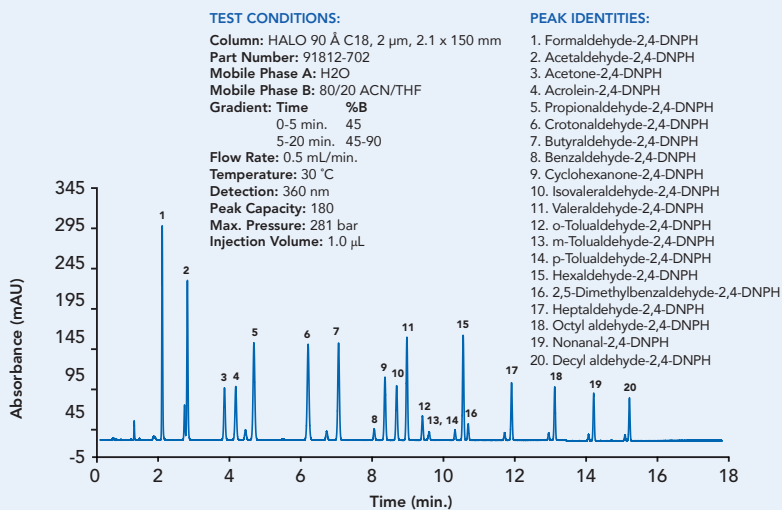
ULTRAFAST BALLISTIC GRADIENT USING HALO 5 μm

Figure G. Many researchers have found HALO 5 μm columns in 2.1 mm ID to be very useful for high-throughput, ballistic separations by LC and LC-MS.



CARBONYL-DNPH HIGH RESOLUTION SEPARATION

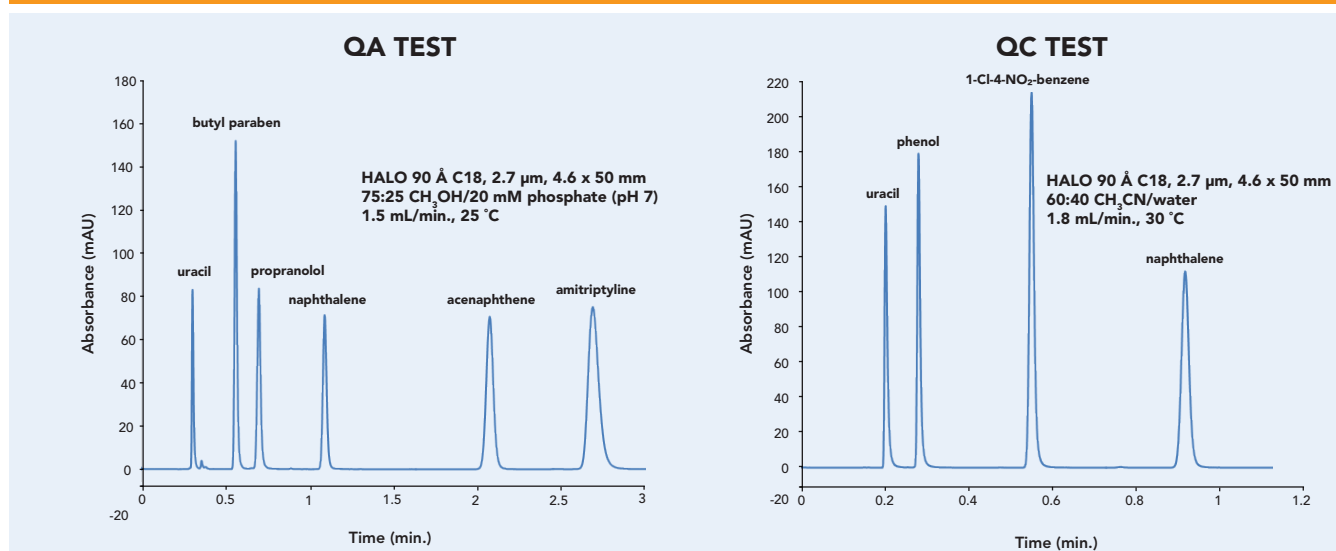
Figure H. Environmental samples can be quite complex as demonstrated by this gradient separation of dinitrophenylhydrazone (DNPH) carbonyl compound derivatives using a HALO 90 Å C18, 2 μm, 2.1 x 150 mm column.



HALO QUALITY PROMISE: PERFORMANCE AND REPRODUCIBILITY – EVERY TIME

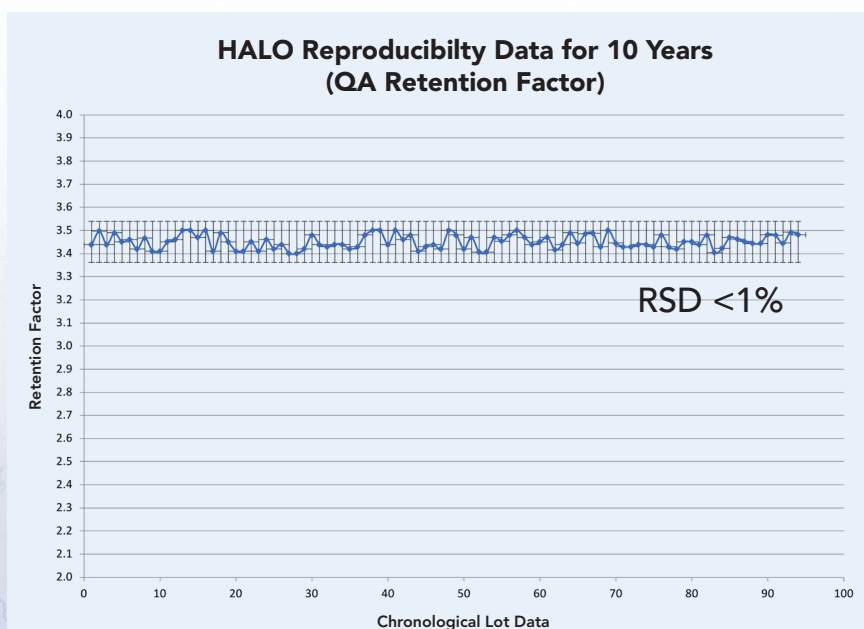
As the originators of Fused-Core particles, Advanced Materials Technology incorporates the most knowledge in the industry to bring high-quality, innovative products to our customers. Our principal scientists have over 150 years of combined experience in liquid chromatography, particle synthesis and column manufacturing.

Figure I. Consistent reproducible performance from column to column and lot to lot is ensured because of well-designed processes and practices in the manufacture of HALO Fused-Core particles, HALO phases and HALO columns. Representative chromatograms of QA and QC tests are shown below, along with a historical plot of selectivity between a neutral and basic analyte.



REPRODUCIBLE PERFORMANCE OVER TIME

Figure J. Advanced Materials Technology (AMT) is one of only a few HPLC column manufacturers that completes the entire column manufacturing process in-house. The scientists and engineers at AMT have expertise in every aspect of the column development process. Every step that comprises the creation of a HALO column is monitored and controlled. From the solid silica cores to the bonded Fused-Core particles to the final loaded and QC-tested column, customers can be confident that the HALO products they receive are reliable and reproducible. The graph demonstrates the superior reproducibility of the retention of HALO 90 Å C18, 2.7 µm columns over a 10-year period.



SELECTING THE APPROPRIATE PORE SIZE

AMT tailors pore sizes to your challenging separations. So how do you choose the correct one?

- ♦ Match the column pore size according to your molecule size and the range of molecular weights (MWs) of the analytes in your sample (Table A)
- ♦ Small molecules (< 5000 Da) are usually analyzed using HALO 90 Å columns
 - Packing materials with smaller pores have greater surface area, which allows improved retention and loading capacity for lower MW analytes
 - When an analyte is too large for the pores, restricted diffusion can occur, which can lead to peak broadening and reduced retention
- ♦ For macrocyclic antibiotics and biomolecules such as peptides and proteins, use larger pore sizes such as HALO 160 Å Peptide and HALO 400 Å Protein BioClass columns
- ♦ For mAbs and intact proteins of molecular sizes > 50 kDa, consider the HALO 1000 Å products

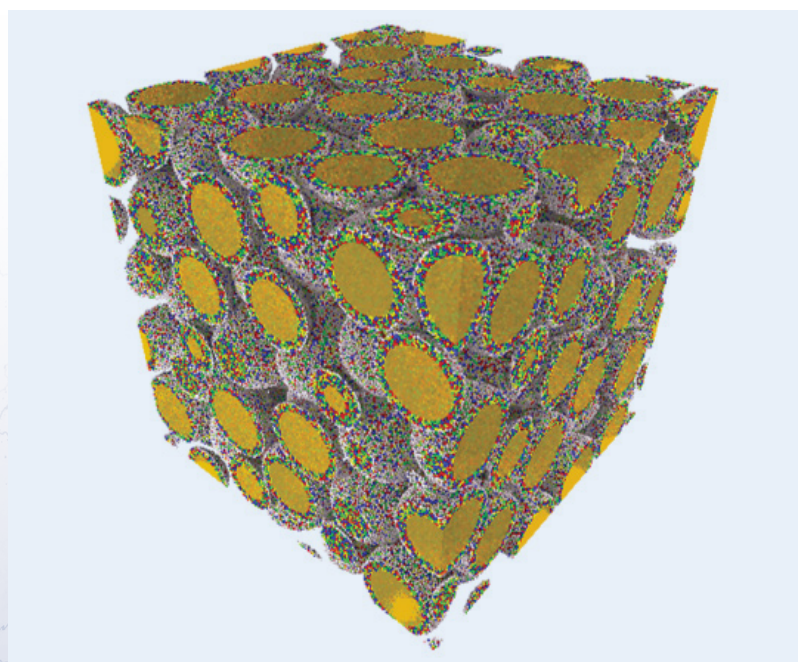
Molecule Size	Pore Size (Å)	Application	Particle Sizes (µm)	Column Family
SMALL (<5000 Da)	90	Small Molecules	2, 2.7, 5	HALO
SMALL (< 20 kDa*)	90	Glycan	2.7	HALO BIOCLASS
MEDIUM (100 Da < MW < 15 kDa)	160	Peptide	2, 2.7, 5	
LARGE (2 kDa < MW < 500 kDa)	400	Protein	3, 4	
LARGE (> 50 kDa)	1000		2.7	

* for glycans, glycopeptides and glycoproteins

QUALITY BY DESIGN

Figure K. HALO particles are manufactured with quality by design in mind. AMT tightly controls the manufacturing process through the use of control charts and in-process monitoring. The particles are designed with target core sizes, shell thicknesses and pore sizes that have been determined to be the best compromise of each of these variables. The narrow particle size distribution of HALO Fused-Core particles is one of the features that sets the columns apart from columns of fully porous particles. This image shows a simulation of a packed bed of HALO wide pore particles. Notice the solid silica cores in yellow and the porous shell in multicolors.

M. R. Schure, R. S. Maier, T. J. Shields, C. M. Wunder, B. M. Wagner Intraparticle and interstitial flow in wide-pore superficially porous and fully porous particles, *Chemical Engineering Science* 174 (2017) 445–458.



HALO COLUMNS FOR SMALL MOLECULE ANALYSES

Of the three variables in the general resolution equation, including efficiency (N) and retention (k), **selectivity (α) is the most powerful parameter** for adjusting and improving resolution between peaks in a chromatographic separation.

EFFICIENCY

SELECTIVITY

RETENTION

$$R_s = \left(\frac{\sqrt{N}}{4} \right) \times \left[\frac{(\alpha - 1)}{\alpha} \right] \times \left[\frac{k_2}{(1 + k)} \right]$$

where

$$\bar{k} = \frac{(k_1 + k_2)}{2}, \alpha = \frac{k_2}{k_1} \text{ and } N = \frac{L}{H} = \frac{L}{h \times d_p}$$

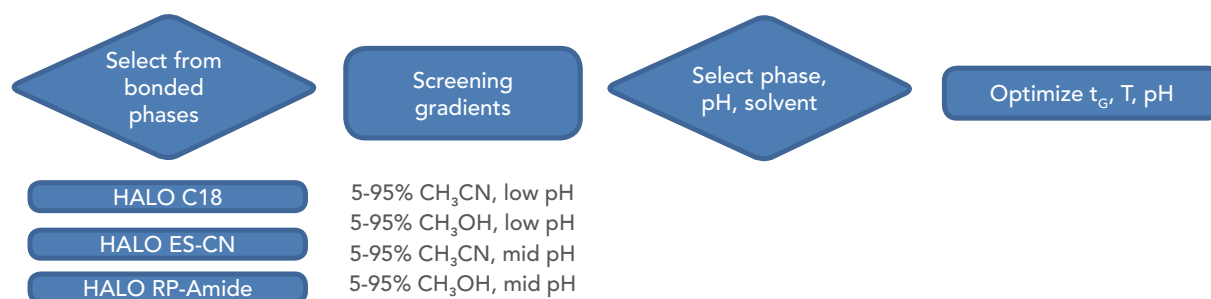
Moreover, column phase selectivity is one of the four most powerful and useful parameters for adjusting HPLC separation selectivity (see Table B). For ionizable analytes, mobile phase pH is, by far, the most effective parameter. However, column stationary phase is comparable to organic modifier choice (acetonitrile vs. methanol) and percent organic modifier/gradient steepness in its ability to change relative retention for UHPLC and HPLC separations. When developing a method, there are multiple ways to achieve a separation that meets specific resolution and retention requirements. One way is to take a systematic approach and screen multiple phases. HALO columns are available in several different stationary phases for various types of analyses. The HALO phases that are available for reversed-phase separations of small molecules are shown in Table C, and the phases are listed according to their differences in selectivity compared to HALO C18 at both pH 2.8 and pH 7. For example, if you were looking for a column with a different selectivity to a HALO C18 column at low pH, you might consider Table C and select a HALO PFP

column as one most likely to be orthogonal to C18. However, the other available HALO phases (Phenyl-Hexyl, ES-CN, Biphenyl, RP-Amide) also retain and separate analytes via retention mechanisms different from HALO C18, HALO C8 and HALO AQ-C18, so it might be prudent to consider one or more of the former phases as part of a comprehensive column screening or method development strategy (Figure L). Another approach to method development is to use trial and error with columns that have similar bonded phases, such as HALO C18 and HALO AQ-C18. According to Table C, these phases are not very orthogonal to each other, but the polar aspects of HALO AQ-C18 may be needed for retention of polar analytes.

Table B. Parameters That Affect HPLC Selectivity in Order of Increasing Effectiveness (Refs. 1 and 2)

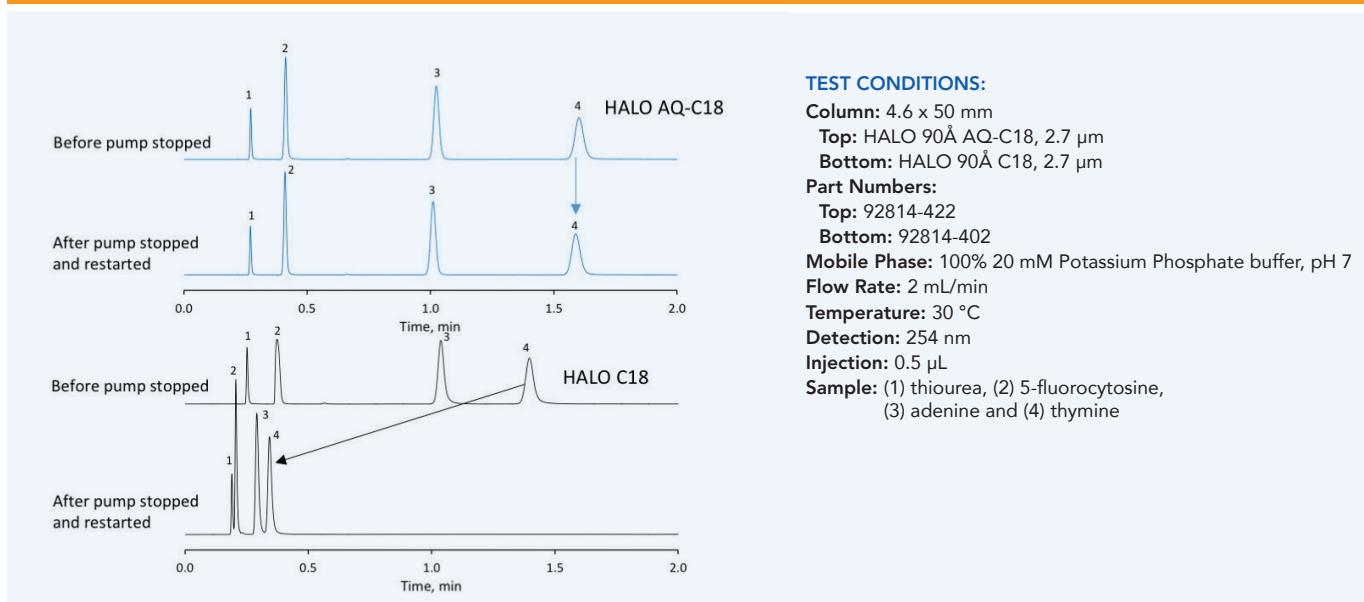
HPLC Parameter	Effectiveness for Changing Selectivity
Mobile phase pH (ionizable analytes only)	Most Effective ↑ Least Effective
Organic modifier choice	
Percent organic modifier or gradient steepness	
Column stationary phase	
Column temperature	
Buffer choice	
Buffer concentration	

Figure L. Example Strategy for Comprehensive Method Development Using Multiple HALO Stationary Phases and Column/Condition Screening, Followed by Optimization of Gradient Time, Temperature and pH



RESISTANCE TO DEWETTING

Figure M. The unique polar modified bonded phase of HALO AQ-C18 enables it to be run in 100% aqueous mobile phase without experiencing loss in retention due to dewetting when pressure is relieved. The retention is nearly 100% maintained compared to the HALO C18 after the pump is stopped and restarted.



Another item that must be considered during method development is phase dewetting. Dewetting occurs when the stationary phase is highly hydrophobic and the mobile phase is changed from one with a high amount of organic solvent component (> 40% ACN or MeOH) to one that is entirely aqueous or mostly aqueous. When the column is under pressure, the aqueous mobile phase is forced into the porous structure where most of the retention occurs. When the pump is stopped, the aqueous mobile phase is no longer forced into the packing pores and is expelled from the interior of the particles. Restarting the pump will not force the aqueous mobile phase

back into the pores since the phase is hydrophobic. The retention of the sample components drastically decreases and resolution is lost. Figure M demonstrates what happens to a separation when dewetting occurs with HALO C18. In contrast, HALO AQ-C18 phase has an added amount of polar characteristic that prevents it from dewetting as shown in Figure M. Even when the pump is stopped and restarted, the retention and resolution are both maintained with the HALO AQ-C18 column. All of the HALO phases except HALO C18 may be used under 100% aqueous conditions without dewetting.

Table C. Orthogonality of HALO Phases

	pH 2.8	pH 7
Most Similar	HALO C18	HALO C18
	HALO C8	HALO C8
	HALO AQ-C18	HALO AQ-C18
	HALO Phenyl-Hexyl	HALO PFP
	HALO ES-CN	HALO Phenyl-Hexyl
	HALO Biphenyl	HALO Biphenyl
	HALO RP-Amide	HALO ES-CN
Most Orthogonal	HALO PFP	HALO RP-Amide

HALO COLUMNS FOR SMALL MOLECULE SEPARATIONS

Table D. HALO Small Molecule Column Specifications

Bonded Phase	USP Designation	Particle Size(s) (µm)	Pore Size (Å)	Carbon Load (%)	Surface Area (m ² /g)	Low pH/T Limit	High pH/T Limit	Endcapped
AQ-C18	L1	2 2.7 5	90	6.5 6.7	135	2/60 °C	9/40 °C	Yes
C8	L7	2 2.7 5	90	4.8 5.4 3.7	120 135 90	2/60 °C	9/40 °C	Yes
C18	L1	2 2.7 5	90	7.2 7.7 5.4	120 135 90	2/60 °C	9/40 °C	Yes
C30	L62	2.7	160	4.5	90	2/60 °C	9/40 °C	Yes
Phenyl-Hexyl	L11	2 2.7 5	90	6.3 7.1 5.2	120 135 90	2/60 °C	9/40 °C	Yes
Biphenyl	L11	2.7	90	7.0	135	2/60 °C	9/40 °C	Yes
PFP	L43	2 2.7 5	90	5.3 5.5 3.9	120 135 90	2/60 °C	8/40 °C	Yes
ES-CN	L10	2 2.7 5	90	3.4 3.5 2.5	120 135 90	1/80 °C	8/40 °C	Yes
RP-Amide	L60	2 2.7 5	90	7.3 8.2 5.1	120 135 90	2/60 °C	9/40 °C	Yes
HILIC	L3	2 2.7 5	90	Unbonded	120 135 90	1/60 °C	8/40 °C	N.A.
Penta-HILIC	L95	2 2.7 5	90	2.8 3.2 2.1	120 135 90	2/60 °C	9/40 °C	No

HALO COLUMNS FOR SMALL MOLECULE SEPARATIONS

Table E. HALO Phases: Features and Benefits, Target Analytes and Best Applications

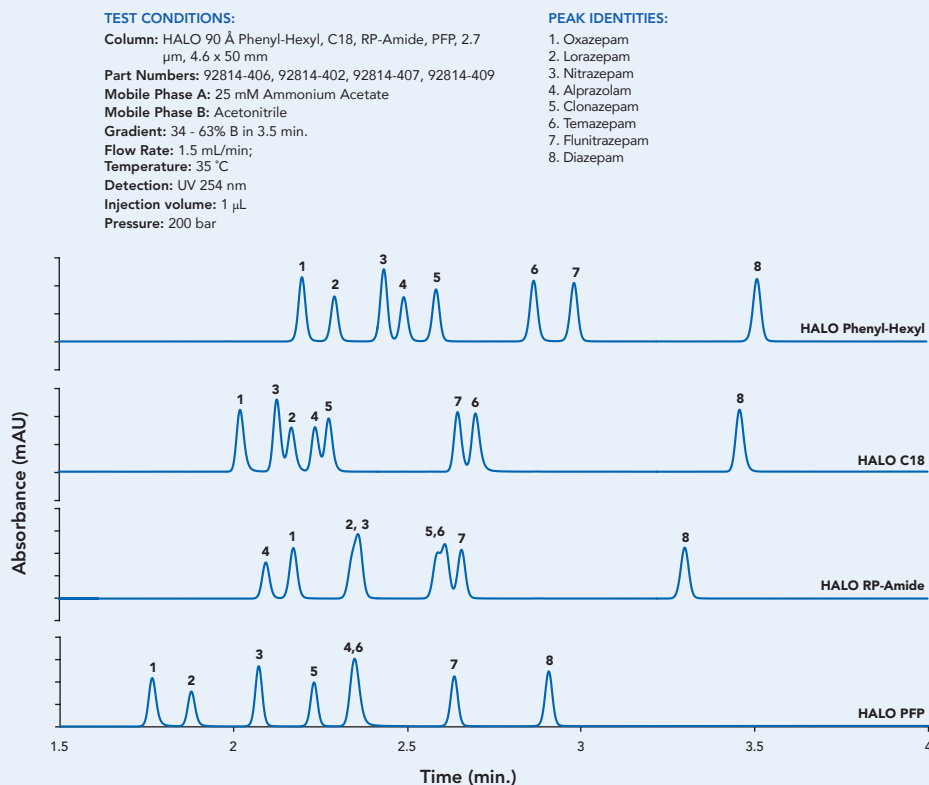
Bonded Phase	Features and Benefits	Target Analytes	Best Applications
C18 (dimethyloctadecylsilane)	<ul style="list-style-type: none"> ♦ Excellent performance for broad range of analyte polarities 	Diverse analytes ranging from polar to non-polar	<ul style="list-style-type: none"> ♦ Pharmaceutical ♦ Environmental ♦ Cannabinoid ♦ General purpose
AQ-C18 (Polar Modified)	<ul style="list-style-type: none"> ♦ Resistant to dewetting, making it 100% aqueous mobile phase compatible ♦ Enhanced retention for polar molecules 	Acids, bases, polar analytes	<ul style="list-style-type: none"> ♦ Pesticides ♦ Nucleobases ♦ Neurotransmitters ♦ Polar acids
C8 (dimethyloctylsilane)	<ul style="list-style-type: none"> ♦ Excellent performance for broad range of analyte polarities 	Diverse analytes ranging from polar to non-polar	<ul style="list-style-type: none"> ♦ Pharmaceutical ♦ Environmental ♦ Higher hydrophobic compounds
C30 (triacontyldimethyl)	<ul style="list-style-type: none"> ♦ High shape selectivity for hydrophobic, long chain, structurally related isomers 	Fat soluble vitamins (A, D, E, K) Hydrophobic analytes Lipids Carotenoids	<ul style="list-style-type: none"> ♦ Foods ♦ Lipids
Phenyl-Hexyl (dimethylphenyl-hexylsilane)	<ul style="list-style-type: none"> ♦ Complementary selectivity to alkyl phases ♦ Enhanced selectivity for aromatic compounds 	Electron-poor molecules, aromatic or unsaturated compounds (ketones, nitriles, alkenes)	<ul style="list-style-type: none"> ♦ Benzodiazepines ♦ Aromatics ♦ Drugs of abuse
Biphenyl (dimethylbiphenyl)	<ul style="list-style-type: none"> ♦ Complementary selectivity to alkyl phases ♦ Enhanced selectivity for aromatic compounds 	Electron-poor molecules, aromatic or unsaturated compounds (ketones, nitriles, alkenes)	<ul style="list-style-type: none"> ♦ Aromatic ♦ Heterocycles ♦ Drugs of abuse ♦ Pain management drugs ♦ Highly aqueous conditions
PPF (pentafluorophenylpropylsilane)	<ul style="list-style-type: none"> ♦ Complementary selectivity to alkyl phases ♦ Enhanced selectivity for stereoisomers ♦ Can be used in RPLC and HILIC modes 	Electron-rich compounds, aromatics, unsaturated compounds with double and/or triple bonds	<ul style="list-style-type: none"> ♦ Steroids ♦ Isomeric compounds ♦ Substituted aromatics
ES-CN (diisopropylcyanopropylsilane)	<ul style="list-style-type: none"> ♦ Complementary selectivity to alkyl phases ♦ More retention for polar analytes and much less retention for non-polar analytes 	Polar and very polar bases, acids and neutrals	<ul style="list-style-type: none"> ♦ Explosives ♦ Aromatics ♦ Polar compounds
RP-Amide (C16 Amide)	<ul style="list-style-type: none"> ♦ Complementary selectivity to alkyl phases ♦ Enhanced stability for minimum bleed and long life 	Alcohols, Acids, Phenols and Catechins	<ul style="list-style-type: none"> ♦ Phenols ♦ Alcohols ♦ Catechins
HILIC (Bare Silica)	<ul style="list-style-type: none"> ♦ Can be used in HILIC and normal-phase modes 	Polar and very polar bases, acids and neutrals, especially with log P < 0.5	<ul style="list-style-type: none"> ♦ Polar compounds
Penta-HILIC (proprietary penta-hydroxy ligand)	<ul style="list-style-type: none"> ♦ Ideal for separation of highly polar compounds that are poorly retained in RPLC 	Polar analytes with Log P values near or less than 0	<ul style="list-style-type: none"> ♦ Polar Basic Compounds

REVERSED-PHASE SEPARATIONS WITH HALO

To illustrate the selectivity differences among the various HALO RPLC phases, the following examples are provided.

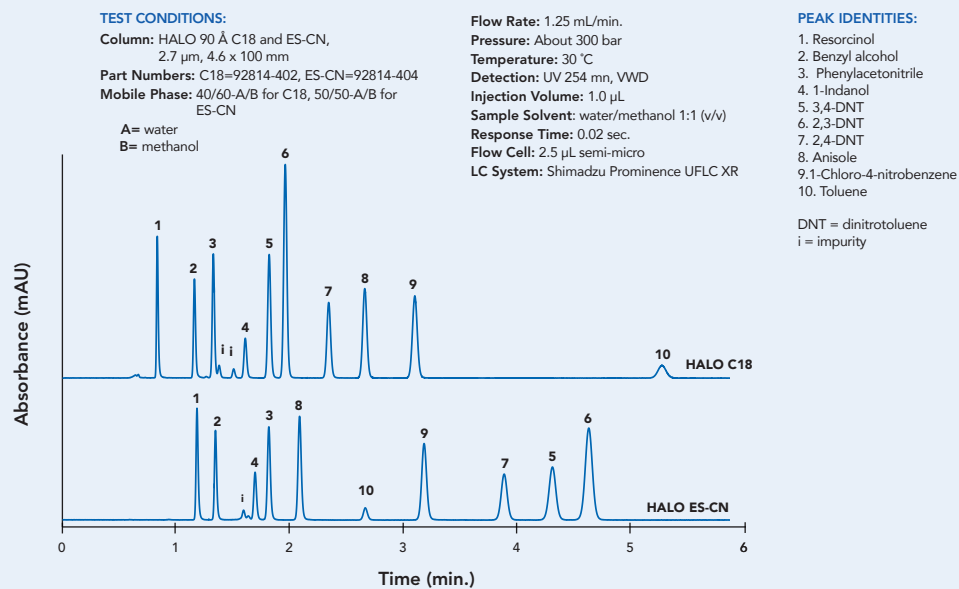
BENZODIAZEPINES ON HALO FUSED-CORE BONDED PHASES

Figure N. HALO Phenyl-Hexyl is the most retentive phase for these anti-anxiety drugs due to its propensity for π - π interactions.



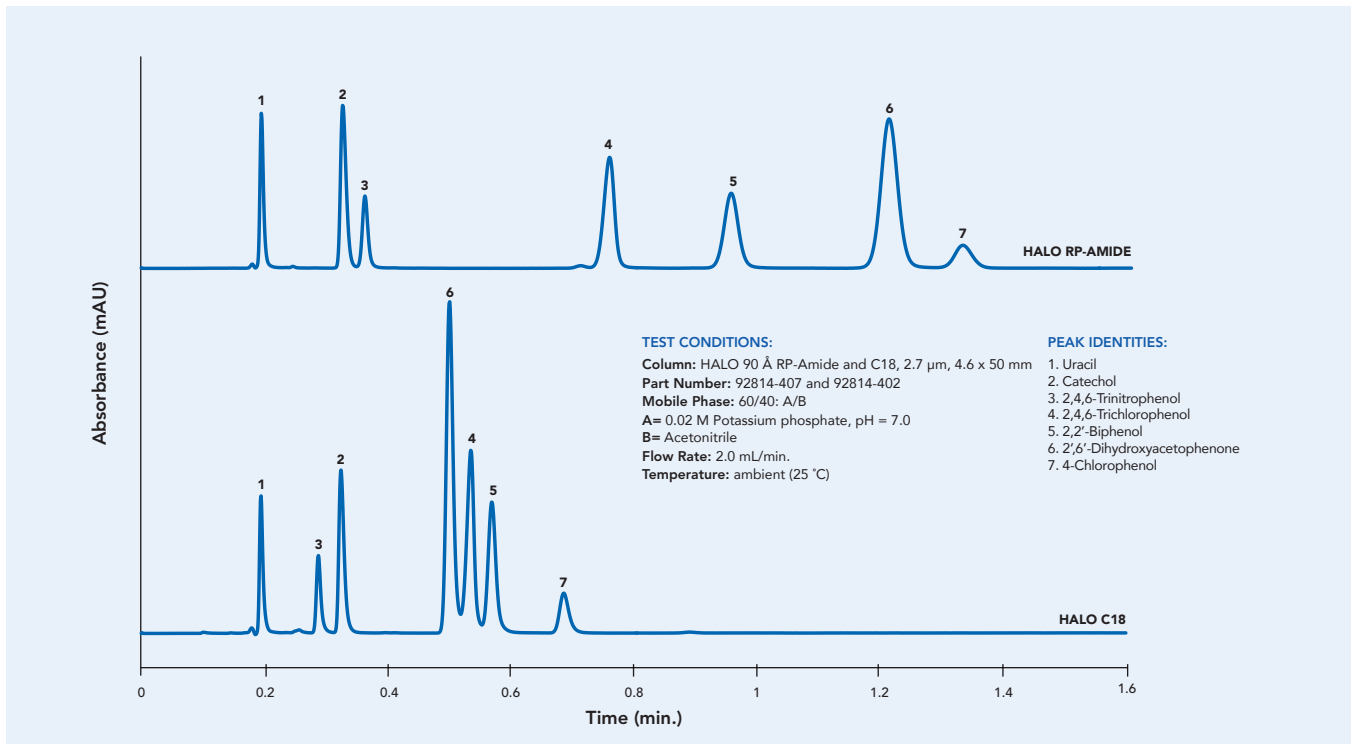
AROMATIC AND NITROAROMATIC COMPOUNDS

Figure O. HALO C18 and HALO ES-CN columns may be used as orthogonal confirmatory columns for explosives analysis.



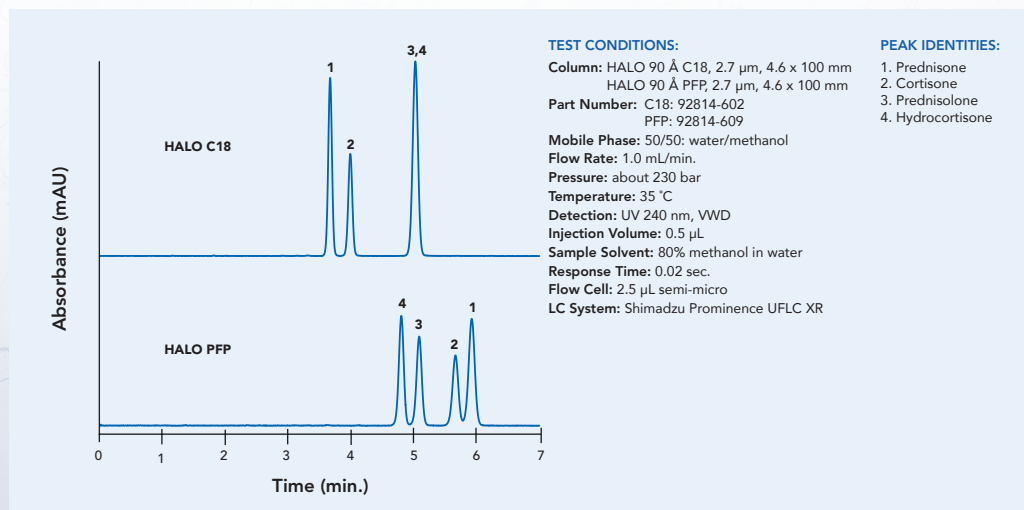
HALO C18 VS. RP-AMIDE FOR PHENOLICS

Figure P. HALO RP-Amide provides greater retention and resolution compared to HALO C18 for this phenol mixture.



SEPARATION OF STRUCTURALLY SIMILAR STEROIDS ON HALO C18 AND PFP

Figure Q. HALO PFP delivers improved resolution and different elution order compared to HALO C18 for this mixture of steroids.



HILIC SEPARATIONS WITH HALO

Hydrophilic interaction liquid chromatography (HILIC) is a useful UHPLC and HPLC mode for the following situations:

- † Polar analytes that are poorly or not retained in RPLC
- † Basic analytes that have poor peak shape (overloading) and/or poor retention at low pH in RPLC
- † Analytes that have log P values near or less than zero
- † When conditions orthogonal to RPLC mode are needed (elution order change)

HALO columns are currently available in two different phases for HILIC separations:

- † HALO HILIC
- † HALO Penta-HILIC

HALO HILIC is a Fused-Core silica phase that can be used either in HILIC mode or in normal-phase mode with water-immiscible solvents (NPLC).

HALO Penta-HILIC is a bonded silica phase, which has a highly polar ligand with 5 hydroxyl groups tethered via novel proprietary linkage chemistry to Fused-Core silica particles.

Some Typical Analytes for HILIC Separations

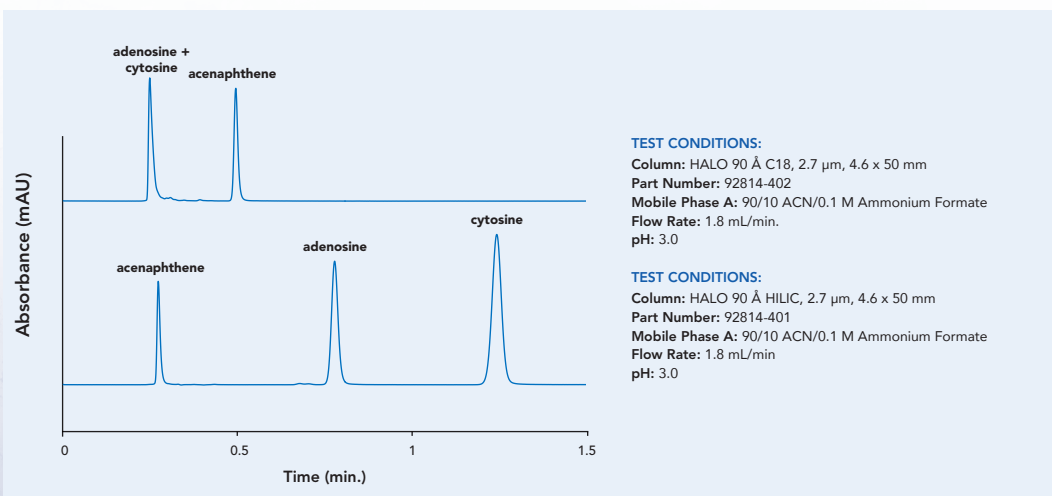
- † Basic pharmaceuticals
- † Peptides
- † Polar organic acids
- † Catecholamines and other neurotransmitters
- † Nucleosides and nucleobases
- † Drug glycoside and glycuronide metabolites
- † Mono-, di-, tri- and other oligosaccharides
- † Opiates
- † Glycosylceramides
- † Polar triazines and pyrimidines
- † Analytes from metabolomic profiling

For more information on HILIC separations, please see references 7-10 on page 31.

RETENTION ORDER REVERSAL AND IMPROVED RETENTION WITH HILIC

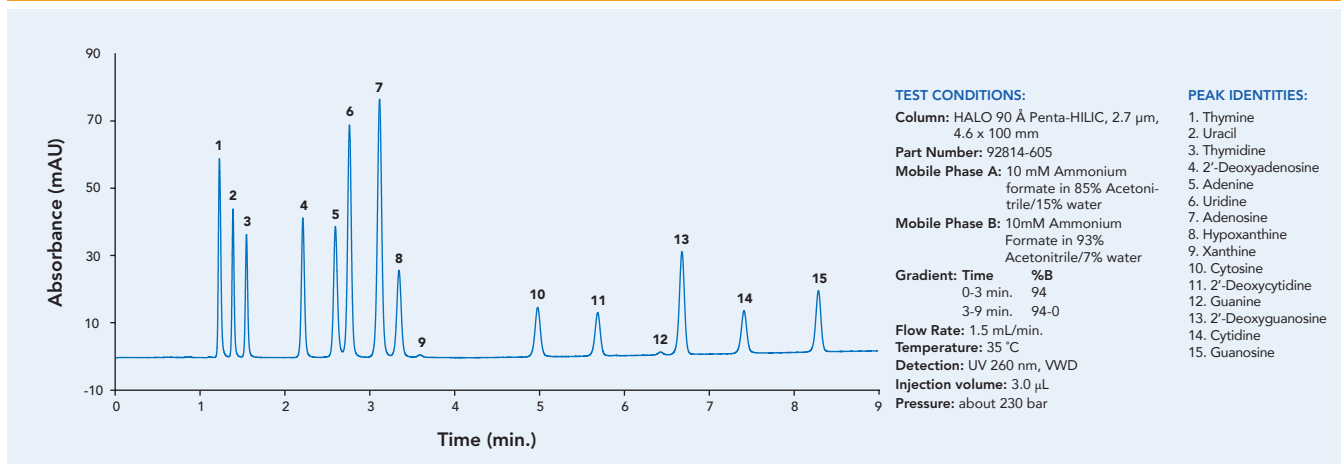
Figure R.

You can often obtain a complete reversal in elution order and different selectivity using HILIC mode compared to reversed-phase mode under the same or appropriate conditions.



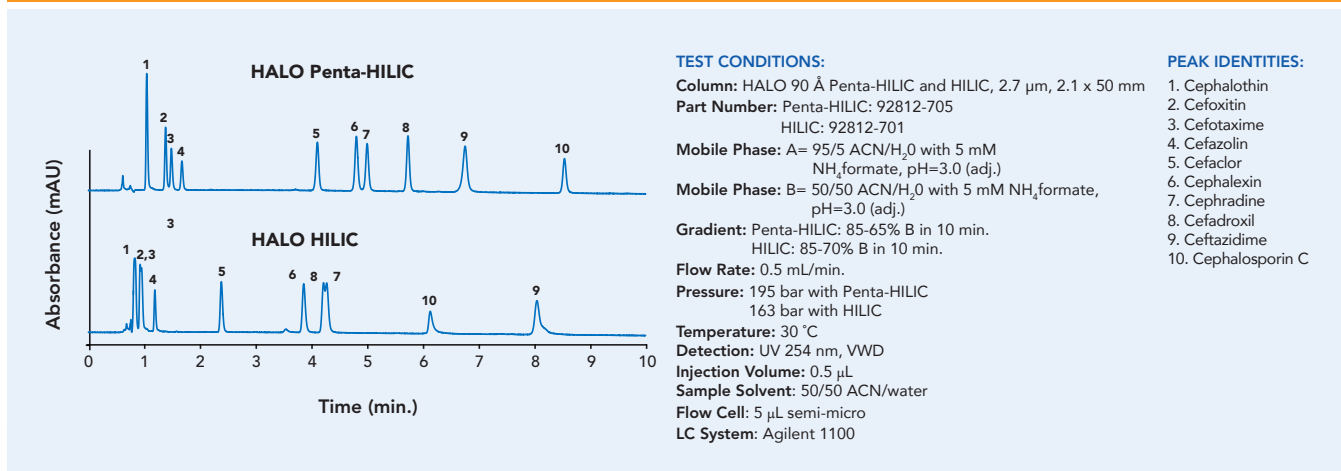
NUCLEOSIDES AND NUCLEOBASES ON HALO PENTA-HILIC

Figure S. These 15 nucleosides and nucleobases are separated in under 10 minutes using a HALO Penta-HILIC column.



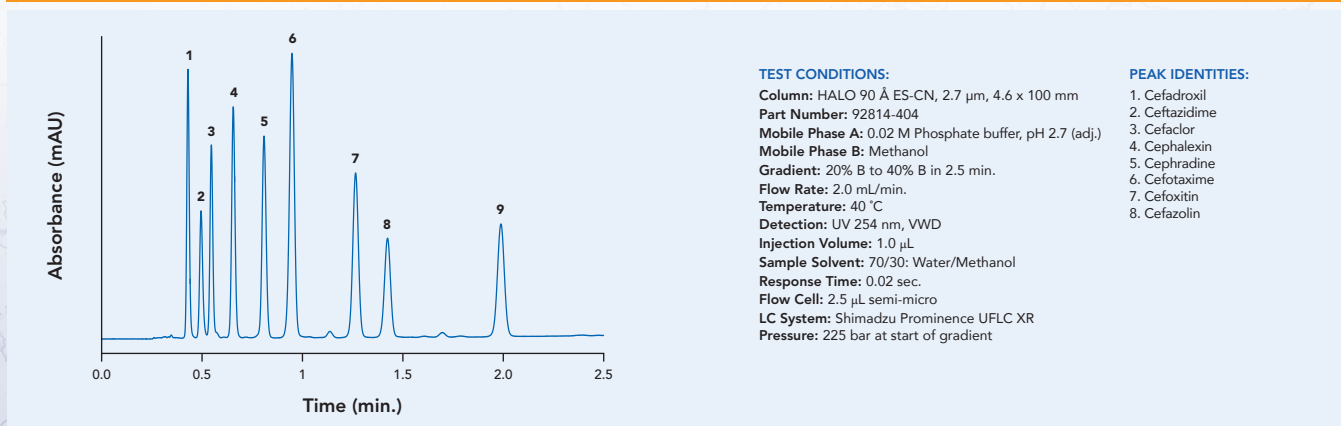
CEPHALOSPORINS ON HALO PENTA-HILIC AND HALO HILIC

Figure T. HALO Penta-HILIC shows increased retention and different selectivity vs. HALO HILIC for these 10 cephalosporins.



REVERSED-PHASE SEPARATION OF CEPHALOSPORINS USING HALO ES-CN

Figure U. HALO HILIC and Penta-HILIC columns often offer an orthogonal separation relative to reversed-phase separations, as shown here for HALO ES-CN for a subset of the same cephalosporins shown in Figure T.

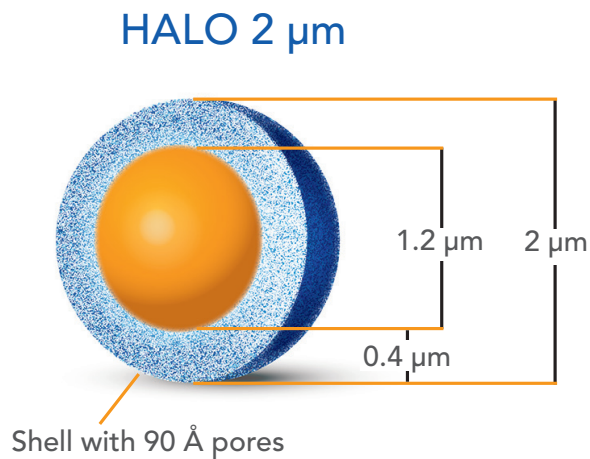


HALO 90 Å 2 µm (UHPLC)

Highest UHPLC performance possible without the disadvantages of sub-2 µm columns

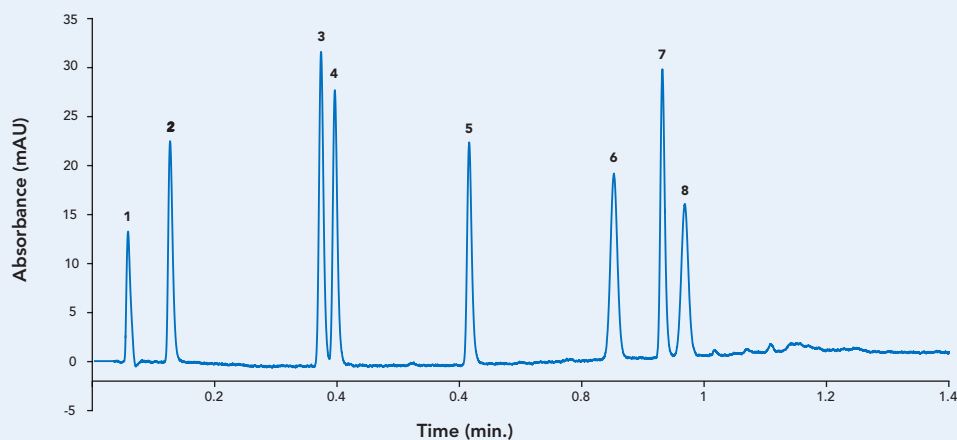
- † Use when the highest efficiency is needed
- † Excellent for fast method development and column/condition screening
- † Best performance obtained with instrumentation having extracolumn volume (IBW < 10 µL)
- † Ruggedness for R&D
- † 1 µm inlet frit
- † Pressure limit, 1000 bar/14,500 psi

Extremely high efficiency columns such as the HALO 90 Å 2 µm columns require minimal band dispersion to see the greatest benefit.



ULTRA-FAST SEPARATION OF ANTICOAGULANTS USING HALO 90 Å C18, 2 µm

Figure V. This separation of anticoagulants is completed in one minute using a short 2.1 x 30 mm HALO C18 column using a Shimadzu Nexera UHPLC system.



TEST CONDITIONS:

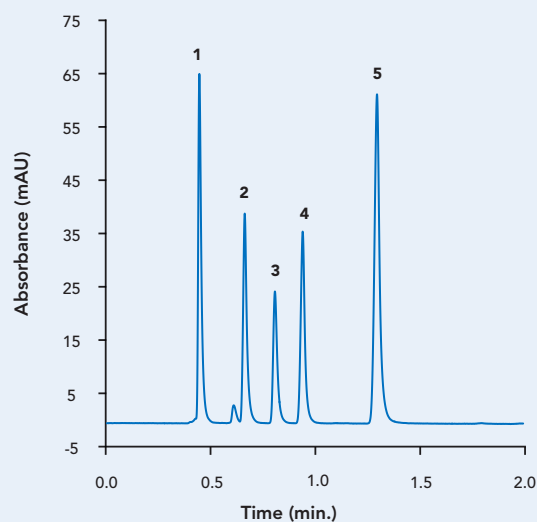
Column: HALO 90 Å C18, 2 µm, 2.1 x 30 mm
 Part Number: 91812-302
 Mobile Phase A: 20 mM Formic acid
 Mobile Phase B: 50/50 Acetonitrile/Methanol
 Gradient: Time %B
 0-0.06 20
 0.06-1.06 20-75
 Flow Rate: 1.1 mL/min.
 Temperature: 45 °C
 Detection: 254 nm
 Injection Volume: 0.2 µL
 Maximum Pressure: 430 bar

PEAK IDENTITIES:

1. Uracil (t_r)
2. 6,7-Dihydroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 6-Chloro-4-hydroxycoumarin
6. Warfarin
7. Coumatetralyl
8. Coumachlor

FAST LOCAL ANESTHETIC SEPARATION USING HALO 2 μ m PENTA-HILIC

Figure W. This mixture of five local anesthetics is resolved isocratically in 1.5 minutes using a HALO 2 μ m Penta-HILIC column.



TEST CONDITIONS:

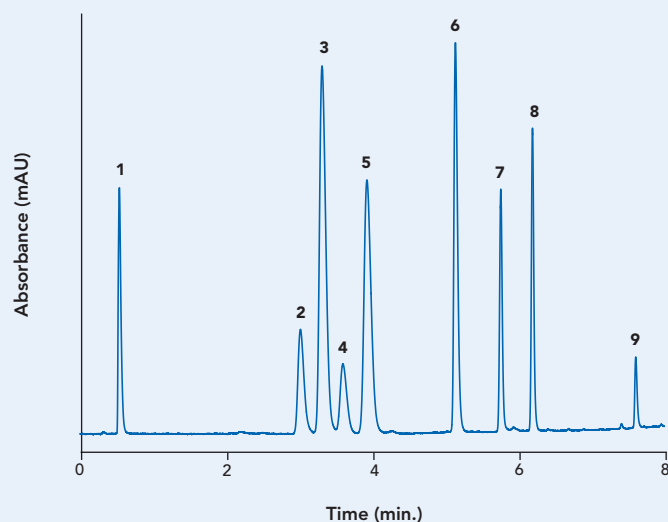
Column: HALO 90 Å Penta-HILIC, 2 μ m, 2.1 x 100 mm
 Part Number: 91812-605
 Isocratic: 92/8: ACN/water with 5mM Ammonium Formate buffer, pH 3
 Flow Rate: 0.5 mL/min.
 Temperature: 30 °C
 Detection: UV 245 nm, photodiode array detector
 Injection Volume: 1.0 μ L
 Sample Solvent: 90/10 ACN/0.1 M ammonium formate buffer pH3
 Data Rate: 40 Hz
 Response Time: 0.1 sec.
 Flow Cell: 2.5 μ L semi-micro
 Pressure: 229 bar
 LC System: Agilent 1200 SL

PEAK IDENTITIES:

1. Benzocaine
2. Lidocaine
3. Tetracaine
4. Procaine
5. Procainamide

STEROID SEPARATION USING HALO 2 μ m PFP

Figure X. HALO PFP columns often show excellent selectivity for steroids. HALO 2 μ m PFP is able to readily separate a mixture of 9 steroids in less than 8 minutes in gradient mode.



TEST CONDITIONS:

Column: HALO 90 Å PFP, 2 μ m, 3.0 x 50 mm
 Part Number: 91813-409
 Mobile Phase A: water
 Mobile Phase B: methanol

Gradient: Time	%B
0 min.	47
3 min.	47
8 min.	88

 Flow Rate: 0.4 mL/min.
 Temperature: 35 °C
 Pressure: 180 bar initial
 Detection: UV 280 nm, WVD
 Injection volume: 2 μ L
 Sample Solvent: methanol
 Response Time: 0.02 sec.
 Flow Cell: 2.5 μ L semi-micro
 LC System: Shimadzu Prominence UFLC XR

PEAK IDENTITIES:

1. Uracil
2. Hydrocortisone
3. Prednisolone
4. Cortisone
5. Prednisone
6. Dexamethasone
7. β -Estradiol
8. Estrone
9. Halcinonide

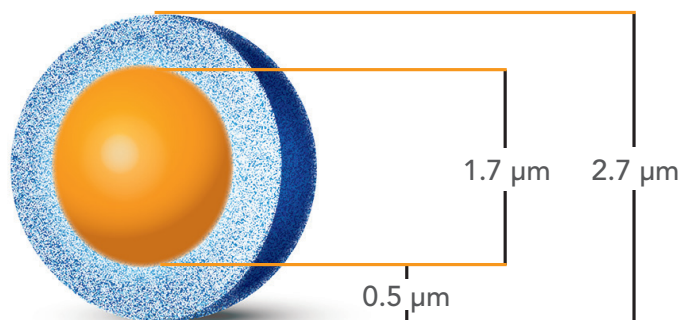
HALO 90 Å 2.7 µm (UHPLC AND HPLC)

Reliable, efficient performance with lower back pressure compared to all sub-2 µm columns

- + Use for high speed or high resolution with UHPLC or HPLC applications
- + Excellent for R&D and routine analyses
- + 2 µm inlet frit
- + Pressure limit, 600 bar/9000 psi

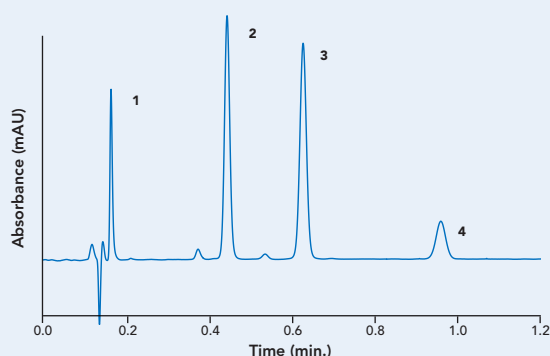
* HALO C30 160 Å, 2.7 µm

HALO 2.7 µm



ULTRAFAST SEPARATION OF STATIN DRUGS

Figure Y. These common statin drugs are separated in 1 minute using a 4.6 x 50 mm HALO Phenyl-Hexyl column.



TEST CONDITIONS:

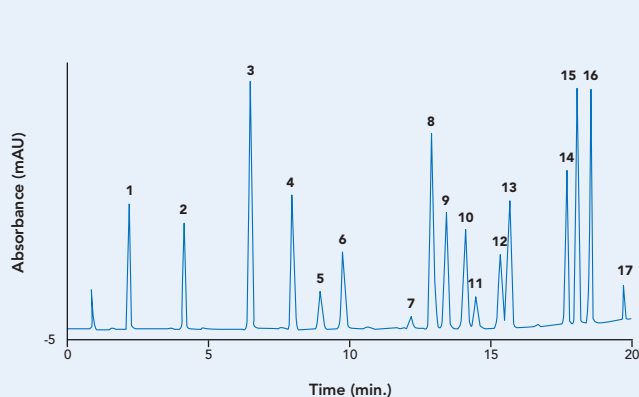
Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
 Part Number: 92814-406
 Mobile Phase: 43/57: A/B
 A: 0.02 M formic acid in water
 B: Acetonitrile
 Flow Rate: 2.5 mL/min.
 Pressure: 228 Bar
 Temperature: 26 °C
 Detection: UV 240 nm, WWD
 Injection Volume: 0.5 µL
 Sample Solvent: 80/20 methanol/water (20 mM formic acid)
 Response Time: 0.02 sec.
 Flow Cell: 2.5 µL semi-micro
 LC System: Shimadzu Prominence UFLC XR

PEAK IDENTITIES:

1. Pravastatin
2. Atorvastatin
3. Mevastatin
4. Simvastatin

HIGH RESOLUTION SEPARATION OF EXPLOSIVES

Figure Z. In this example, a 4.6 x 150 mm HALO C18 column is used to resolve 17 explosives in 20 minutes. This separation is quite sensitive to temperature, and was optimized using gradient time x temperature ($t_g \times T$) computer modeling and simulation using DryLab® software.



TEST CONDITIONS:

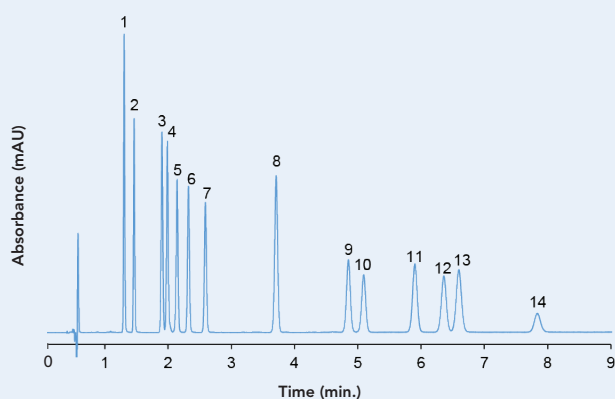
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
 Part Number: 92814-702
 Mobile Phase A: Water
 Mobile Phase B: Methanol
 Gradient: Time %B
 0.0 25
 14.0 35
 20.0 62
 Flow Rate: 1.5 mL/min.
 Temperature: 43 °C
 Detection: UV 220 nm, WWD
 Injection Volume: 40 µL
 Sample Solvent: 50/50: Water/methanol
 Response Time: 0.02 sec.
 Data rate: 25 Hz
 Pressure: 366 bar to start, max. 405 bar
 Flow Cell: 2.5 µL semi-micro
 LC System: Shimadzu Prominence UFLC XR

PEAK IDENTITIES:

1. HMX
2. RDX
3. 1,3,5-Trinitrotoluene
4. 1,3-Dinitrotoluene
5. 3,5-Dinitroaniline
6. Nitrobenzene
7. Nitroglycerin
8. Tetryl
9. 2-Amino-4,6-Dinitrotoluene
10. 4-Amino-2,6-Dinitrotoluene
11. 2,4-Dinitrotoluene
12. 2,6-Dinitrotoluene
13. 2-Nitrotoluene
14. 2-Nitrotoluene
15. 4-Nitrotoluene
16. 3-Nitrotoluene
17. PETN (pentaerythritol tetranitrate)

EFFICIENT CANNABINOID SEPARATION ON HALO 90 Å C18

Figure AA. Fourteen cannabinoids are resolved in less than eight minutes using a HALO 90 Å C18 column.



TEST CONDITIONS:

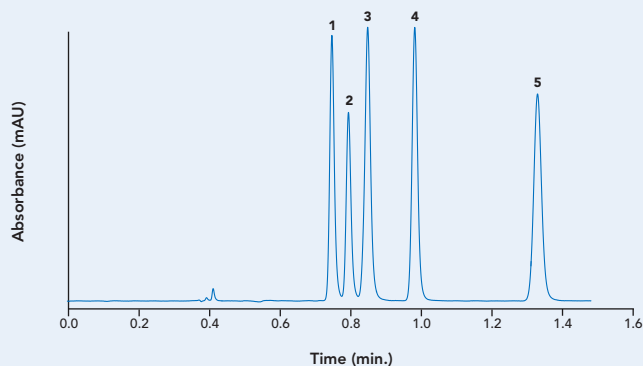
Column: HALO 90 Å C18, 2.7 μ m, 3.0 x 150 mm
 Part Number: 92813-702
 Mobile Phase: 25/75 A/B
 A: Water/0.1% formic acid
 B: Acetonitrile/0.085% formic acid
 Flow Rate: 1.0 mL/min
 Pressure: 350 bar
 Temperature: 30 °C
 Detection: UV 220 nm, PDA
 Injection: 0.6 μ L
 Sample Solvent: 75/25 methanol/ water
 Response Time: 0.025 sec.
 Data Rate: 100 Hz
 Flow Cell: 1 μ L
 LC System: Shimadzu Nexera X2

PEAK IDENTITIES:

1. Cannabidivarinic acid (CBDVA)
2. Cannabidvarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Cannabinol (CBN)
9. delta-9- Tetrahydrocannabinol (Δ 9-THC)
10. delta-8-Tetrahydrocannabinol (Δ 8-THC)
11. Cannabicyclol (CBL)
12. Cannabichromene (CBC)
13. delta-9-Tetrahydrocannabinolic acid A (THCA)
14. Cannabichromenic acid (CBCA)

ULTRAFAST SEPARATION OF TRICYCLIC ANTIDEPRESSANTS

Figure BB. These basic tricyclic antidepressants are separated in less than two minutes, with excellent peak shape, using a HALO Penta-HILIC column.



TEST CONDITIONS:

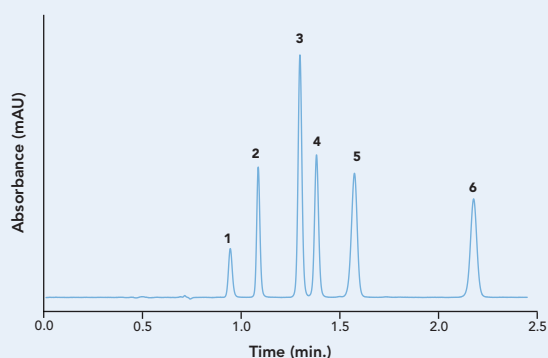
Column: HALO 90 Å Penta HILIC, 2.7 μ m, 4.6 x 100 mm
 Part Number: 92814-605
 Mobile Phase: 7/93: A/B
 A: 0.1 M Ammonium formate, pH=3.5 (adj.)
 B: Acetonitrile
 Flow Rate: 2.5 mL/min.
 Temperature: 30 °C
 Detection: UV 254 nm, WWD
 Injection Volume: 0.5 μ L
 Sample Solvent: 10/90: Water/acetonitrile
 Response Time: 0.02 sec.
 Maximum Pressure: 165 Bar
 Flow Cell: 2.5 μ L semi-micro
 LC System: Shimadzu Prominence UFLC XR

PEAK IDENTITIES:

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

HIGH RESOLUTION OF NEONICOTINOIDS ON HALO 2.7 μ m ES-CN

Figure CC. Six neonicotinoids are separated using a HALO 2.7 μ m ES-CN column. The sub-3 μ m Fused-Core silica-based packing allows rapid separations at modest pressures.



TEST CONDITIONS:

Column: HALO 90 Å ES-CN, 2.7 μ m, 4.6 x 100 mm
 Part Number: 92814-604
 Mobile Phase: 70/30: A/B
 A: 0.1% Formic acid in water
 B: Acetonitrile
 Flow Rate: 1.5 mL/min.
 Pressure: 205 Bar
 Temperature: 35 °C
 Detection: UV 254 nm, WWD
 Injection Volume: 0.5 μ L
 Sample Solvent: Acetonitrile
 Response Time: 0.02 sec.
 Flow Cell: 2.5 μ L semi-micro
 LC System: Shimadzu Prominence UFLC XR

PEAK IDENTITIES:

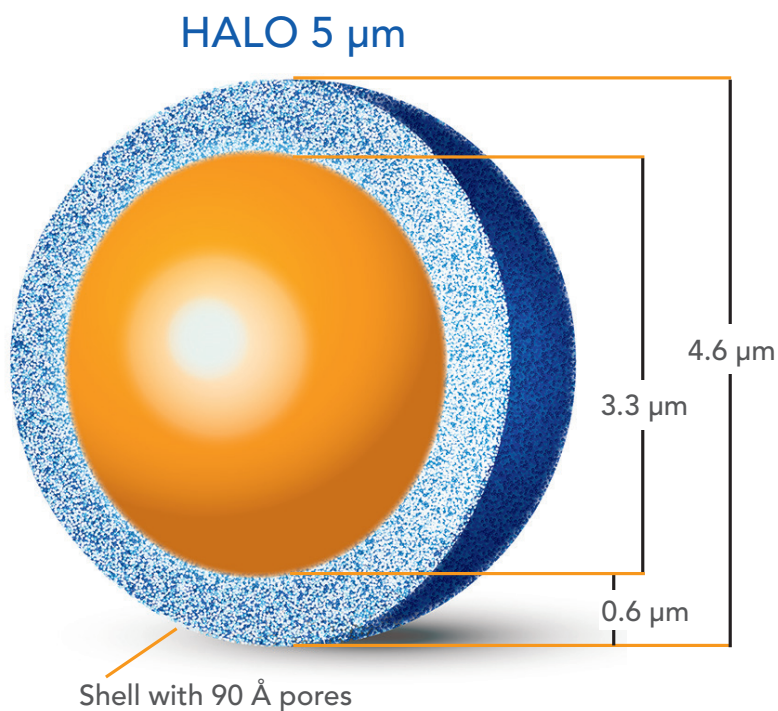
1. Nitenpyram
2. Thiamethoxam
3. Clothianidin
4. Imidacloprid
5. Acetamiprid
6. Thiacloprid

HALO 90 Å 5 µm (HPLC)

Performance of 3 µm non-core column
at 5 µm column pressures

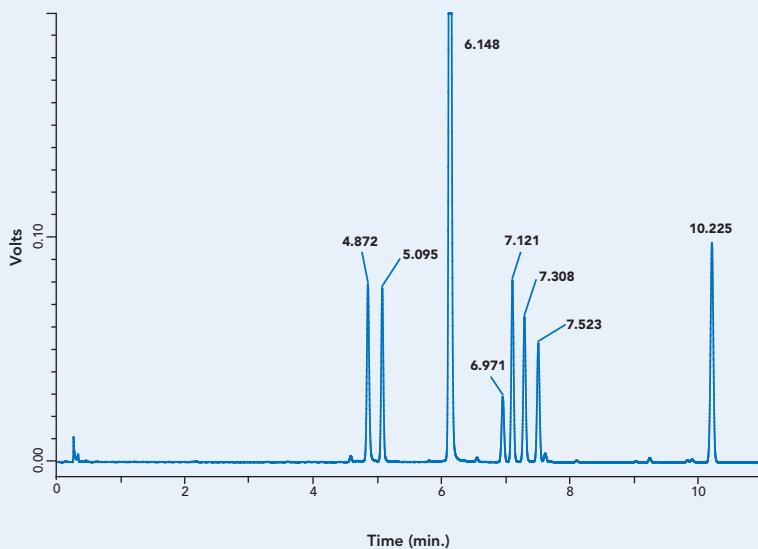
Ideal for:

- QC laboratories
- Dirty samples
- High throughput, ballistic gradient and isocratic applications
- High resolution at HPLC back pressures (using columns in series)
- 2 µm inlet frit
- Pressure limit, 600 bar/9000 psi



FAST, HIGH RESOLUTION GRADIENT FLAVONOID SEPARATION

Figure DD.
This mixture of 8 flavonoids is baseline resolved in less than 11 minutes using a 2.1 x 150 mm HALO 5 µm C18 column with a fast 1.0-mL/min. flow rate with an LC-MS-compatible mobile phase.



SAMPLE:

Mixture of 8 flavonoids, 1 µL in MeOH

TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm, 2.1 x 150 mm
 Part Number: 95812-702
 Flow Rate: 1.0 mL/min.
 Temperature: 40 °C
 Gradient: 5% CH₃CN for 0.5 min.
 5-60% CH₃CN/10 mM NH₄COO
 (0.1% HCOOH) in 15 min.
 Max. Pressure: 280 bar

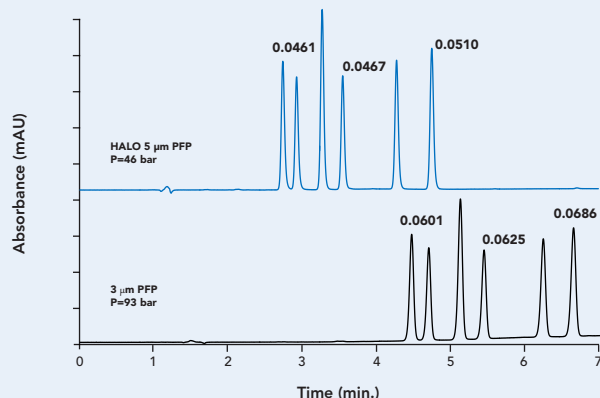
ANALYTES:

1. Hesperidin
2. Myricetin
3. Quercetin
4. Naringenin
5. Apigenin
6. Hesperetin
7. Kaempferol
8. Biochanin

BENZODIAZEPINE SEPARATION USING HALO 5 µm PFP

Figure EE.

These six benzodiazepine drugs are separated in 5 minutes with better performance than a 3 µm non-core column at ½ the pressure.



TEST CONDITIONS:

Column: HALO 90 Å PFP, 5 µm, 4.6 x 100 mm
 Part Number: 95814-609
 Mobile Phase A: 25 mM Ammonium Acetate, pH 5.5
 Mobile Phase B: ACN, 36-65% B in 7 min.
 Temperature: 35 °C
 Flow: 0.75mL/min.
 Detector: UV at 254 nm
 Injection: 1 µL

PEAK IDENTITIES:

1. Oxazepam
2. Lorazepam
3. Nitrazepam
4. Clonazepam
5. Flunitrazepam
6. Diazepam

NOTE:

Peak widths at half height are labeled for comparable peaks on both columns.

Comparative results presented here may not be representative for all applications.

LC-MS ANALYSIS OF STEVIA GLYCOSIDES USING HALO PENTA-HILIC

Figure FF.

LC-MS analysis of stevia glycosides from a Stevia natural sweetener extract is easily accomplished using the HALO 5 µm Penta-HILIC column due to its unique bonded phase containing five OH groups.

TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 5 µm, 3.0 x 250 mm
 Part Number: 95813-905
 Mobile Phase A: 50/50 Water/acetonitrile with 5 mM Ammonium formate, pH 3
 Mobile Phase B: 5/95 Water/acetonitrile with 5 mM Ammonium formate, pH 3
 Gradient: 90% B to 67% B over 30 min.

Flow Rate: 0.5 mL/min.

Pressure: 60 bar

Temperature: Ambient

Injection Volume: 5 µL

Sample Solvent: 80/20: Acetonitrile/water

LC System: Shimadzu Nexera

MS: Shimadzu LCMS 2020 (single quadrupole)

ESI: +4.5 kV

Scan Range: 200-1200 m/z

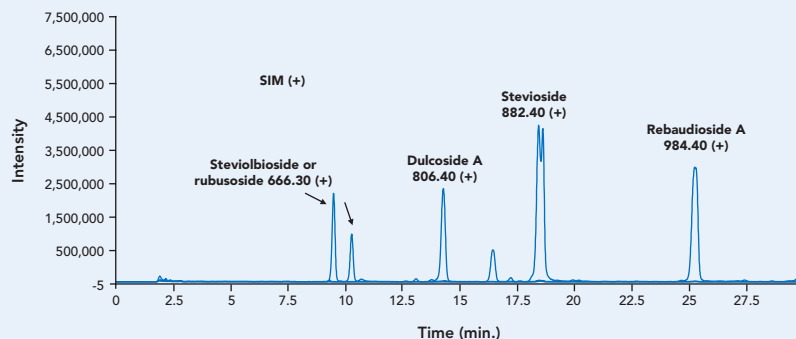
Scan Rate: 2 pps

Capillary: 250 °C

Heat Block: 350 °C

Nebulizing Gas Flow: 1.5 L/min.

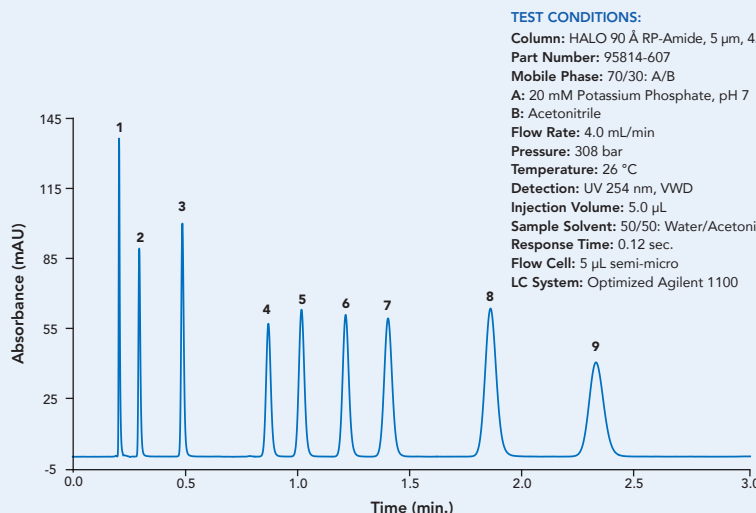
Drying Gas Flow: 15 L/min.



POLAR AROMATIC COMPOUNDS ON HALO 5 µm RP-AMIDE

Figure GG.

HALO 5 µm RP-Amide shows excellent resolution and peak shape for this mixture of polar aromatic compounds.



TEST CONDITIONS:

Column: HALO 90 Å RP-Amide, 5 µm, 4.6 x 100 mm

Part Number: 95814-607

Mobile Phase: 70/30: A/B

A: 20 mM Potassium Phosphate, pH 7

B: Acetonitrile

Flow Rate: 4.0 mL/min

Pressure: 308 bar

Temperature: 26 °C

Detection: UV 254 nm, VWD

Injection Volume: 5.0 µL

Sample Solvent: 50/50: Water/Acetonitrile

Response Time: 0.12 sec.

Flow Cell: 5 µL semi-micro

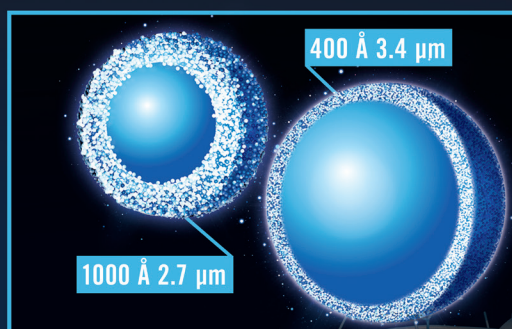
LC System: Optimized Agilent 1100

PEAK IDENTITIES:

1. Uracil
2. Benzamide
3. Aniline
4. Cinnamyl Alcohol
5. Dimethyl Phthalate
6. 2-Nitroaniline
7. 4'-Bromoacetanilide
8. 2,2'-Biphenol
9. 4,4'-Biphenol

Table F. HALO BioClass Column Specifications

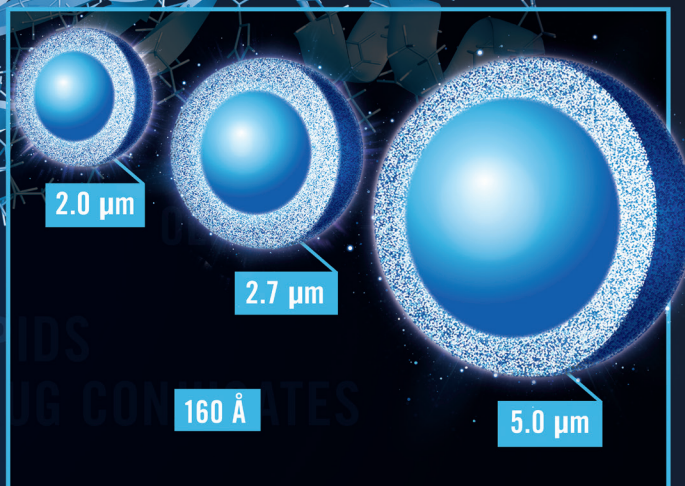
Bonded Phase	USP Designation	Particle Sizes (s) (μm)	Pore Size (\AA)	Carbon Load (%)	Surface Area (m^2/g)	Low pH/T Limit	High pH/T Limit	Endcapped	
Protein	C4	L26	2.7	1000	0.6	22	2/90 °C	9/40 °C	Yes
	ES-C18	L1	2.7	1000	1.4	22	1/90 °C	8/40 °C	Yes
	Diphenyl	L11	2.7	1000	1.0	22	2/90 °C	9/40 °C	Yes
	C4	L26	3.4	400	0.4	15	2/90 °C	9/40 °C	Yes
	ES-C18	L1	3.4	400	1.0	15	1/90 °C	8/40 °C	Yes
Peptide	ES-C18	L1	2	160	4.0	65	1/90 °C	8/40 °C	No
			2.7		4.6	90			
			5		4.0	60			
Peptide	ES-CN	L10	2.7	160	2.2	90	1/90 °C	8/40 °C	Yes
			5		1.5	60			
Peptide	Phenyl-Hexyl	L11	2.7	160	4.7	90	2/90 °C	9/40 °C	Yes
Glycan	Proprietary Ligand	L95	2.7	90	3.2	135	2/65 °C	9/40 °C	No



PROTEIN SEPARATIONS



GLYCAN SEPARATIONS



PEPTIDE SEPARATIONS

PROTEIN
ES-C18
MABS

PHENYL-HEXYL
FRAGMENTS
PEPTIDE

GLYCAN

GLYCOLIPIDS
ANTIBODY-DRUG CONJUGATES
VARIANTS

POLYPEPTIDES

Table G. HALO BioClass Features & Benefits

Bonded Phase		Features and Benefits	Target Analytes	Best Applications
1000 Å Protein	C4 (dimethylbutylsilane)	<ul style="list-style-type: none"> Outstanding high temperature stability at low pH Unrestricted access to bonded phase Exceptional mass transfer kinetics Compatible with UHPLC and HPLC Low LC-MS bleed 	Monoclonal antibodies, antibody-drug conjugates, antibody fragments and large proteins with MWs ≤ 500 kDa	High resolution separations of monoclonal antibodies and their variants and antibody-drug conjugates
	ES-C18 (diisobutyloctadecylsilane)	<ul style="list-style-type: none"> Even better stability up to 90°C Can elute very large proteins with good peak shape and recovery Compatible with UHPLC and HPLC Very low LC-MS bleed 	Monoclonal antibodies, antibody-drug conjugates, antibody fragments and large proteins with MWs ≤ 500 kDa	High resolution separations of monoclonal antibodies and their variants and antibody-drug conjugates
	Diphenyl (diphenylmethyl)	<ul style="list-style-type: none"> Outstanding temperature stability from 40°C to 90°C Exceptional low temperature performance without peak area loss Compatible with UHPLC and HPLC Low LC-MS bleed 	Monoclonal antibodies, antibody-drug conjugates, antibody fragments and large proteins with MWs ≤ 500 kDa	High resolution separations of monoclonal antibodies and their variants and antibody-drug conjugates
400 Å Protein	C4 (dimethylbutylsilane)	<ul style="list-style-type: none"> Stability up to 90°C Can elute very large proteins with good peak shape and recovery Compatible with UHPLC and HPLC Low LC-MS bleed 	Monoclonal antibodies, proteins and polypeptides < 500 kDa	Monoclonal antibodies and mid-to-high molecular weight proteins and polypeptides
	ES-C18 (diisobutyloctadecylsilane)	<ul style="list-style-type: none"> Even better stability up to 90°C Can elute very large proteins with good peak shape and recovery Compatible with UHPLC and HPLC Very low LC-MS bleed 	Proteins and polypeptides < 500 kDa	Mid-to-high molecular weight proteins and polypeptides
160 Å Peptide	ES-C18 (diisobutyloctadecylsilane)	<ul style="list-style-type: none"> Fast separations High peak capacity Rugged, reliable performance Use with either UHPLC or HPLC 	Peptides and polypeptides < 20 kDa	Intermediate molecular weight proteins and polypeptides
	ES-CN (diisopropylcyanopropylsilane)	<ul style="list-style-type: none"> Alternative selectivity to ES-C18 and Phenyl-Hexyl for peptide mapping and proteomic applications 	Peptides and polypeptides < 20 kDa	Intermediate molecular weight proteins and polypeptides
	Phenyl-Hexyl (dimethylphenyl-hexylsilane)	<ul style="list-style-type: none"> Alternative selectivity to ES-C18 and ES-CN for peptide mapping and proteomic applications 	Peptides and polypeptides < 20 kDa	Intermediate molecular weight proteins and polypeptides
Glycan	Proprietary hydrophilic ligand	<ul style="list-style-type: none"> Improved retention of acids and zwitterions Very low sensitivity to buffer concentration Able to separate isobaric oligosaccharides with different linkages 	Glycans (< 20 kDa), glycopeptides and polar peptides	Provides orthogonal HILIC selectivity to HALO Peptide ES-C18

HALO ENABLED LARGE MOLECULE ANALYSIS

Today, researchers are keenly interested in both fast and high-resolution separations of numerous biomolecules. The HALO Fused-Core technology supports the development of novel therapeutic proteins and peptides in pharmaceutical drug development to advance understanding in modern university laboratories, enabling researchers to characterize protein post-translational modifications and fully assess subtle differences in biosimilars and other products of bioengineering and manufacture. HALO BioClass columns have been specifically designed to accomplish these bioseparation goals with a simplified and more effective solution.

With both tailored particle and pore size options, HALO BioClass offers application specific solutions for:

- ✦ Intact proteins, monoclonal antibodies (mAbs), biosimilars, and other large biomolecules such as pegylated proteins, antibody drug conjugates (ADCs), etc.
- ✦ Peptide mapping (analysis of enzyme digests) for characterization and monitoring of synthetic protein drugs
- ✦ Analysis of therapeutic peptides and peptide biomarkers (protein surrogates)
- ✦ High resolution separations of complex mixtures of glycans released from N- and O-linked glycoproteins

PROTEIN SOLUTIONS

† As the first manufacturer of the 1000 Å fused-core particle, AMT recognizes the benefit of unrestricted pore access and offers both 400 Å and 1000 Å products to tailor the perfect large molecule solution.

† Benefits of HALO protein solutions include:

- Provides narrower peaks and better recoveries for large biomolecules (vs. smaller pore sizes and non-core particles)
- Allows HALO Protein columns to be used with both UHPLC and HPLC instrumentation for fast bioseparations at moderate back pressures

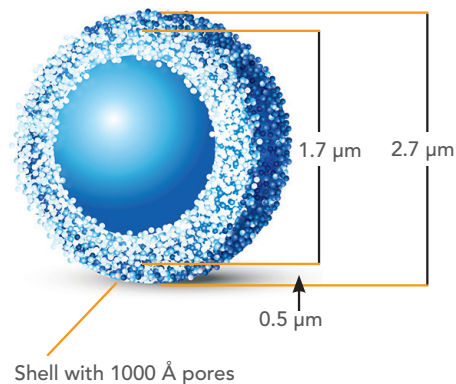
† C4, Diphenyl and sterically-protected ES-C18 phases

- Excellent high temperature stability (up to 90 °C) for improved peak shape and recovery

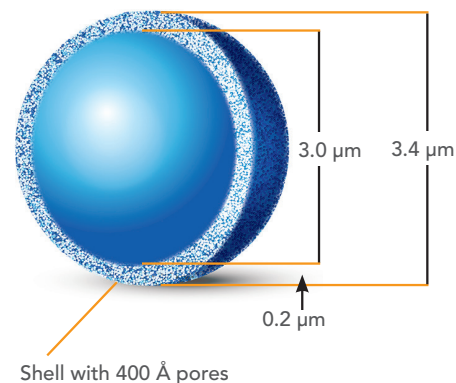
† 2 µm inlet frit

† Pressure limit, 600 bar/9000 psi

HALO 1000 Å 2.7 µm

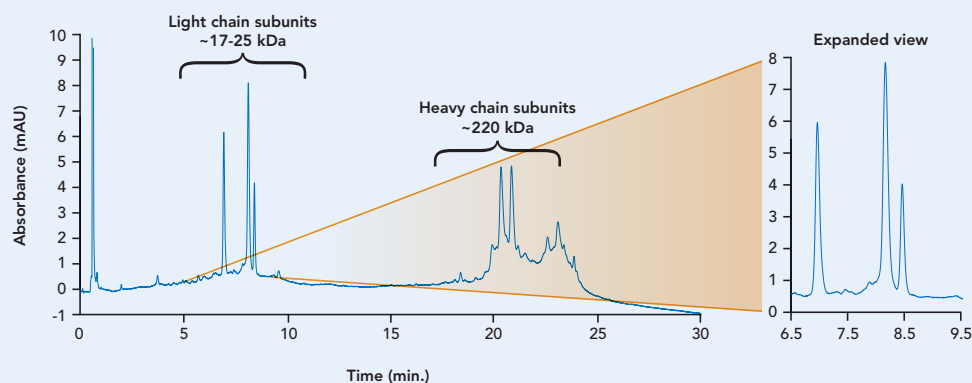


HALO 400 Å 3.4 µm



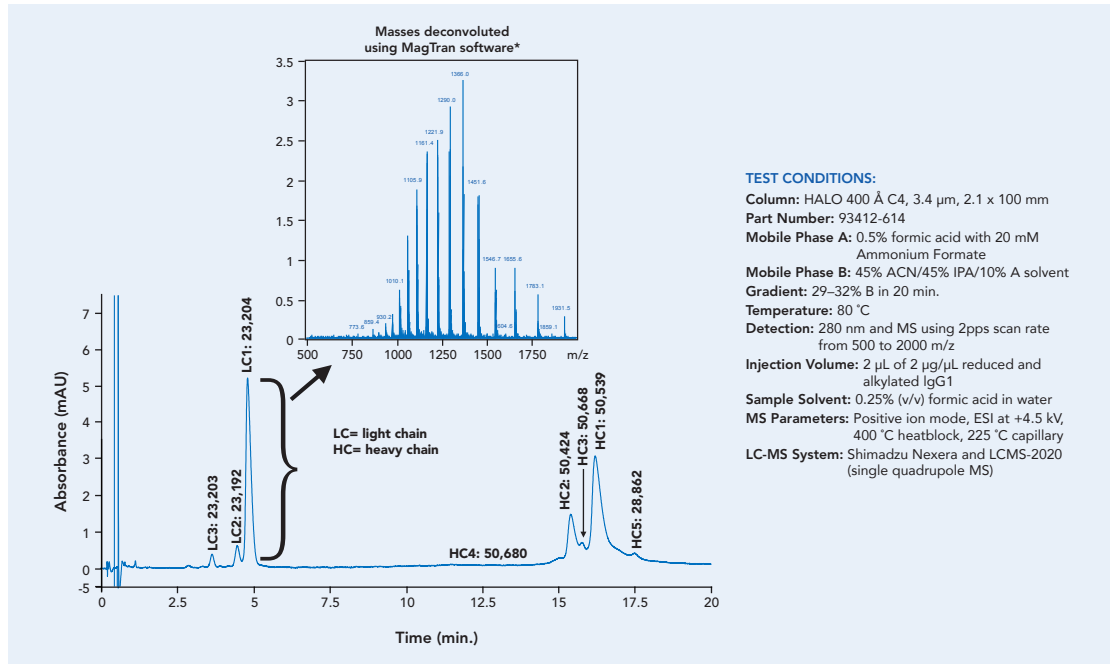
LARGE PROTEIN SEPARATION USING HALO PROTEIN C4 FUSED-CORE COLUMN

Figure HH. High resolution separation of light and heavy chains of a denatured contractile protein (whole myosin from purified rabbit skeletal muscle) using HALO 400 Å Protein C4 at 80 °C.



HIGH RESOLUTION OF LIGHT AND HEAVY CHAIN VARIANTS OF IgG1

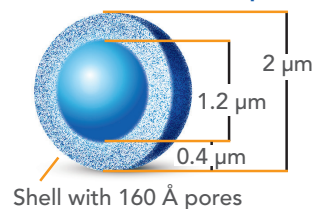
Figure II. Very high resolution is obtained between variants of light and heavy chains of a reduced and alkylated monoclonal antibody (IgG1) sample using a HALO 400 Å Protein C4 column.



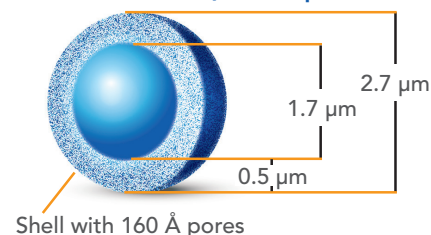
PEPTIDE SOLUTIONS

- ♦ Extremely stable at high temperatures and low pH
- ♦ Ideal for both ultrafast and ultrahigh resolution separations of peptides and polypeptides up to 20 kDa
- ♦ Outperforms non-core 3 μm , 300 Angstrom columns in terms of peak width, peak capacity and peak height (Figure MM)
- ♦ Offers comparable peak capacity to sub-2 μm non-core columns at 40–50% back pressure (2.7 μm)
- ♦ ~ 20% higher peak capacity than sub-2 μm non-core columns at comparable back pressure (2 μm)
- ♦ Columns (Peptide 2.7 and 5 μm) can be used in series to increase peak capacity for UHPLC and HPLC analyses of complex tryptic digest samples (Figure NN)
- ♦ HALO Peptide ES-CN (2.7 and 5 μm) offers different selectivity and improved retention for polar peptides (Figure OO)
- ♦ 2 μm inlet frit (2.7 and 5 μm); 1 μm inlet frit (2 μm) provides extra protection from plugging

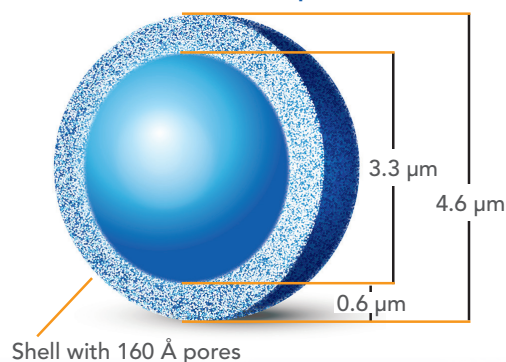
HALO 2 μm Peptide



HALO 2.7 μm Peptide

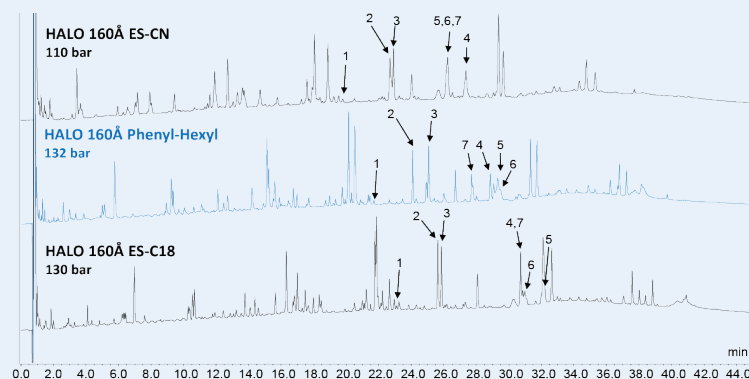


HALO 5 μm Peptide



ENHANCED SELECTIVITY WITH HALO 160 Å PHENYL-HEXYL FOR A TRYPTIC DIGEST

Figure LL. The HALO 160 Å Phenyl-Hexyl column provided improved resolution between tryptic digest fragments 2 and 3 compared to the 160 Å ES-CN column and the 160 Å ES-C18 column. Peptide identification was accomplished by using MS-MS fragmentation spectra.



TEST CONDITIONS:

Columns: HALO 160Å ES-CN, 2.7 μm , 2.1 x 100 mm
 Part Number: 92122-604
 HALO 160Å Phenyl-Hexyl, 2.7 μm , 2.1 x 100 mm
 Part Number: 92112-606
 HALO 160Å ES-C18, 2.7 μm , 2.1 x 100 mm
 Part Number: 92122-602

Mobile Phase:

A = water + 10 mM difluoroacetic acid (DFA)

B = ACN + 10 mM difluoroacetic acid

Flow Rate: 0.3 mL/min

Gradient: 2–50 %B in 60 min

Temperature: 60 °C

Detection: UV 220 nm, VWD

Injection Volume: 5 μL of 0.2 mg/mL digest

Sample Solvent: 50 mM Tris-HCl/1.5 M Guanidine-HCl with 0.25% formic acid

LC System: Shimadzu Nexera

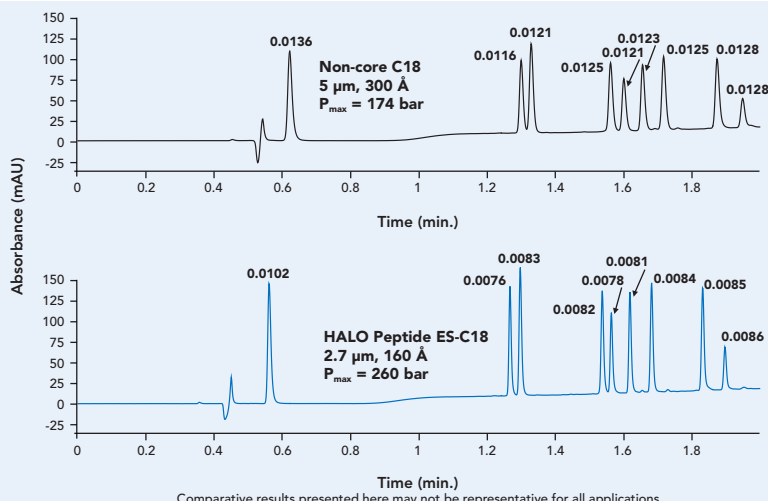
Flow Cell: 2.5 μL semi-micro

PEAK IDENTITIES:

1. FTISADTSKNTAYLQMNLSR (754 m/z)
2. LScAASGFNIKDTYIHWVR (747 m/z)
3. GFYPDIAVEWESNGQPENNYK (849 m/z)
4. LLIYSASFLYSGVPSR (592 m/z)
5. SGTASWcLLNMFYPR (899 m/z)
6. ScDKTHtPPcPAPELLGGPSVFLFPPKPK (834 m/z)
7. VSVLTVLHODWLNQKEYK (1115 m/z)

COMPARISON OF FUSED-CORE TO NON-CORE COLUMNS FOR PEPTIDE SEPARATIONS

Figure MM. HALO Peptide 160 Å 2.7 µm column produces significantly taller peaks and higher peak capacity than a non-core 3 µm column.



TEST CONDITIONS:

Columns: HALO 160 Å ES-C18, 2.7 µm, 4.6 x 100 mm and non-core C18, 3 µm, 4.6 x 100 mm
Part Number: 92124-602
Mobile Phase A: 90% water/10% ACN/0.1% TFA
Mobile Phase B: 30% water/70% ACN/0.1% TFA
Gradient: 0-87.5% B in 2 min.
Flow Rate: 2.5 mL/min.
Temperature: 60 °C
Injection Volume: 5 µL
LC System: Agilent 1100

PEAK IDENTITIES:

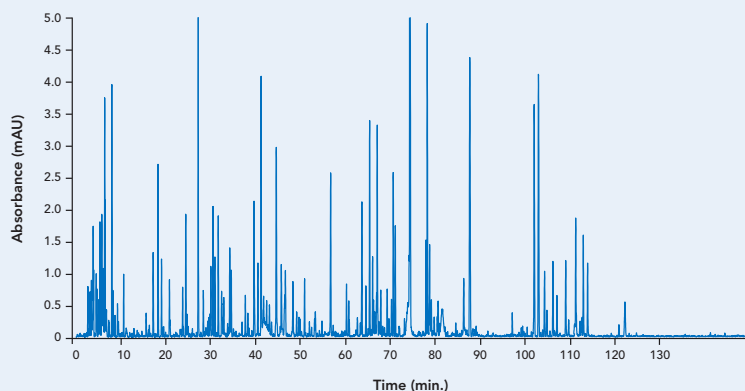
1. Gly-Tyr
2. Angiotensin 1/2 (1-7) amide
3. Val-Tyr-Val
4. Met-Enk
5. Angiotensin 1/2 (1-8) amide
6. Angiotensin II
7. Leu-Enk
8. Angiotensin (1-12) human
9. Angiotensin (1-12) mouse

Peak widths at half height are shown above respective peaks.

Comparative results presented here may not be representative for all applications.

COUPLED HALO PEPTIDE COLUMNS FOR MAXIMUM PEAK CAPACITY

Figure NN. Three HALO Peptide 160 Å ES-C18, 2.7 µm 150-mm columns (450 mm total length) were connected in series to achieve a peak capacity of 560 for this mixture of tryptic digests of α-1-glycoprotein and apotransferrin.

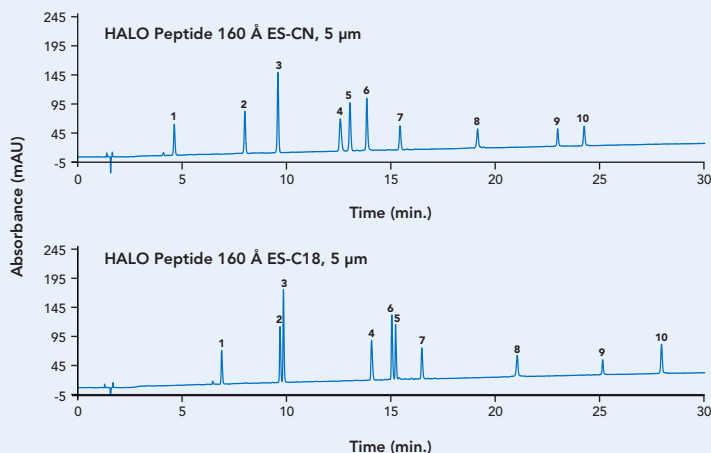


TEST CONDITIONS:

Columns: HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm (3)
Part Number: 3 of 92122-702
Mobile Phase A: water/0.1% formic acid/20 mM ammonium formate
Mobile Phase B: A with 80% acetonitrile
Gradient: 5-55% B in 150 min.
Flow Rate: 0.5 mL/min.
Temperature: 70 °C
Detection: 220 nm
Injection Volume: 50 µL [25 µg each] of α-1-glycoprotein tryptic digest and apotransferrin tryptic digest

ALTERNATE SELECTIVITY USING HALO 160 Å 5 µm PEPTIDE ES-CN

Figure OO. HALO Peptide 160 Å ES-CN, 5 µm ES-CN offers alternative selectivity to HALO Peptide 160 Å ES-C18, 5 µm for this mixture of 10 peptides and polypeptides.



TEST CONDITIONS:

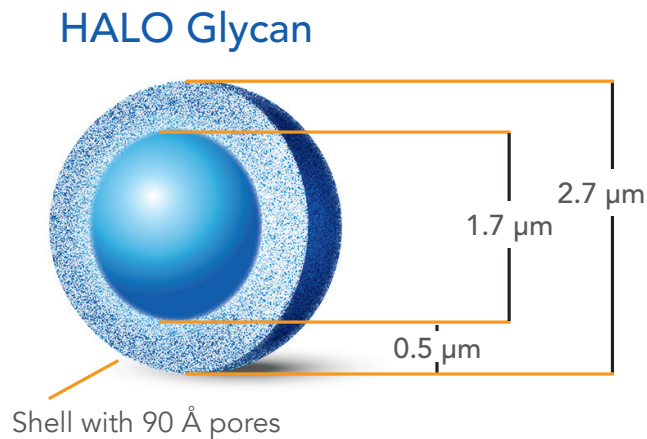
Column: HALO 160 Å ES-CN, 5 µm
HALO 160 Å ES-C18, 5 µm
Part Numbers: ES-CN: 95124-704
ES-C18: 95124-702
Instrument: Optimized Agilent 1100
Injection Volume: 10 µL
Detection: 215 nm
Temperature: 40 °C
Flow Rate: 1.0 mL/min
Mobile Phase A: water/0.1% TFA
Mobile Phase B: ACN/0.1% TFA
Gradient: 5-50% B in 30 min.

PEAK IDENTITIES:

1. Asp-Phe
2. Angiotensin (1-7) amide
3. Tyr-Tyr-Tyr
4. Bradykinin
5. Leu-Enk
6. Angiotensin II
7. Neurotensin
8. β-endorphin
9. Sauvagine
10. Mellitin

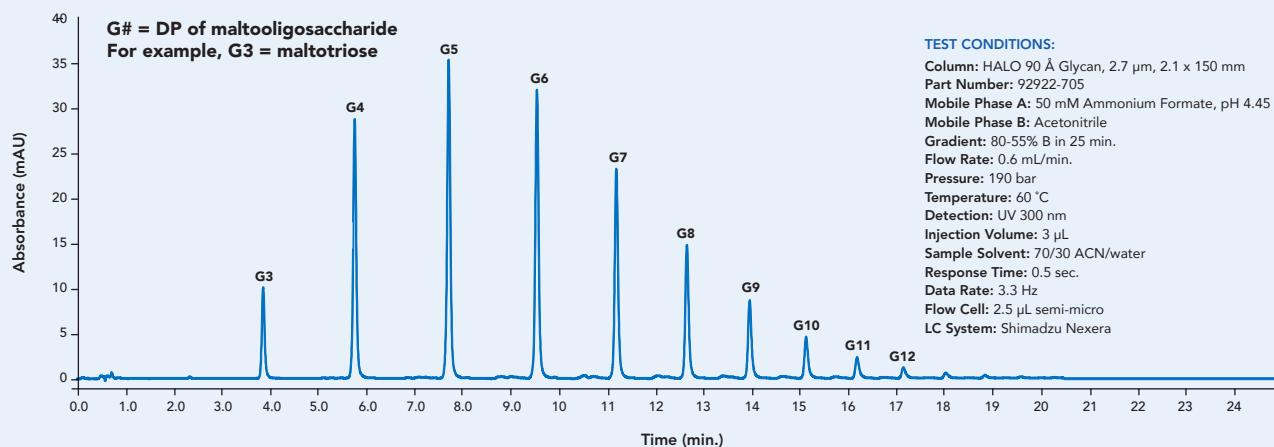
GLYCAN SOLUTIONS

- ✦ 90 Ångstrom pore size
- ✦ Incorporates a highly polar ligand that contains 5 hydroxyl groups tethered to 2.7 µm Fused-Core silica particles via novel, proprietary linkage chemistry
- ✦ Ideal for hydrophilic interaction liquid chromatography (HILIC) separations of oligosaccharides, and particularly, of released and labeled glycans from glycoproteins and proteoglycans
- ✦ Mobile phases typically consist of acetonitrile and aqueous ammonium formate buffer (50 mM, pH 4.4) used to form a gradient of increasing water content during elution
- ✦ Each lot of HALO Glycan material is tested for quality assurance (Figure PP) by separation of a procainamide-reducing-end-labeled glycan ladder of oligosaccharides having 2–25 glucose units (GU).
 - Peaks for oligosaccharides composed of 5 and 10 GU must meet tight specifications for retention and peak width before lot is approved for glycan analysis
- ✦ 2 µm inlet frit
- ✦ Pressure limit, 600 bar/9000 psi



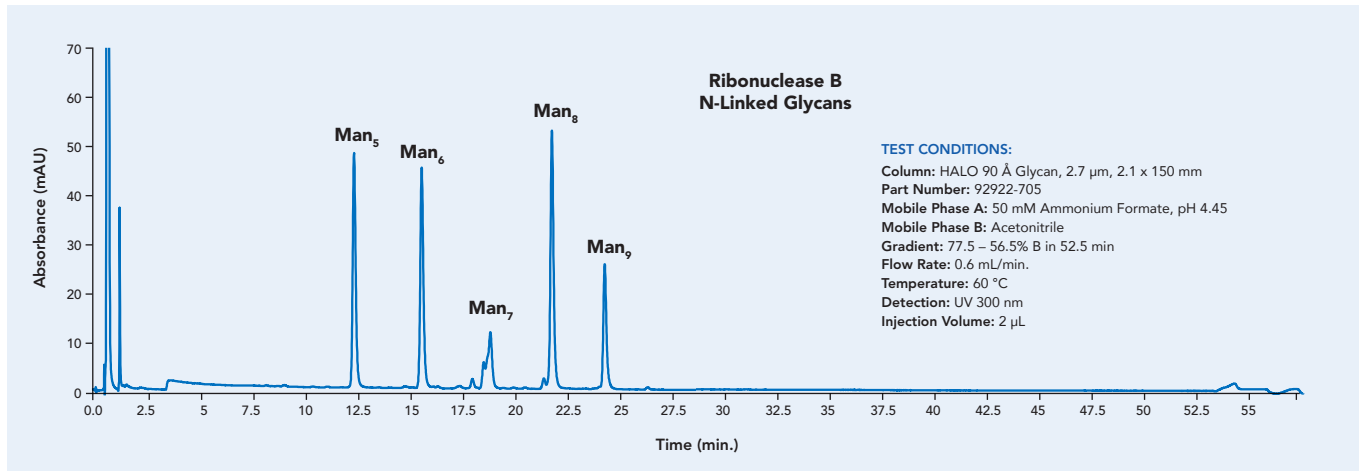
QA ANALYSIS OF HALO GLYCAN

Figure PP. Example QA Chromatogram for HALO Glycan column. Each HALO Glycan packing lot is tested using this glycan ladder mixture to assess and ensure lot-to-lot reproducibility.



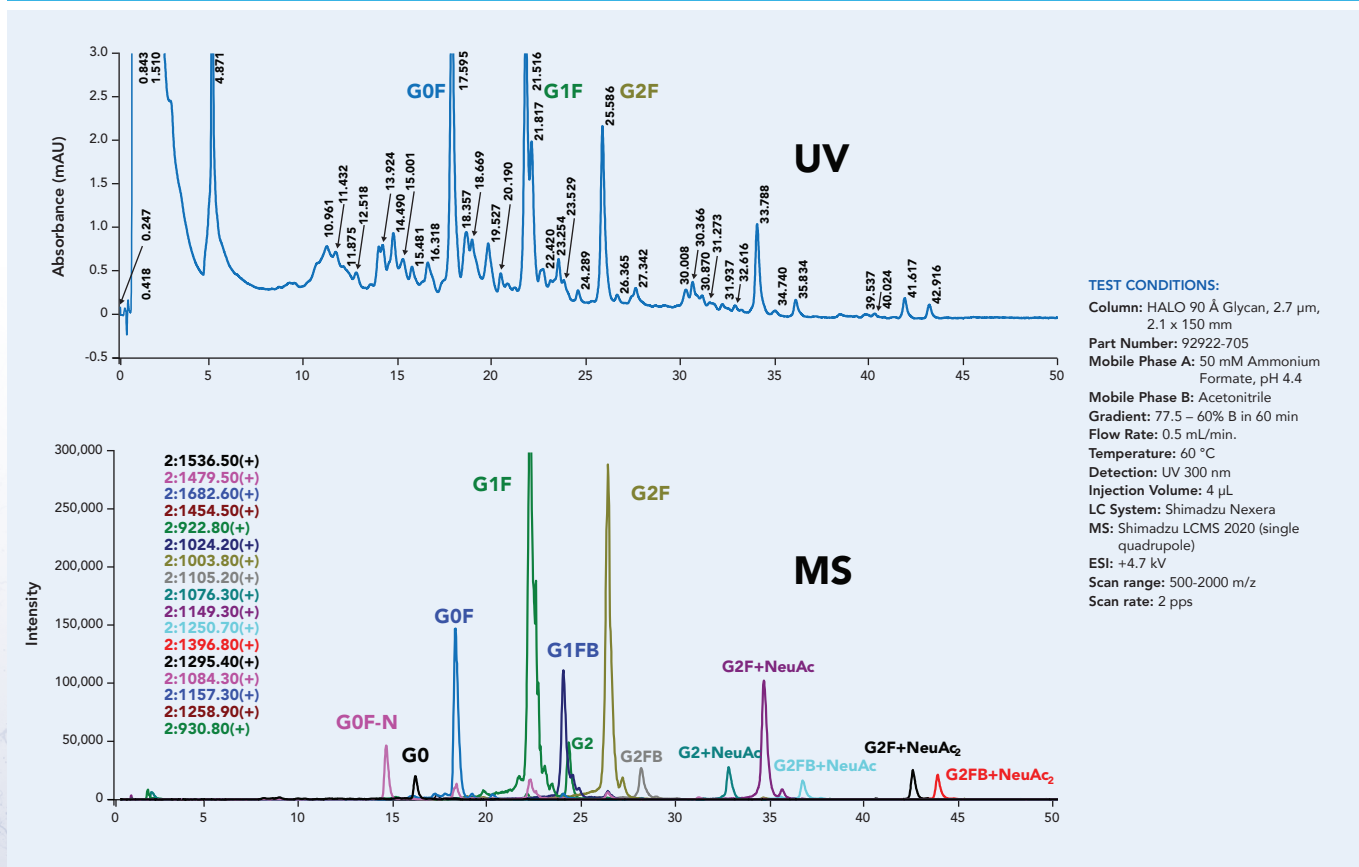
SEPARATION OF N-LINKED GLYCANS FROM RIBONUCLEASE B

Figure QQ. Gradient HILIC-MS separation of N-linked glycans, which had been released using PNGase from ribonuclease B, using the HALO Glycan column.



SEPARATION OF N-LINKED GLYCANS FROM HUMAN IgG

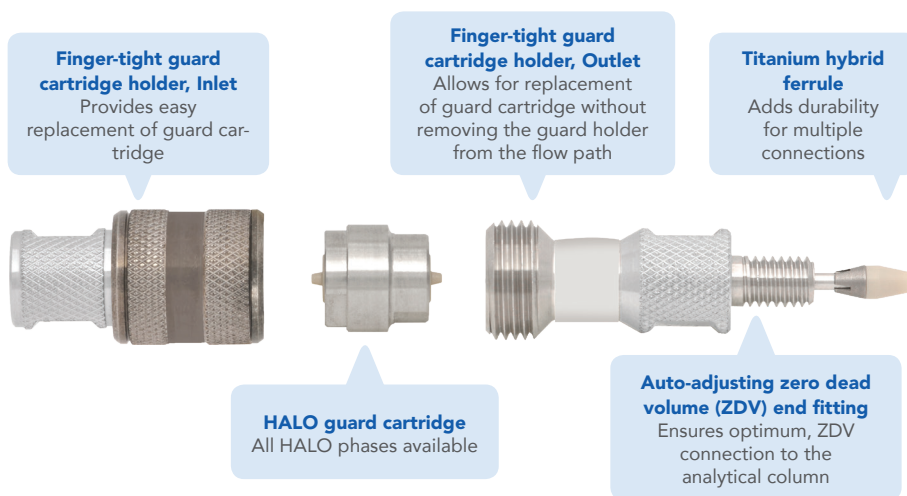
Figure RR. Released- and procainamide-labeled glycans from human IgG were separated using a 2.1 x 150 mm HALO Glycan column and detected using UV and selected-ion-monitoring MS detection.



HALO UHPLC AND HPLC GUARD COLUMNS

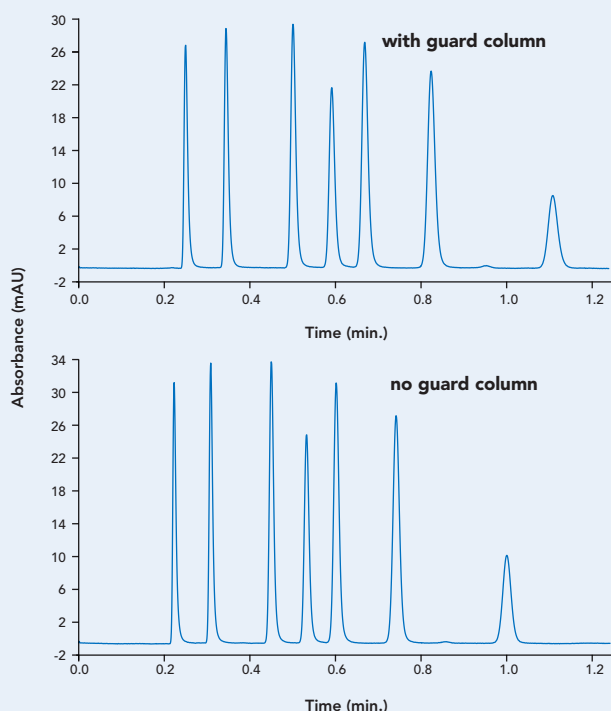
- Collect strongly retained compounds from the sample and minimizes column fouling
- Ultra-low dispersion, easy to use, operate at pressures up to 1000 bar
- Finger-tight, direct-connect units that auto-adjust to any column with a 10–32 inlet port
- Easily replace guard cartridge without removing guard holder from the flow path
- Available for all HALO analytical geometries (2.1, 3.0 and 4.6 mm ID) and phases

See below for an exploded view of the HALO guard cartridge and guard holder. Please see pages 32–36 for ordering information.



HALO GUARD COLUMNS: PROTECTION + PERFORMANCE

Figure S5. HALO guard columns provide optimum protection for your HALO HPLC and UHPLC column without sacrificing column efficiency.



TEST CONDITIONS:
 Column: HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm
 Mobile Phase: 60/40 ACN/water
 Flow Rate: 1.8 mL/min.
 Temperature: 30 °C
 Detection: 254 nm
 Injection Volume: 1 µL
 Pressure: 158 bar with guard column
 146 bar without guard column
 Instrument: Optimized Agilent 1100
 bypassed semi-micro flow cell
 0.05" ID tubing
 14 Hz data rate

The Optimize Technologies EXP® Direct Connect Holder: U.S. Patent No. 8,201,854 & 8,696,902 and Foreign Patents Pending.

REFERENCES

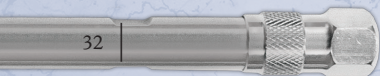
1. Adapted from book, "Introduction to Modern Liquid Chromatography", 3rd Edition, L. R. Snyder, J. J. Kirkland and J. W. Dolan, 2010, p.29, Wiley & Sons.
2. "Orthogonal" separations for reversed-phase liquid chromatography; J. Pellett, P. Lukulay, Y. Mao, W. Bowen, R. Reed, M. Ma, R.C. Munger, J.W. Dolan, L. Wrisley, K. Medwid, N.P. Tolti, C.C. Chan, M. Skibic, K. Biswas, K. A. Wells, and L.R. Snyder; Journal of Chromatography A, 1101 (2006) 122–135.
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8. A.J. Alpert. Anal. Chem. 80, 62–76 (2008).
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10. A.J. Alpert et al., Anal. Chem. 82, 5253–5259 (2010).



HALO 90 Å 2 μm COLUMNS

The part numbers for HALO 90 Å 2 μm columns are presented below and available in 2.1 and 3.0 mm internal diameters. Guard columns are also available for these IDs for UHPLC to provide additional protection when necessary.

<i>Dimensions ID x Length (in mm)</i>	C18	AQ-C18	C8	Phenyl-Hexyl	RP-Amide	PPF	ES-CN	Penta-HILIC	HILIC
2.1 x 20	91812-202	91812-222	91812-208	91812-206	91812-207	91812-209	91812-204	91812-205	91812-201
2.1 x 30	91812-302	91812-322	91812-308	91812-306	91812-307	91812-309	91812-304	91812-305	91812-301
2.1 x 50	91812-402	91812-422	91812-408	91812-406	91812-407	91812-409	91812-404	91812-405	91812-401
2.1 x 75	91812-502	91812-522	91812-508	91812-506	91812-507	91812-509	91812-504	91812-505	91812-501
2.1 x 100	91812-602	91812-622	91812-608	91812-606	91812-607	91812-609	91812-604	91812-605	91812-601
2.1 x 150	91812-702	91812-722	91812-708	91812-706	91812-707	91812-709	91812-704	91812-705	91812-701
3.0 x 20	91813-202	91813-222	91813-208	91813-206	91813-207	91813-209	91813-204	91813-205	91813-201
3.0 x 30	91813-302	91813-322	91813-308	91813-306	91813-307	91813-309	91813-304	91813-305	91813-301
3.0 x 50	91813-402	91813-422	91813-408	91813-406	91813-407	91813-409	91813-404	91813-405	91813-401
3.0 x 75	91813-502	91813-522	91813-508	91813-506	91813-507	91813-509	91813-504	91813-505	91813-501
3.0 x 100	91813-602	91813-622	91813-608	91813-606	91813-607	91813-609	91813-604	91813-605	91813-601
3.0 x 150	91813-702	91813-722	91813-708	91813-706	91813-707	91813-709	91813-704	91813-705	91813-701
2 μm, 90 Å Guard Columns, 3-Pack									
<i>Dimensions ID x Length (in mm)</i>	C18	AQ-C18	C8	Phenyl-Hexyl	RP-Amide	PPF	ES-CN	Penta-HILIC	HILIC
2.1 x 5	91812-102	91812-122	91812-108	91812-106	91812-107	91812-109	91812-104	91812-105	91812-101
3.0 x 5	91813-102	91813-122	91813-108	91813-106	91813-107	91813-109	91813-104	91813-105	91813-101
Guard Column Holder	94900-001								



HALO 2.7 μm COLUMNS

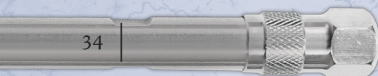
HALO 2.7 μm columns are available in nano, capillary and analytical diameters, as well as in a 10 mm semi-preparative diameter. Guard columns are available for analytical diameters of 2.1, 3.0 and 4.6 mm to provide additional protection when necessary.

Dimensions ID x L (in mm)	C18	AQ-C18	C8	C30	Phenyl-Hexyl	Biphenyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
0.075 x 50	98219-402	98219-422	98219-408	91219-402	98219-406	98219-411	98219-407	98219-409	98219-404	98219-405	98219-401
0.075 x 100	98219-602	98219-622	98219-608	91219-602	98219-606	98219-611	98219-607	98219-609	98219-604	98219-605	98219-601
0.075 x 150	98219-702	98219-722	98219-708	91219-702	98219-706	98219-711	98219-707	98219-709	98219-704	98219-705	98219-701
0.1 x 50	98218-402	98218-422	98218-408	91218-402	98218-406	98218-411	98218-407	98218-409	98218-404	98218-405	98218-401
0.1 x 100	98218-602	98218-622	98218-608	91218-602	98218-606	98218-611	98218-607	98218-609	98218-604	98218-605	98218-601
0.1 x 150	98218-702	98218-722	98218-708	91218-702	98218-706	98218-711	98218-707	98218-709	98218-704	98218-705	98218-701
0.2 x 50	98217-402	98217-422	98217-408	91217-402	98217-406	98217-411	98217-407	98217-409	98217-404	98217-405	98217-401
0.2 x 100	98217-602	98217-622	98217-608	91217-602	98217-606	98217-611	98217-607	98217-609	98217-604	98217-605	98217-601
0.2 x 150	98217-702	98217-722	98217-708	91217-702	98217-706	98217-711	98217-707	98217-709	98217-704	98217-705	98217-701
0.3 x 50	98216-402	98216-422	98216-408	91216-402	98216-406	98216-411	98216-407	98216-409	98216-404	98216-405	98216-401
0.3 x 100	98216-602	98216-622	98216-608	91216-602	98216-606	98216-611	98216-607	98216-609	98216-604	98216-605	98216-601
0.3 x 150	98216-702	98216-722	98216-708	91216-702	98216-706	98216-711	98216-707	98216-709	98216-704	98216-705	98216-701
0.5 x 50	98215-402	98215-422	98215-408	91215-402	98215-406	98215-411	98215-407	98215-409	98215-404	98215-405	98215-401
0.5 x 100	98215-602	98215-622	98215-608	91215-602	98215-606	98215-611	98215-607	98215-609	98215-604	98215-605	98215-601
0.5 x 150	98215-702	98215-722	98215-708	91215-702	98215-706	98215-711	98215-707	98215-709	98215-704	98215-705	98215-701
1.0 x 30	92811-302	92811-322	92811-308	92111-302	92811-306	92811-311	92811-307	92811-309	92811-304	92811-305	92811-301
1.0 x 50	92811-402	92811-422	92811-408	92111-402	92811-406	92811-411	92811-407	92811-409	92811-404	92811-405	92811-401
1.0 x 75	92811-502	92811-522	92811-508	92111-502	92811-506	92811-511	92811-507	92811-509	92811-504	92811-505	92811-501
1.0 x 100	92811-602	92811-622	92811-608	92111-602	92811-606	92811-611	92811-607	92811-609	92811-604	92811-605	92811-601
1.0 x 150	92811-702	92811-722	92811-708	92111-702	92811-706	92811-711	92811-707	92811-709	92811-704	92811-705	92811-701
2.1 x 20	92812-202	92812-222	92812-208	92112-230	92812-206	92812-211	92812-207	92812-209	92812-204	92812-205	92812-201
2.1 x 30	92812-302	92812-322	92812-308	92112-330	92812-306	92812-311	92812-307	92812-309	92812-304	92812-305	92812-301
2.1 x 50	92812-402	92812-422	92812-408	92112-430	92812-406	92812-411	92812-407	92812-409	92812-404	92812-405	92812-401
2.1 x 75	92812-502	92812-522	92812-508	92112-530	92812-506	92812-511	92812-507	92812-509	92812-504	92812-505	92812-501
2.1 x 100	92812-602	92812-622	92812-608	92112-630	92812-606	92812-611	92812-607	92812-609	92812-604	92812-605	92812-601
2.1 x 150	92812-702	92812-722	92812-708	92112-730	92812-706	92812-711	92812-707	92812-709	92812-704	92812-705	92812-701
2.1 x 250	92812-902	92812-922	92812-908	92112-930	92812-906	92812-911	92812-907	92812-909	92812-904	92812-905	92812-901
3.0 x 20	92813-202	92813-222	92813-208	92113-230	92813-206	92813-211	92813-207	92813-209	92813-204	92813-205	92813-201
3.0 x 30	92813-302	92813-322	92813-308	92113-330	92813-306	92813-311	92813-307	92813-309	92813-304	92813-305	92813-301
3.0 x 50	92813-402	92813-422	92813-408	92113-430	92813-406	92813-411	92813-407	92813-409	92813-404	92813-405	92813-401
3.0 x 75	92813-502	92813-522	92813-508	92113-530	92813-506	92813-511	92813-507	92813-509	92813-504	92813-505	92813-501
3.0 x 100	92813-602	92813-622	92813-608	92113-630	92813-606	92813-611	92813-607	92813-609	92813-604	92813-605	92813-601
3.0 x 150	92813-702	92813-722	92813-708	92113-730	92813-706	92813-711	92813-707	92813-709	92813-704	92813-705	92813-701
3.0 x 250	92813-902	92813-922	92813-908	92113-930	92813-906	92813-911	92813-907	92813-909	92813-904	92813-905	92813-901
4.6 x 20	92814-202	92814-222	92814-208	92114-230	92814-206	92814-211	92814-207	92814-209	92814-204	92814-205	92814-201
4.6 x 30	92814-302	92814-322	92814-308	92114-330	92814-306	92814-311	92814-307	92814-309	92814-304	92814-305	92814-301
4.6 x 50	92814-402	92814-422	92814-408	92114-430	92814-406	92814-411	92814-407	92814-409	92814-404	92814-405	92814-401
4.6 x 75	92814-502	92814-522	92814-508	92114-530	92814-506	92814-511	92814-507	92814-509	92814-504	92814-505	92814-501
4.6 x 100	92814-602	92814-622	92814-608	92114-630	92814-606	92814-611	92814-607	92814-609	92814-604	92814-605	92814-601
4.6 x 150	92814-702	92814-722	92814-708	92114-730	92814-706	92814-711	92814-707	92814-709	92814-704	92814-705	92814-701
4.6 x 250	92814-902	92814-922	92814-908	92114-930	92814-906	92814-911	92814-907	92814-909	92814-904	92814-905	92814-901
10.0 x 50	92810-402	92810-422	92810-408	92110-402	92810-406	92810-411	92810-407	92810-409	92810-404	92810-405	92810-401
10.0 x 75	92810-502	92810-522	92810-508	92110-502	92810-506	92810-511	92810-507	92810-509	92810-504	92810-505	92810-501
10.0 x 100	92810-602	92810-622	92810-608	92110-602	92810-606	92810-611	92810-607	92810-609	92810-604	92810-605	92810-601
10.0 x 150	92810-702	92810-722	92810-708	92110-702	92810-706	92810-711	92810-707	92810-709	92810-704	92810-705	92810-701
2.7 μm, 90 Å Guard Columns, 3-Pack											
Dimensions ID x Length (in mm)	C18	AQ-C18	C8	C30	Phenyl-Hexyl	Biphenyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 5	92812-102	92812-122	92812-108	92112-130	92812-106	92812-111	92812-107	92812-109	92812-104	92812-105	92812-101
3.0 x 5	92813-102	92813-122	92813-108	92113-130	92813-106	92813-111	92813-107	92813-109	92813-104	92813-105	92813-101
4.6 x 5	92814-102	92814-122	92814-108	92114-130	92814-106	92814-111	92814-107	92814-109	92814-104	92814-105	92814-101
Guard Column Holder 94900-001											

HALO 90 Å 5 µm COLUMNS

HALO 90 Å 5 µm columns are available in nano, capillary and analytical diameters, and in a 10 mm semi-preparative diameter. Guard columns are available for analytical diameters of 2.1, 3.0 and 4.6 mm.

<i>Dimensions ID x Length (in mm)</i>	C18	AQ-C18	C8	Phenyl-Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
0.075 x 50	98519-402	98519-422	98519-408	98519-406	98519-407	98519-409	98519-404	98519-405	98519-401
0.075 x 100	98519-602	98519-622	98519-608	98519-606	98519-607	98519-609	98519-604	98519-605	98519-601
0.075 x 150	98519-702	98519-722	98519-708	98519-706	98519-707	98519-709	98519-704	98519-705	98519-701
0.1 x 50	98518-402	98518-422	98518-408	98518-406	98518-407	98518-409	98518-404	98518-405	98518-401
0.1 x 100	98518-602	98518-622	98518-608	98518-606	98518-607	98518-609	98518-604	98518-605	98518-601
0.1 x 150	98518-702	98518-722	98518-708	98518-706	98518-707	98518-709	98518-704	98518-705	98518-701
0.2 x 50	98517-402	98517-422	98517-408	98517-406	98517-407	98517-409	98517-404	98517-405	98517-401
0.2 x 100	98517-602	98517-622	98517-608	98517-606	98517-607	98517-609	98517-604	98517-605	98517-601
0.2 x 150	98517-702	98517-722	98517-708	98517-706	98517-707	98517-709	98517-704	98517-705	98517-701
0.3 x 50	98516-402	98516-422	98516-408	98516-406	98516-407	98516-409	98516-404	98516-405	98516-401
0.3 x 100	98516-602	98516-622	98516-608	98516-606	98516-607	98516-609	98516-604	98516-605	98516-601
0.3 x 150	98516-702	98516-722	98516-708	98516-706	98516-707	98516-709	98516-704	98516-705	98516-701
0.5 x 50	98515-402	98515-422	98515-408	98515-406	98515-407	98515-409	98515-404	98515-405	98515-401
0.5 x 100	98515-602	98515-622	98515-608	98515-606	98515-607	98515-609	98515-604	98515-605	98515-601
0.5 x 150	98515-702	98515-722	98515-708	98515-706	98515-707	98515-709	98515-704	98515-705	98515-701
1.0 x 30	95811-302	95811-322	95811-308	95811-306	95811-307	95811-309	95811-304	95811-305	95811-301
1.0 x 50	95811-402	95811-422	95811-408	95811-406	95811-407	95811-409	95811-404	95811-405	95811-401
1.0 x 75	95811-502	95811-522	95811-508	95811-506	95811-507	95811-509	95811-504	95811-505	95811-501
1.0 x 100	95811-602	95811-622	95811-608	95811-606	95811-607	95811-609	95811-604	95811-605	95811-601
1.0 x 150	95811-702	95811-722	95811-708	95811-706	95811-707	95811-709	95811-704	95811-705	95811-701
2.1 x 20	95812-202	95812-222	95812-208	95812-206	95812-207	95812-209	95812-204	95812-205	95812-201
2.1 x 30	95812-302	95812-322	95812-308	95812-306	95812-307	95812-309	95812-304	95812-305	95812-301
2.1 x 50	95812-402	95812-422	95812-408	95812-406	95812-407	95812-409	95812-404	95812-405	95812-401
2.1 x 75	95812-502	95812-522	95812-508	95812-506	95812-507	95812-509	95812-504	95812-505	95812-501
2.1 x 100	95812-602	95812-622	95812-608	95812-606	95812-607	95812-609	95812-604	95812-605	95812-601
2.1 x 150	95812-702	95812-722	95812-708	95812-706	95812-707	95812-709	95812-704	95812-705	95812-701
2.1 x 250	95812-902	95812-922	95812-908	95812-906	95812-907	95812-909	95812-904	95812-905	95812-901
3.0 x 20	95813-202	95813-222	95813-208	95813-206	95813-207	95813-209	95813-204	95813-205	95813-201
3.0 x 30	95813-302	95813-322	95813-308	95813-306	95813-307	95813-309	95813-304	95813-305	95813-301
3.0 x 50	95813-402	95813-422	95813-408	95813-406	95813-407	95813-409	95813-404	95813-405	95813-401
3.0 x 75	95813-502	95813-522	95813-508	95813-506	95813-507	95813-509	95813-504	95813-505	95813-501
3.0 x 100	95813-602	95813-622	95813-608	95813-606	95813-607	95813-609	95813-604	95813-605	95813-601
3.0 x 150	95813-702	95813-722	95813-708	95813-706	95813-707	95813-709	95813-704	95813-705	95813-701
3.0 x 250	95813-902	95813-922	95813-908	95813-906	95813-907	95813-909	95813-904	95813-905	95813-901
4.6 x 20	95814-202	95814-222	95814-208	95814-206	95814-207	95814-209	95814-204	95814-205	95814-201
4.6 x 30	95814-302	95814-322	95814-308	95814-306	95814-307	95814-309	95814-304	95814-305	95814-301
4.6 x 50	95814-402	95814-422	95814-408	95814-406	95814-407	95814-409	95814-404	95814-405	95814-401
4.6 x 75	95814-502	95814-522	95814-508	95814-506	95814-507	95814-509	95814-504	95814-505	95814-501
4.6 x 100	95814-602	95814-622	95814-608	95814-606	95814-607	95814-609	95814-604	95814-605	95814-601
4.6 x 150	95814-702	95814-722	95814-708	95814-706	95814-707	95814-709	95814-704	95814-705	95814-701
4.6 x 250	95814-902	95814-922	95814-908	95814-906	95814-907	95814-909	95814-904	95814-905	95814-901
10.0 x 50	95810-402	95810-422	95810-408	95810-406	95810-407	95810-409	95810-404	95810-405	95810-401
10.0 x 75	95810-502	95810-522	95810-508	95810-506	95810-507	95810-509	95810-504	95810-505	95810-501
10.0 x 100	95810-602	95810-622	95810-608	95810-606	95810-607	95810-609	95810-604	95810-605	95810-601
10.0 x 150	95810-702	95810-722	95810-708	95810-706	95810-707	95810-709	95810-704	95810-705	95810-701
10.0 x 250	95810-902	95810-922	95810-908	95810-906	95810-907	95810-909	95810-904	95810-905	95810-901
5 µm, 90Å Guard Columns, 3-Pack									
<i>Dimensions ID x Length (in mm)</i>	C18	AQ-C18	C8	Phenyl-Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 5	95812-102	95812-122	95812-108	95812-106	95812-107	95812-109	95812-104	95812-105	95812-101
3.0 x 5	95813-102	95813-122	95813-108	95813-106	95813-107	95813-109	95813-104	95813-105	95813-101
4.6 x 5	95814-102	95814-122	95814-108	95814-106	95814-107	95814-109	95814-104	95814-105	95814-101
Guard Column Holder	94900-001								



HALO 1000 Å AND 400 Å PROTEIN COLUMNS

Part numbers for nano, capillary, analytical and semi-preparative HALO 1000 and 400 Å in 2.7 and 3.4 µm phases are provided below. Guard columns are available in 2.1, 3.0 and 4.6 mm IDs for UHPLC and HPLC applications to provide additional column protection when desired.

Dimensions ID x Length (in mm)	400 Å, 3.4 µm		1000 Å, 2.7 µm		
	C4	ES-C18	C4	ES-C18	Diphenyl
0.075 x 50	94319-414	94319-402	97219-414	97219-402	97219-426
0.075 x 100	94319-614	94319-602	97219-614	97219-602	97219-626
0.075 x 150	94319-714	94319-702	97219-714	97219-702	97219-726
0.1 x 50	94318-414	94318-402	97218-414	97218-402	97218-426
0.1 x 100	94318-614	94318-602	97218-614	97218-602	97218-626
0.1 x 150	94318-714	94318-702	97218-714	97218-702	97218-726
0.2 x 50	94317-414	94317-402	97217-414	97217-402	97217-426
0.2 x 100	94317-614	94317-602	97217-614	97217-602	97217-626
0.2 x 150	94317-714	94317-702	97217-714	97217-702	97217-726
0.3 x 50	94316-414	94316-402	97216-414	97216-402	97216-426
0.3 x 100	94316-614	94316-602	97216-614	97216-602	97216-626
0.3 x 150	94316-714	94316-702	97216-714	97216-702	97216-726
0.5 x 50	94315-414	94315-402	97215-414	97215-402	97215-426
0.5 x 100	94315-614	94315-602	97215-614	97215-602	97215-626
0.5 x 150	94315-714	94315-702	97215-714	97215-702	97215-726
1.0 x 30	93411-314	93411-302	92711-314	92711-302	92711-326
1.0 x 50	93411-414	93411-402	92711-414	92711-402	92711-426
1.0 x 75	93411-514	93411-502	92711-514	92711-502	92711-526
1.0 x 100	93411-614	93411-602	92711-614	92711-602	92711-626
1.0 x 150	93411-714	93411-702	92711-714	92711-702	92711-726
2.1 x 20	93412-214	93412-202	92712-214	92712-202	92712-226
2.1 x 30	93412-314	93412-302	92712-314	92712-302	92712-326
2.1 x 50	93412-414	93412-402	92712-414	92712-402	92712-426
2.1 x 75	93412-514	93412-502	92712-514	92712-502	92712-526
2.1 x 100	93412-614	93412-602	92712-614	92712-602	92712-626
2.1 x 150	93412-714	93412-702	92712-714	92712-702	92712-726
2.1 x 250	93412-914	93412-902	92712-914	92712-902	92712-926
3.0 x 20	93413-214	93413-202	92713-214	92713-202	92713-226
3.0 x 30	93413-314	93413-302	92713-314	92713-302	92713-326
3.0 x 50	93413-414	93413-402	92713-414	92713-402	92713-426
3.0 x 75	93413-514	93413-502	92713-514	92713-502	92713-526
3.0 x 100	93413-614	93413-602	92713-614	92713-602	92713-626
3.0 x 150	93413-714	93413-702	92713-714	92713-702	92713-726
3.0 x 250	93413-914	93413-902	92713-914	92713-902	92713-926
4.6 x 20	93414-214	93414-202	92714-214	92714-202	92714-226
4.6 x 30	93414-314	93414-302	92714-314	92714-302	92714-326
4.6 x 50	93414-414	93414-402	92714-414	92714-402	92714-426
4.6 x 75	93414-514	93414-502	92714-514	92714-502	92714-526
4.6 x 100	93414-614	93414-602	92714-614	92714-602	92714-626
4.6 x 150	93414-714	93414-702	92714-714	92714-702	92714-726
4.6 x 250	93414-914	93414-902	92714-914	92714-902	92714-926
10.0 x 50	93410-414	93410-402	92710-414	92710-402	92710-426
10.0 x 75	93410-514	93410-502	92710-514	92710-502	92710-526
10.0 x 100	93410-614	93410-602	92710-614	92710-602	92710-626
10.0 x 150	93410-714	93410-702	92710-714	92710-702	92710-726
Guard Columns, 3-Pack					
Dimensions ID x Length (in mm)	C4	ES-C18	C4	ES-C18	Diphenyl
2.1 x 5	93412-114	93412-102	92712-114	92712-102	92712-126
3.0 x 5	93413-114	93413-102	92713-114	92713-102	92713-126
4.6 x 5	93414-114	93414-102	92714-114	92714-102	92714-126
Guard Column Holder 94900-001					

HALO 90 Å GLYCAN COLUMNS

HALO Glycan columns are available in 2.1 and 4.6 mm diameters in the following lengths as a 2.7 µm particle size. Guard columns are available for UHPLC and HPLC applications if additional protection is desired.

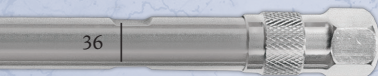
Dimensions ID x Length (in mm)	HALO Glycan
2.1 x 50	92922-405
2.1 x 100	92922-605
2.1 x 150	92922-705
4.6 x 50	92924-405
4.6 x 100	92924-605
4.6 x 150	92924-705
Guard Columns, 3-Pack	
Dimensions ID x Length (in mm)	HALO Glycan
2.1 x 5	92922-105
4.6 x 5	92924-105
Guard Column Holder 94900-001	



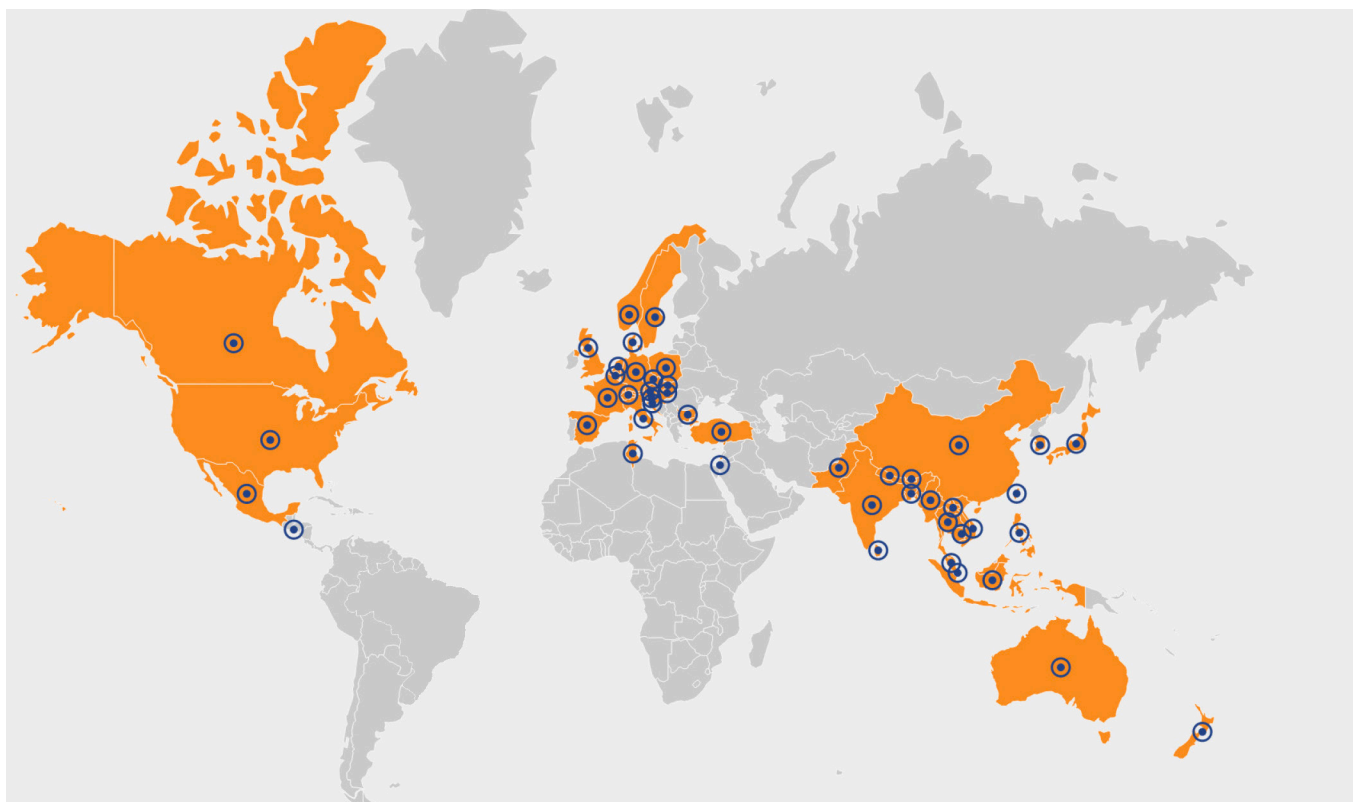
HALO 160 Å PEPTIDE COLUMNS

The part numbers are provided below for the nano, capillary, analytical and semi-preparative HALO 160 Å 2, 2.7 and 5 µm phases. Guard columns are available for 2.1, 3.0 and 4.6 mm internal diameters for UHPLC and HPLC applications, if additional protection is desired.

Dimensions ID x Length (in mm)	160 Å, 2 µm		160 Å, 2.7 µm			160 Å, 5 µm	
	ES-C18	ES-C18	ES-CN	Phenyl-Hexyl	ES-C18	ES-CN	
0.075 x 50	-	91229-402	91229-404	91229-406	91529-402	91529-404	
0.075 x 100	-	91229-602	91229-604	91229-606	91529-602	91529-604	
0.075 x 150	-	91229-702	91229-704	91229-706	91529-702	91529-704	
0.1 x 50	-	91228-402	91228-404	91228-406	91528-402	91528-404	
0.1 x 100	-	91228-602	91228-604	91228-606	91528-602	91528-604	
0.1 x 150	-	91228-702	91228-704	91228-706	91528-702	91528-704	
0.2 x 50	-	91227-402	91227-404	91227-406	91527-402	91527-404	
0.2 x 100	-	91227-602	91227-604	91227-606	91527-602	91527-604	
0.2 x 150	-	91227-702	91227-704	91227-706	91527-702	91527-704	
0.3 x 50	-	91226-402	91226-404	91226-406	91526-402	91526-404	
0.3 x 100	-	91226-602	91226-604	91226-606	91526-602	91526-604	
0.3 x 150	-	91226-702	91226-704	91226-706	91526-702	91526-704	
0.5 x 50	-	91225-402	91225-404	91225-406	91525-402	91525-404	
0.5 x 100	-	91225-602	91225-604	91225-606	91525-602	91525-604	
0.5 x 150	-	91225-702	91225-704	91225-706	91525-702	91525-704	
1.0 x 30	-	92121-302	92121-304	92121-306	95121-302	95121-304	
1.0 x 50	-	92121-402	92121-404	92121-406	95121-402	95121-404	
1.0 x 75	-	92121-502	92121-504	92121-506	95121-502	95121-504	
1.0 x 100	-	92121-602	92121-604	92121-606	95121-602	95121-604	
1.0 x 150	-	92121-702	92121-704	92121-706	95121-702	95121-704	
2.1 x 20	91122-202	92122-202	92122-204	92122-206	95122-202	95122-204	
2.1 x 30	91122-302	92122-302	92122-304	92122-306	95122-302	95122-304	
2.1 x 50	91122-402	92122-402	92122-404	92122-406	95122-402	95122-404	
2.1 x 75	91122-502	92122-502	92122-504	92122-506	95122-502	95122-504	
2.1 x 100	91122-602	92122-602	92122-604	92122-606	95122-602	95122-604	
2.1 x 150	91122-702	92122-702	92122-704	92122-706	95122-702	95122-704	
2.1 x 250	91122-902	92122-902	92122-904	92122-906	95122-902	95122-904	
3.0 x 20	91123-202	92123-202	92123-204	92123-206	95123-202	95123-204	
3.0 x 30	91123-302	92123-302	92123-304	92123-306	95123-302	95123-304	
3.0 x 50	91123-402	92123-402	92123-404	92123-406	95123-402	95123-404	
3.0 x 75	91123-502	92123-502	92123-504	92123-506	95123-502	95123-504	
3.0 x 100	91123-602	92123-602	92123-604	92123-606	95123-602	95123-604	
3.0 x 150	91123-702	92123-702	92123-704	92123-706	95123-702	95123-704	
3.0 x 250	91123-902	92123-902	92123-904	92123-906	95123-902	95123-904	
4.6 x 20	-	92124-202	92124-204	92124-206	95124-202	95124-204	
4.6 x 30	-	92124-302	92124-304	92124-306	95124-302	95124-304	
4.6 x 50	-	92124-402	92124-404	92124-406	95124-402	95124-404	
4.6 x 75	-	92124-502	92124-504	92124-506	95124-502	95124-504	
4.6 x 100	-	92124-602	92124-604	92124-606	95124-602	95124-604	
4.6 x 150	-	92124-702	92124-704	92124-706	95124-702	95124-704	
4.6 x 250	-	92124-902	92124-904	92124-906	95124-902	95124-904	
10.0 x 50	-	92120-402	92120-404	92120-406	95120-402	95120-404	
10.0 x 75	-	92120-502	92120-504	92120-506	95120-502	95120-504	
10.0 x 100	-	92120-602	92120-604	92120-606	95120-602	95120-604	
10.0 x 150	-	92120-702	92120-704	92120-706	95120-702	95120-704	
10.0 x 250	-	-	-	-	95120-902	95120-904	
Guard Columns, 3-pack							
Dimensions ID x Length (in mm)	ES-C18	ES-C18	ES-CN	Phenyl-Hexyl	ES-C18	ES-CN	
2.1 x 5	91122-102	92122-102	92122-104	92122-106	95122-102	95122-104	
3.0 x 5	91123-102	92123-102	92123-104	92123-106	95123-102	95123-104	
4.6 x 5	-	92124-102	92124-104	92124-106	95124-102	95124-104	
Guard Column Holder 94900-001							



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