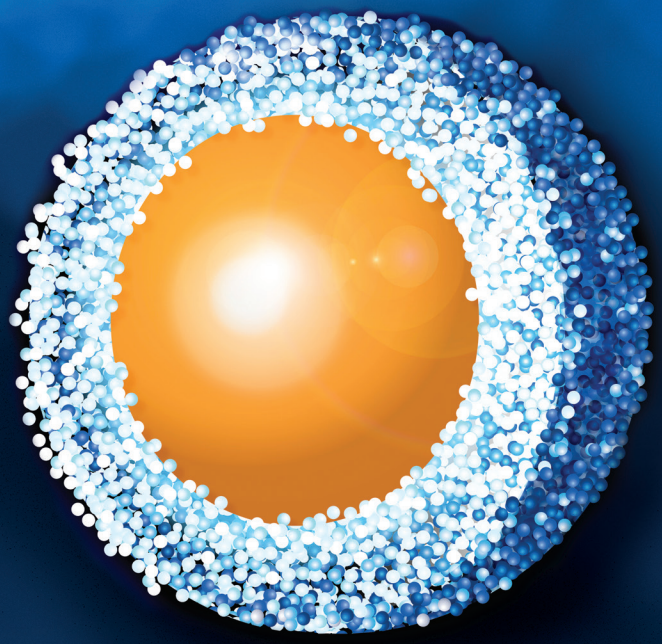

HALO®

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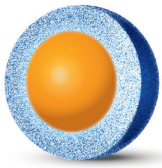
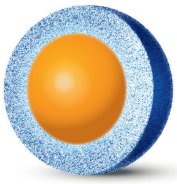
DISCOVER THE ADVANTAGES OF HALO® AND HALO® BIOCLASS FUSED-CORE® COLUMNS

HALO®

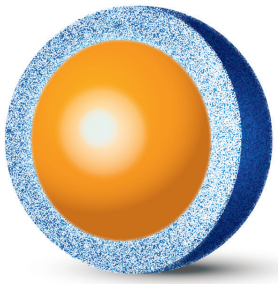
SMALL MOLECULE



2 micron particle

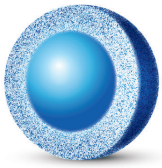


2.7 micron particle

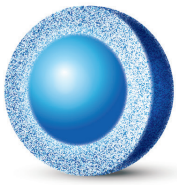


5 micron particle

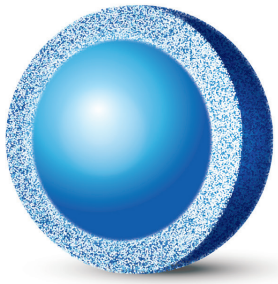
BIOCLASS



2 micron particle

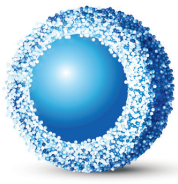


2.7 micron particle

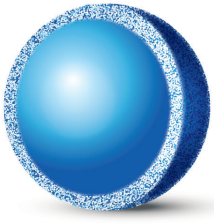


5 micron particle

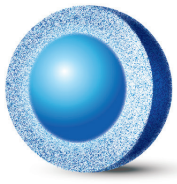
PEPTIDE



2.7 micron particle



3.4 micron particle



2.7 micron particle

PROTEIN

GLYCAN

Table of Contents

MILESTONES IN THE DEVELOPMENT OF FUSED-CORE® PARTICLES

1960 — Superficially porous particles (SPPs) were first proposed by Golay.

1969 — Jack Kirkland (DuPont Company), inspired by Horvath, Preiss, and Lipsky's nucleotide separation using ~50 µm cores containing a thin layer of anion exchange resin, develops Zipax®, a 30 µm particle of silica sol on glass beads.

mid 70s — Kirkland, Permaphase permanently bonded stationary phase on SPP.

HALO® 90 Å 2.7 µm

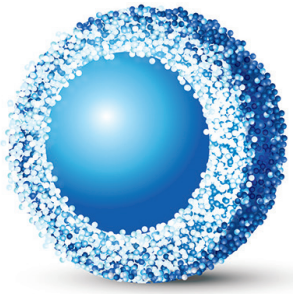
approximately 20 yrs — Smaller totally porous particles develop rapidly and dominate HPLC column market. However, demand for speed and resolution create need for particle innovation that delivers higher performance at lower pressures.

1992 — Kirkland and Boyes described 5 µm SPPs with 300 Å pores.

2006 — Kirkland, Langlois and DeStefano, of Advanced Materials Technology, Inc., were first to commercialize Fused-Core® particles smaller than 3 µm (HALO®® 2.7).

2010 — AMT continues to be a leader in development and commercialization of novel packing materials for both small and large molecules (HALO®® BioClass 160 Å and 400 Å and HALO®® 90 Å 2 µm).

2014



HALO® 1000 Å 2.7 µm

2015 — AMT celebrated its tenth year of innovative solutions for challenging separations.

2017 — First 1000 Å SPP product for large molecule analysis released with development by Boyes and Kirkland.

TODAY THE INNOVATION CONTINUES ...

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 (~4–6% RSD vs. ~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.

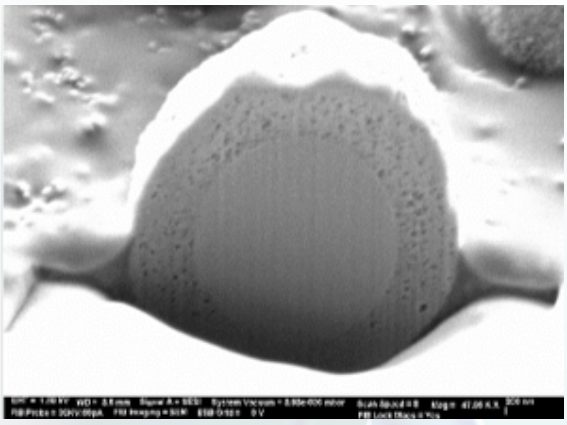


Figure B. SEM image of a focused-ion beam cleaved HALO® 1000 Å 2.7 µm silica particle. This "cut-away" view shows the solid core and shell with large pores allowing unrestricted access of macromolecules to the bonded phase.

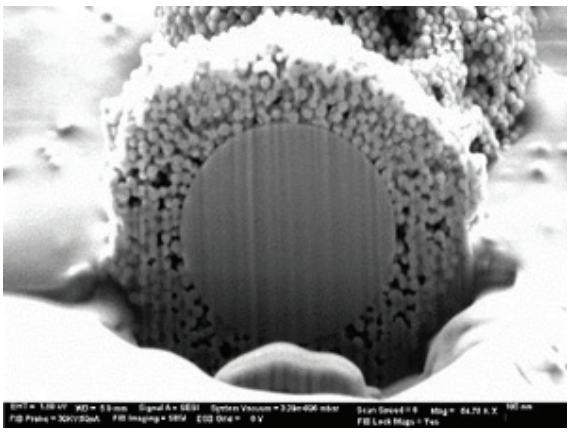
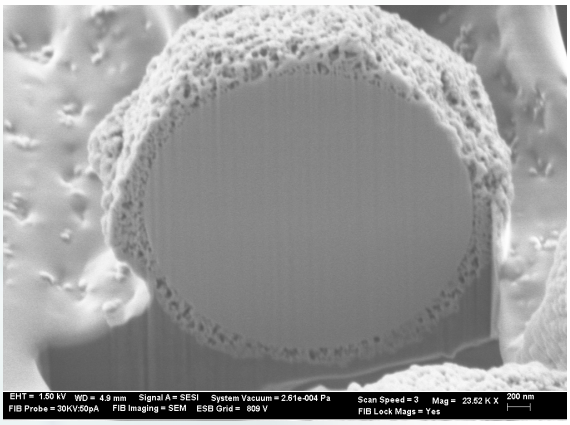


Figure C. SEM image of a focused-ion-beam-cleaved HALO® Protein 3.4 µm silica particle. This "cut-away" view shows the solid core with its very thin 0.2 µm outer porous layer.



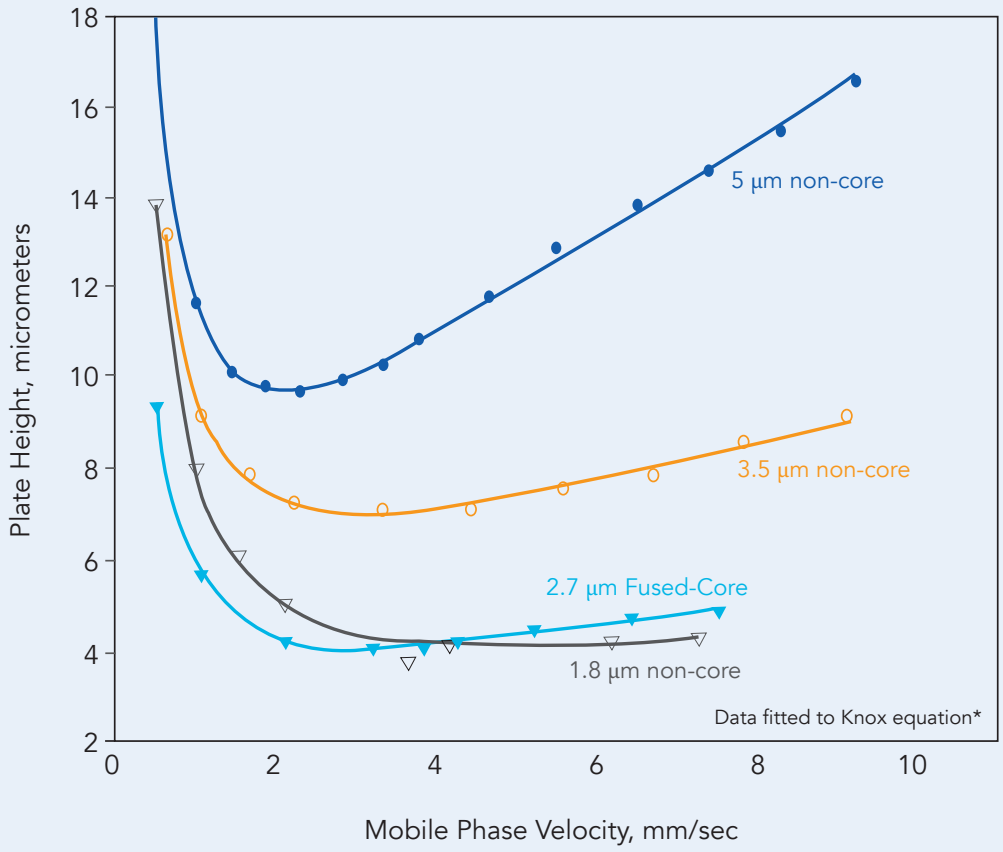
Understanding SPP Performance (Figure D)

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_0)

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

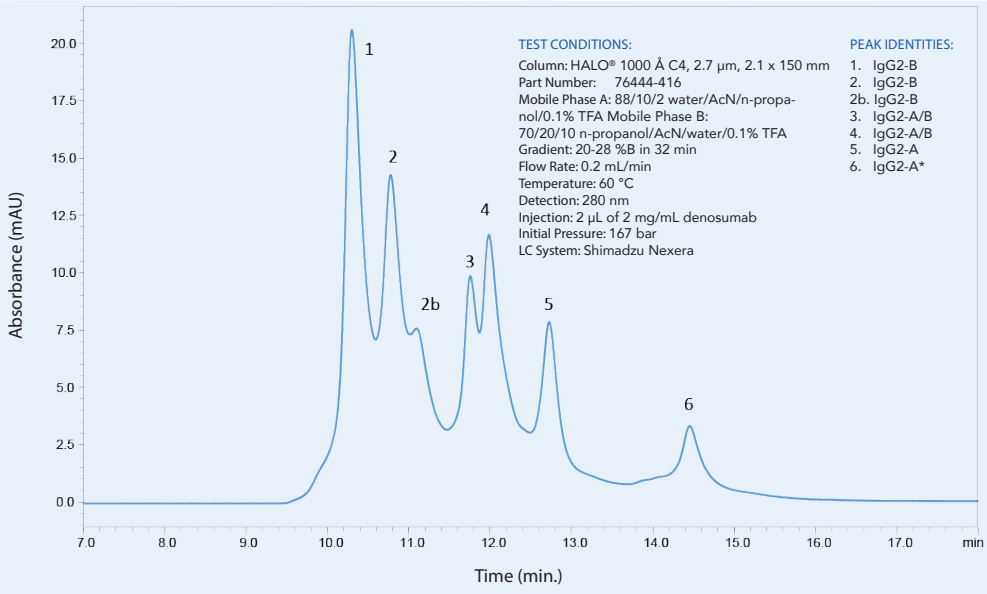
- Less plugging, longer usable column lifetime and greater uptime due to larger porosity frits (vs. sub-2 µm totally porous (non-core) columns)
  - 2 µm frits for HALO® 2.7 µm and 5 µm columns
  - 1 µm frits for HALO® 2 µm columns vs. 0.2–0.5 µm frits for sub-2 µm non-core columns
- Excellent column-to-column and lot-to-lot reproducibility thanks to tight manufacturing controls
- Robust pores in multiple sizes for a tailored application solution (90 Å, 160 Å, 400 Å and 1000 Å)

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 Å pore particles for large molecule enablement

Figure E. High Resolution of IgG2 with HALO® 1000 Å C4
Very high resolution separations are achieved with HALO® 1000 Å C4 for a complex IgG2 such as denosumab. The assignments are based on non-reduced Lys-C digestion mapping.



ULTRAFAST PEPTIDE SEPARATION

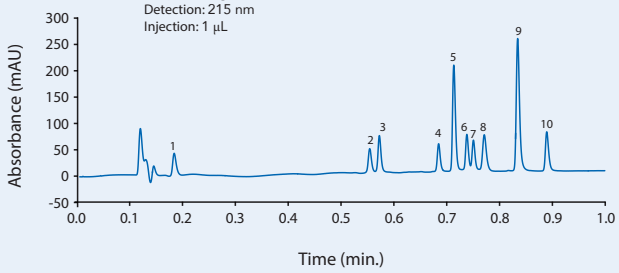
Figure F. Separation of a 10 peptide mixture is accomplished in less than one minute using a HALO® Peptide ES-C18 column on a delay-volume minimized and optimized Agilent 1200 system.

TEST CONDITIONS:

Column: HALO® 160 Å ES-C18, 2.7 µm, 2.1 x 50 mm
Part Number: 76444-264
Mobile Phase A: water/0.1% TFA;
Mobile Phase B: 80/20 ACN/water/0.1% TFA
Gradient: Hold at 12.5% B until 0.15 min.,12.5% to 88% B from 0.15—1.15 min.
Flow Rate: 1.05 mL/min.
Temperature: 60 °C
Pressure: 213 bar
LC System: Agilent 1200 SL
Detection: 215 nm
Injection: 1 µL

PEAK IDENTITIES:

1. Gly-Tyr
2. Val-Tyr-Val
3. Angiotensin 1/2 (1-7) amide
4. Met-enk
5. Angiotensin 1/2 (1-8) amide
6. Angiotensin II
7. Leu-Enk
8. Ribonuclease A
9. Angiotensin (1-12) (mouse)
10. Porcine Insulin



ULTRAFAST BALLISTIC GRADIENT USING HALO® 5 µm

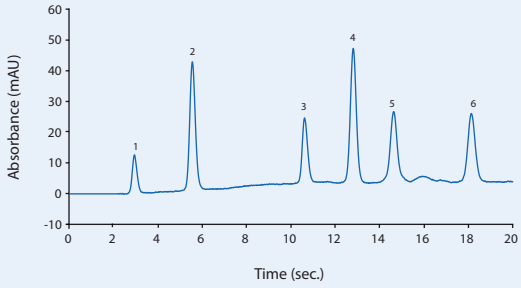
Figure G.
Many researchers have found HALO® 5 µm columns in 2.1 mm ID to be very useful for high-throughput, ballistic separations by LC and LC-MS.

TEST CONDITIONS:

Column: HALO® 90 Å C18, 5 µm, 2.1 x 20 mm
Part Number: 76445-626
Mobile Phase A: Water/0.1% TFA
Mobile Phase B: Acetonitrile/0.1% TFA
Gradient: 5-50% B in 20 sec.
Flow Rate: 6 mL/min.
Pressure: 549 bar
Temperature: 40 °C
Detection: UV 254 nm, PDA
Injection Volume: 0.5 µL
Flow Cell: 1 µL micro
LC System: Shimadzu Nexera

PEAK IDENTITIES:

1. Atenolol
2. Pindolol
3. Propranolol
4. Indoprofen
5. Naproxen
6. Coumatetralyl



CARBONYL-DNPH HIGH RESOLUTION SEPARATION

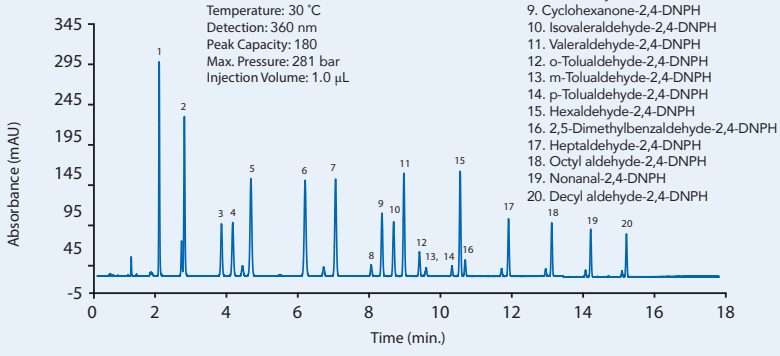
Figure H. Environmental samples can be quite complex as demonstrated by this gradient separation of dinitrophenylhydrazone (DNPH) carbonyl compound derivatives using a HALO® 90 Å C18, 2 µm, 2.1 x 150 mm column.

TEST CONDITIONS:

Column: HALO® 90 Å C18, 2 µm, 2.1 x 150 mm
Part Number:76443-918
Mobile Phase A: H2O
Mobile Phase B: 80/20 ACN/THF
Gradient: Time %B
0-5 min. 45
5-20 min. 45-90
Flow Rate: 0.5 mL/min.
Temperature: 30 °C
Detection: 360 nm
Peak Capacity: 180
Max. Pressure: 281 bar
Injection Volume: 1.0 µL

PEAK IDENTITIES:

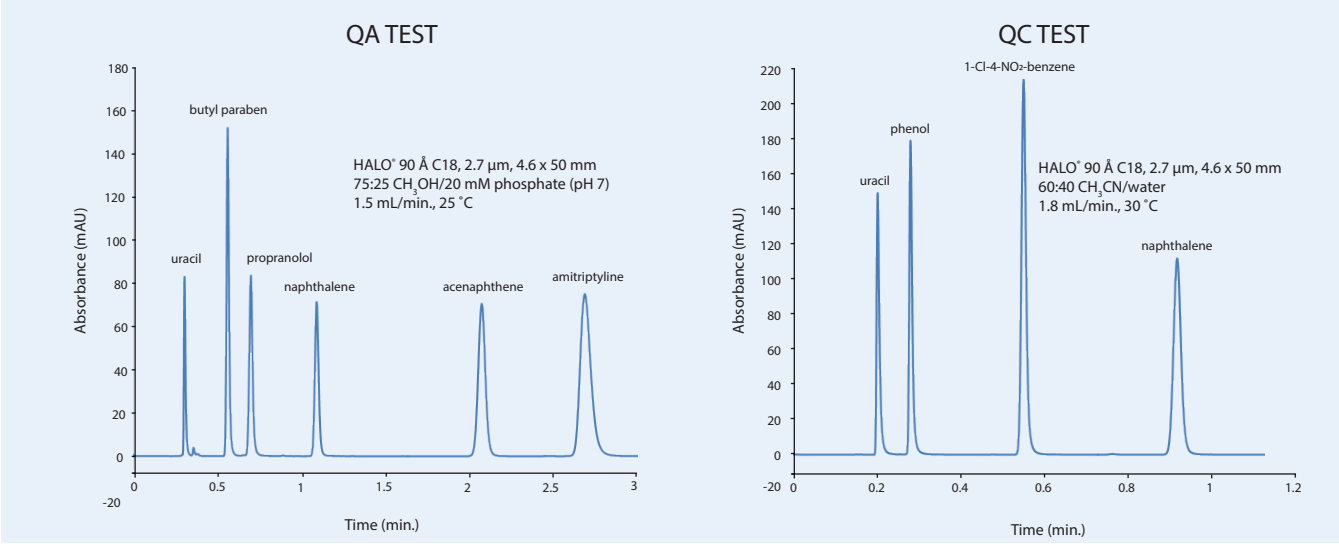
1. Formaldehyde-2,4-DNPH
2. Acetaldehyde-2,4-DNPH
3. Acetone-2,4-DNPH
4. Acrolein-2,4-DNPH
5. Propionaldehyde-2,4-DNPH
6. Crotonaldehyde-2,4-DNPH
7. Butyraldehyde-2,4-DNPH
8. Benzaldehyde-2,4-DNPH
9. Cyclohexanone-2,4-DNPH
10. Isovaleraldehyde-2,4-DNPH
11. Valeraldehyde-2,4-DNPH
12. o-Tolualdehyde-2,4-DNPH
13. m-Tolualdehyde-2,4-DNPH
14. p-Tolualdehyde-2,4-DNPH
15. Hexaldehyde-2,4-DNPH
16. 2,5-Dimethylbenzaldehyde-2,4-DNPH
17. Heptaldehyde-2,4-DNPH
18. Octyl aldehyde-2,4-DNPH
19. Nonanal-2,4-DNPH
20. Decyl aldehyde-2,4-DNPH



HALO® QUALITY PROMISE: PERFORMANCE AND REPRODUCIBILITY – EVERY TIME

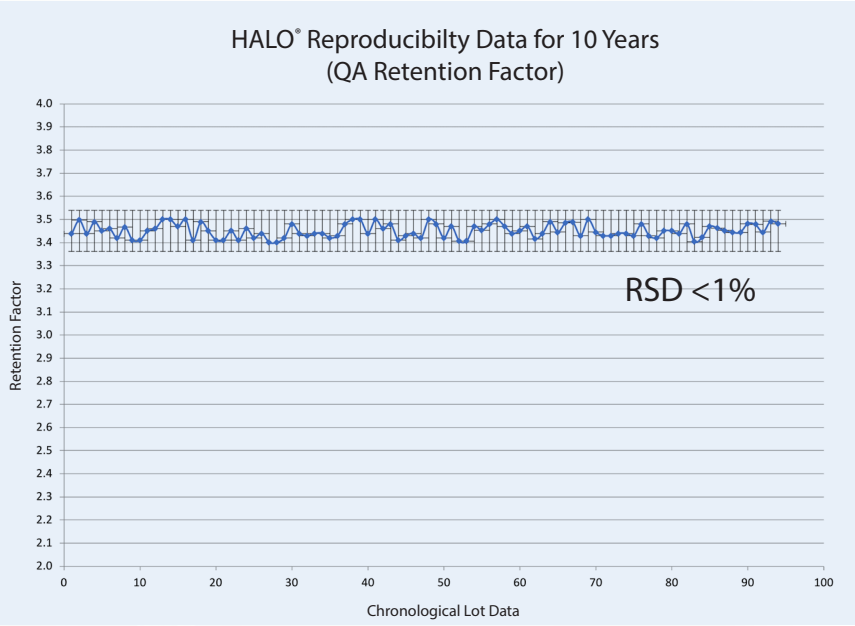
As the originators of Fused-Core® particles, Advanced Materials Technology incorporates the most knowledge in the industry to bring high-quality, innovative products to our customers. Our principal scientists have over 150 years of combined experience in liquid chromatography, particle synthesis and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

Table A. Guidance for Pore Size Selection

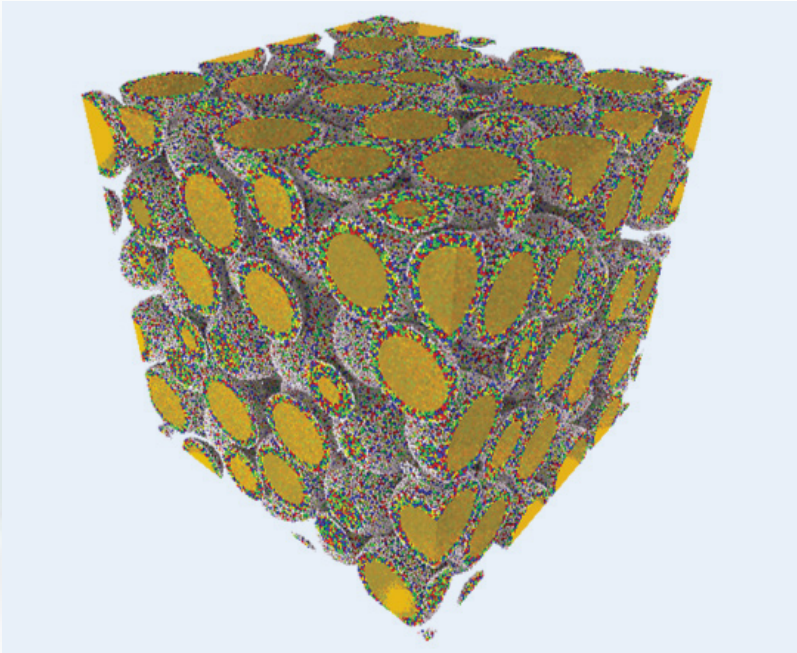
Molecule Size	Pore Size (Å)	Application	Particle Sizes (µm)	Column Family
SMALL (<5000 Da)	90	Small Molecules	2, 2.7, 5	HALO®
SMALL (< 20 kDa*)	90	Glycan	2.7	HALO® BIOCLASS
MEDIUM (100 Da < MW < 15 kDa)	160	Peptide	2, 2.7, 5	
LARGE (2 kDa < MW < 500 kDa)	400	Protein	3.4	
LARGE (> 50 kDa)	1000		2.7	

* 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

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

where

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

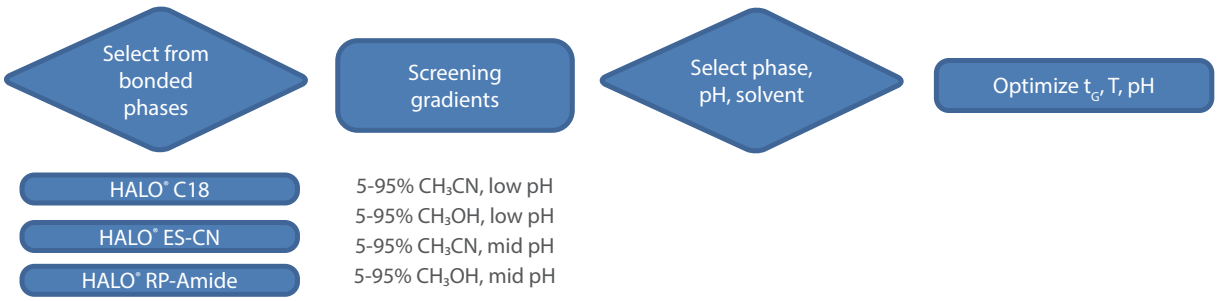
Moreover, column phase selectivity is one of the four most powerful and useful parameters for adjusting HPLC separation selectivity (see Table B). For ionizable analytes, mobile phase pH is, by far, the most effective parameter. However, column stationary phase is comparable to organic modifier choice (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)

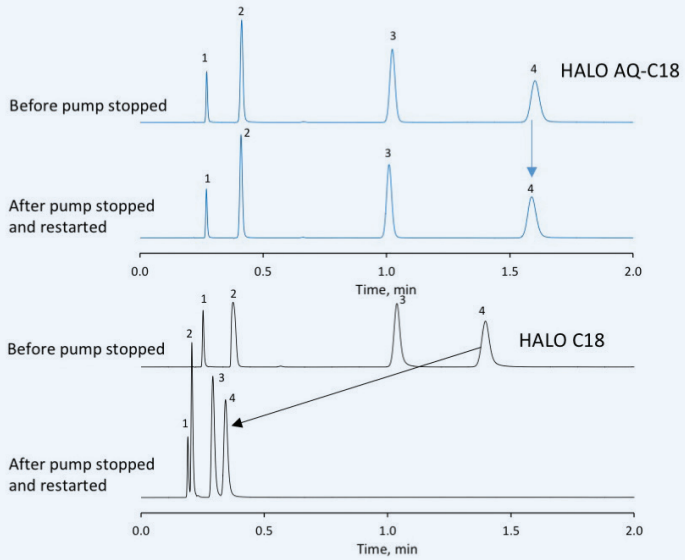
HPLC Parameter	Effectiveness for Changing Selectivity
Mobile phase pH (ionizable analytes only)	Most Effective
Organic modifier choice	
Percent organic modifier or gradient steepness	
Column stationary phase	
Column temperature	
Buffer choice	
Buffer concentration	Least Effective

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.



TEST CONDITIONS:

Column: 4.6 x 50 mm
  Top: HALO® 90Å AQ-C18, 2.7 µm
  Bottom: HALO® 90Å C18, 2.7 µm
Part Numbers:
  Top: 76444-980
  Bottom: 76444-964
Mobile Phase: 100% 20 mM Potassium Phosphate buffer, pH 7
Flow Rate: 2 mL/min
Temperature: 30 °C
Detection: 254 nm
Injection: 0.5 µL
Sample: (1) thiourea, (2) 5-fluorocytosine, (3) adenine and (4) thymine

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 2.7 5	90	6.7 7.0 5.5	120 135 90	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

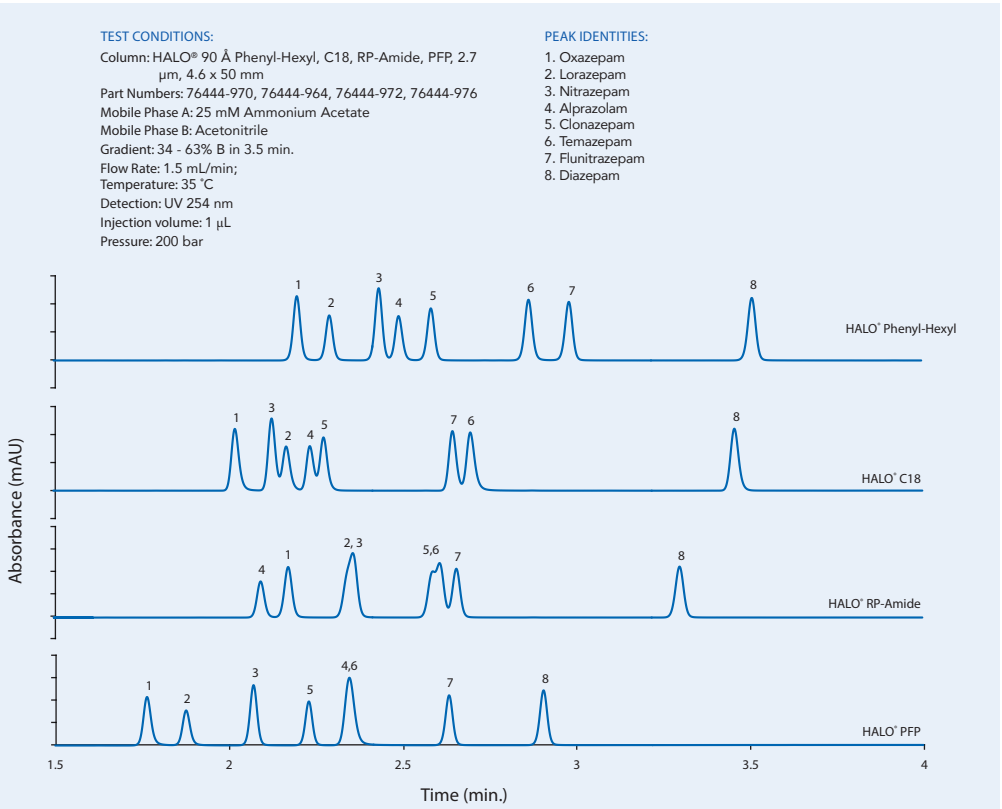
Bonded Phase	Features and Benefits	Target Analytes	Best Applications
C18 (dimethyloctadecylsilane)	• Excellent performance for broad range of analyte polarities	Diverse analytes ranging from polar to non-polar	• Pharmaceutical • Environmental • Cannabinoid • General purpose
AQ-C18 (Polar Modified)	• Resistant to dewetting, making it 100% aqueous mobile phase compatible • Enhanced retention for polar molecules	Acids, bases, polar analytes	• Pesticides • Nucleobases • Neurotransmitters • Polar acids
C8 (dimethyloctylsilane)	• Excellent performance for broad range of analyte polarities	Diverse analytes ranging from polar to non-polar	• Pharmaceutical • Environmental • Higher hydrophobic compounds
C30 (triacontyldimethyl)	• High shape selectivity for hydrophobic, long chain, structurally related isomers	Fat soluable vitamins (A, D, E, K) Hydrophobic analytes Lipids Carotenoids	• Foods • Lipids
Phenyl-Hexyl (dimethylphenyl-hexylsilane)	• Complementary selectivity to alkyl phases • Enhanced selectivity for aromatic compounds	Electron-poor molecules, aromatic or unsaturated compounds (ketones, nitriles, alkenes)	• Benzodiazepines • Aromatics • Drugs of abuse
Biphenyl (dimethylbiphenyl)	• Complementary selectivity to alkyl phases • Enhanced selectivity for aromatic compounds	Electron-poor molecules, aromatic or unsaturated compounds (ketones, nitriles, alkenes)	• Aromatic • Heterocycles • Drugs of abuse • Pain management drugs • Highly aqueous conditions
PFP (pentafluorophenylpropylsi-lane)	• Complementary selectivity to alkyl phases • Enhanced selectivity for stereoisomers • Can be used in RPLC and HILIC modes	Electron-rich compounds, aromatics, unsaturated com-pounds with double and/or triple bonds	• Steroids • Isomeric compounds • Substituted aromatics
ES-CN (diisopropylcyanopropylsilane)	• 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	• Explosives • Aromatics • Polar compounds
RP-Amide (C16 Amide)	• Complementary selectivity to alkyl phases • Enhanced stability for minimum bleed and long life	Alcohols, Acids, Phenols and Catechins	• Phenols • Alcohols • Catechins
HILIC (Bare Silica)	• Can be used in HILIC and normal-phase modes	Polar and very polar bases, acids and neutrals, especially with log P < 0.5	• Polar compounds
Penta-HILIC (proprietary penta-hydroxy ligand)	• Ideal for separation of highly polar compounds that are poorly retained in RPLC	Polar analytes with Log P values near or less than 0	• Polar Basic Compounds

REVERSED-PHASE SEPARATIONS WITH HALO®

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

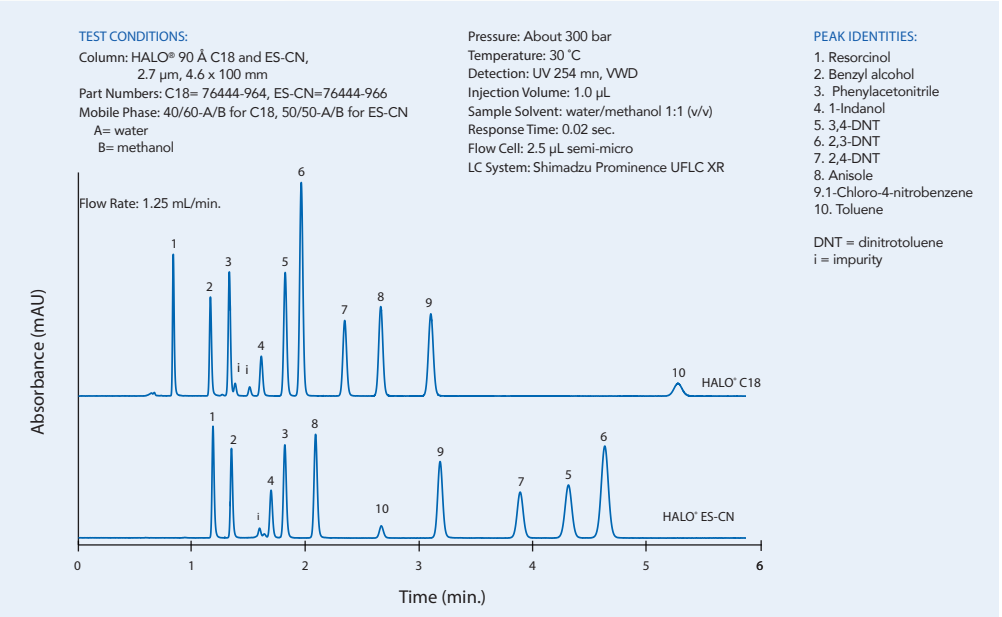
BENZODIAZEPINES ON HALO® FUSED-CORE BONDED PHASES

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



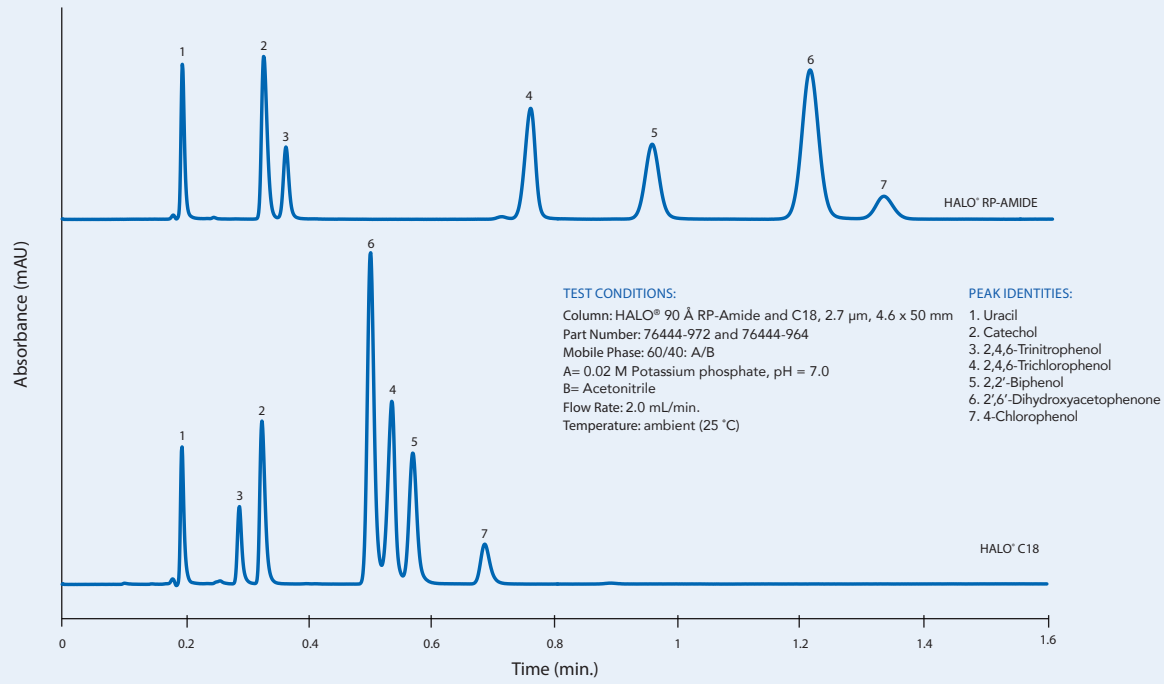
AROMATIC AND NITROAROMATIC COMPOUNDS

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



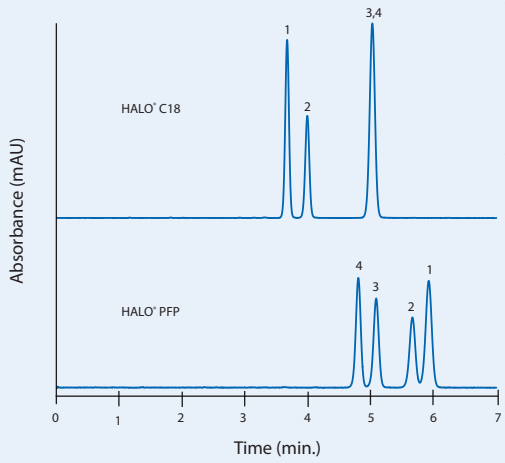
HALO® C18 VS. RP-AMIDE FOR PHENOLICS

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.

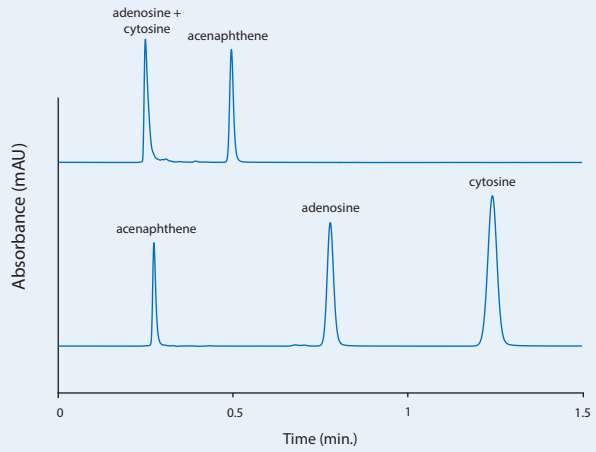
Some Typical Analytes for HILIC Separations

- 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.

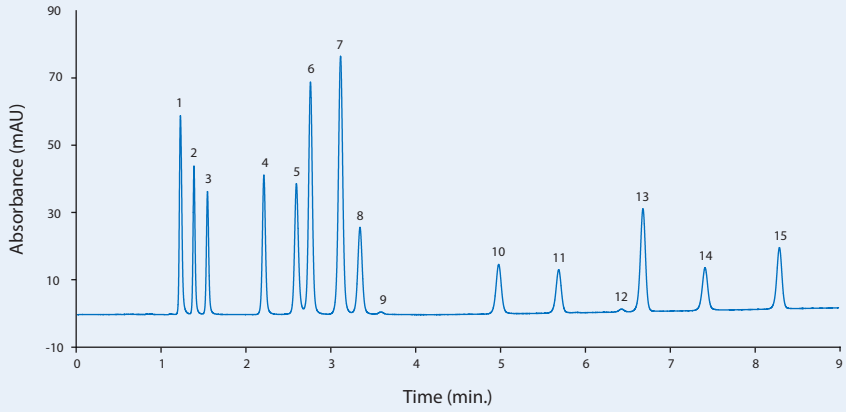


TEST CONDITIONS:
Column: HALO® 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 76444-964
Mobile Phase A: 90/10 ACN/0.1 M Ammonium Formate
Flow Rate: 1.8 mL/min.
pH: 3.0

TEST CONDITIONS:
Column: HALO® 90 Å HILIC, 2.7 µm, 4.6 x 50 mm
Part Number: 76444-962
Mobile Phase A: 90/10 ACN/0.1 M Ammonium Formate
Flow Rate: 1.8 mL/min
pH: 3.0

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.



TEST CONDITIONS:

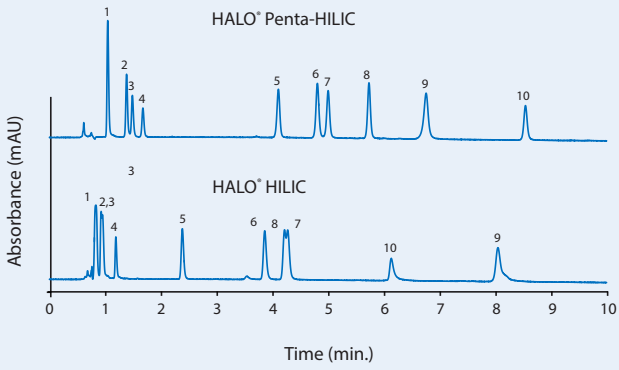
Column: HALO® 90 Å Penta-HILIC, 2.7 µm, 4.6 x 100 mm
Part Number:76443-510
Mobile Phase A: 10 mM Ammonium formate in 85% Acetonitrile/15% water
Mobile Phase B: 10mM Ammonium Formate in 93% Acetonitrile/7% water
Gradient: Time %B
0-3 min. 94
3-9 min. 94-0
Flow Rate: 1.5 mL/min.
Temperature: 35 °C
Detection: UV 260 nm, VWD
Injection volume: 3.0 µL
Pressure: about 230 bar

PEAK IDENTITIES:

1. Thymine
2. Uracil
3. Thymidine
4. 2'-Deoxyadenosine
5. Adenine
6. Uridine
7. Adenosine
8. Hypoxanthine
9. Xanthine
10. Cytosine
11. 2'-Deoxycytidine
12. Guanine
13. 2'-Deoxyguanosine
14. Cytidine
15. Guanosine

CEPHALO®SPORINS ON HALO® PENTA-HILIC AND HALO® HILIC

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



TEST CONDITIONS:

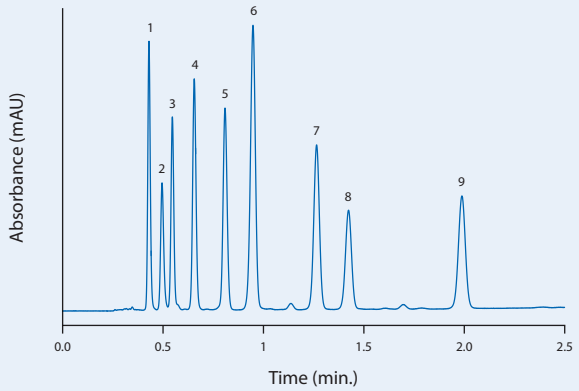
Column: HALO® 90 Å Penta-HILIC and HILIC, 2.7 µm, 2.1 x 50 mm
Part Number: Penta-HILIC: 76443-450
HILIC: 76444-762
Mobile Phase: A= 95/5 ACN/$H_2$0 with 5 mM NH_4formate, pH=3.0 (adj.)
Mobile Phase: B= 50/50 ACN/$H_2$0 with 5 mM NH_4formate, pH=3.0 (adj.)
Gradient: Penta-HILIC: 85-65% B in 10 min.
HILIC: 85-70% B in 10 min.
Flow Rate: 0.5 mL/min.
Pressure: 195 bar with Penta-HILIC
163 bar with HILIC
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 50/50 ACN/water
Flow Cell: 5 µL semi-micro
LC System: Agilent 1100

PEAK IDENTITIES:

1. CepHALO®thin
2. Cefoxitin
3. Cefotaxime
4. Cefazolin
5. Cefaclor
6. Cephalexin
7. Cephradine
8. Cefadroxil
9. Ceftazidime
10. CepHALO®sporin C

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.



TEST CONDITIONS:

Column: HALO® 90 Å ES-CN, 2.7 µm, 4.6 x 100 mm
Part Number: 76444-966
Mobile Phase A: 0.02 M Phosphate buffer, pH 2.7 (adj.)
Mobile Phase B: Methanol
Gradient: 20% B to 40% B in 2.5 min.
Flow Rate: 2.0 mL/min.
Temperature: 40 °C
Detection: UV 254 nm, VWD
Injection Volume: 1.0 µL
Sample Solvent: 70/30: Water/Methanol
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
Pressure: 225 bar at start of gradient

PEAK IDENTITIES:

1. Cefadroxil
2. Ceftazidime
3. Cefaclor
4. Cephalexin
5. Cephradine
6. Cefotaxime
7. Cefoxitin
8. Cefazolin

HALO®

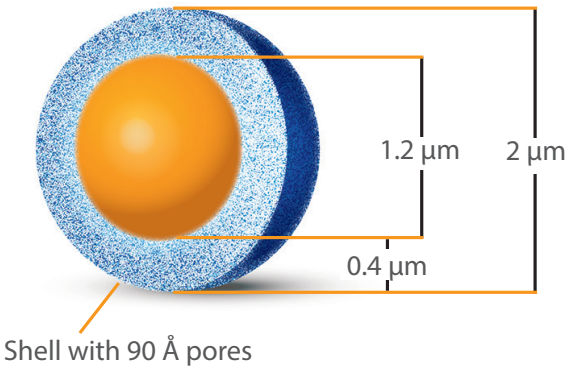
HALO® 90 Å 2 µm (UHPLC)

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

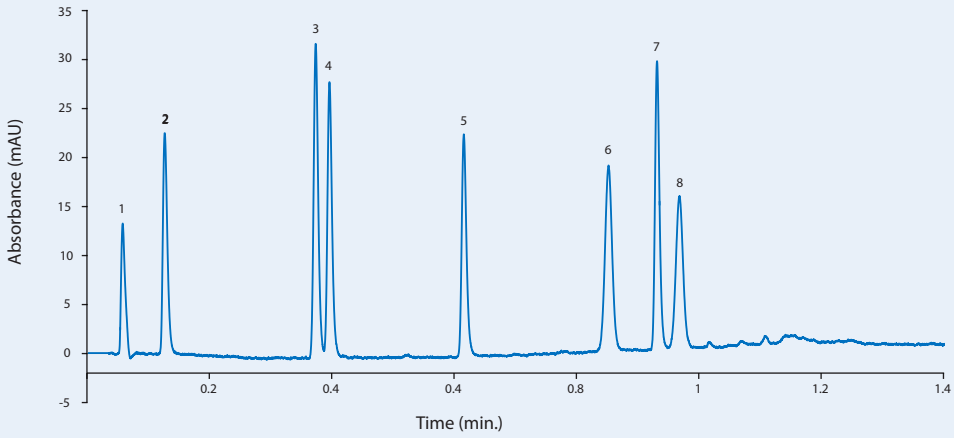
Extremely high efficiency columns such as the HALO® 90 Å 2 µm columns require minimal band dispersion to see the greatest benefit.

HALO® 2 µm



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: 76443-838
Mobile Phase A: 20 mM Formic acid
Mobile Phase B: 50/50 Acetonitrile/Methanol
Gradient:

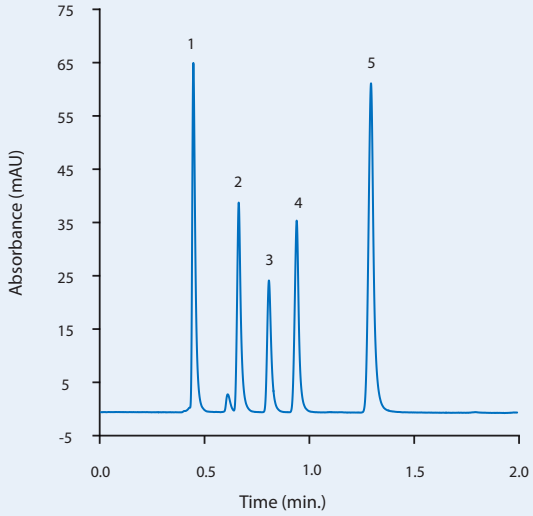
Time	%B
0-0.06	20
0.06-1.06	20-75

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_0)
2. 6,7-Dihdroxycoumarin
3. 4-Hydroxycoumarin
4. Coumarin
5. 6-Chloro-4-hydroxycoumarin
6. Warfarin
7. Coumatetralyl
8. Coumachlor

FAST LOCAL ANESTHETIC SEPARATION USING HALO® 2 µm PENTA-HILIC

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

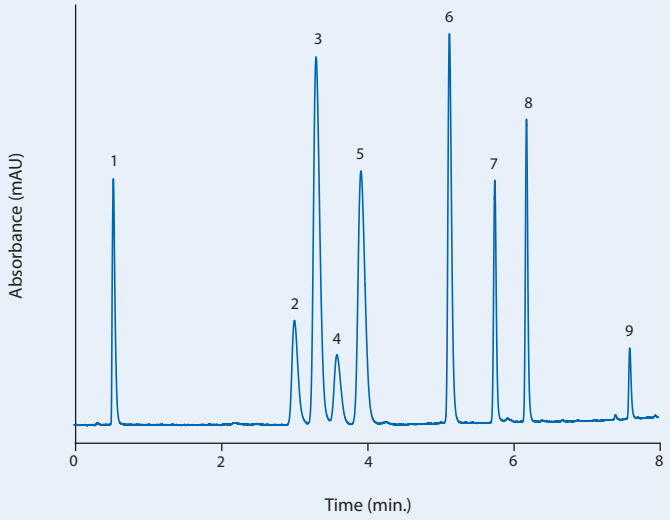


TEST CONDITIONS:
Column: HALO® 90 Å Penta-HILIC, 2 µm, 2.1 x 100 mm
Part Number: 76443-902
Isocratic: 92/8: ACN/water with 5mM Ammonium Formate buffer, pH 3
Flow Rate: 0.5 mL/min.
Temperature: 30 °C
Detection: UV 245 nm, photodiode array detector
Injection Volume: 1.0 µL
Sample Solvent: 90/10 ACN/0.1 M ammonium formate buffer pH3
Data Rate: 40 Hz
Response Time: 0.1 sec.
Flow Cell: 2.5 µL 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 µm PFP

Figure X. HALO® PFP columns often show excellent selectivity for steroids. HALO® 2 µm 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 µm, 3.0 x 50 mm
Part Number: 76444-010
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, VWD
Injection volume: 2 µL
Sample Solvent: methanol
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

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

See page 32 for full list of HALO® 2 µm part numbers.

HALO®

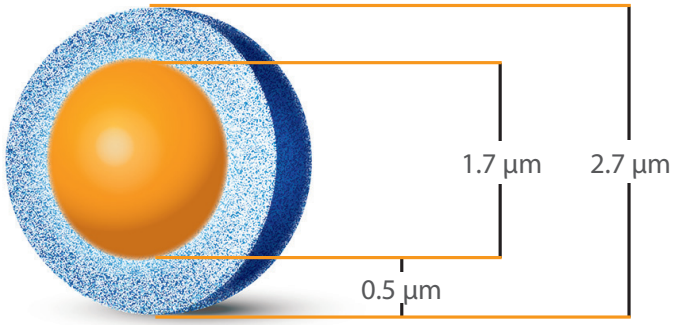
HALO® 90 Å 2.7 µm (UHPLC AND HPLC)

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

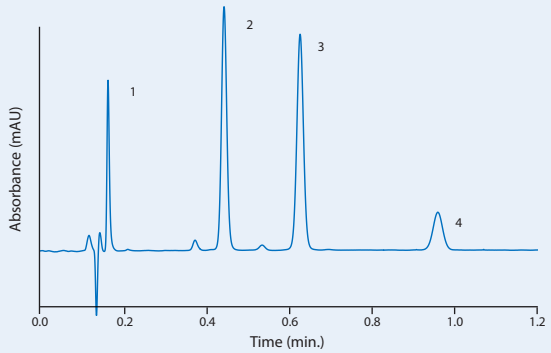
* HALO® C30 160 Å, 2.7 µm

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:

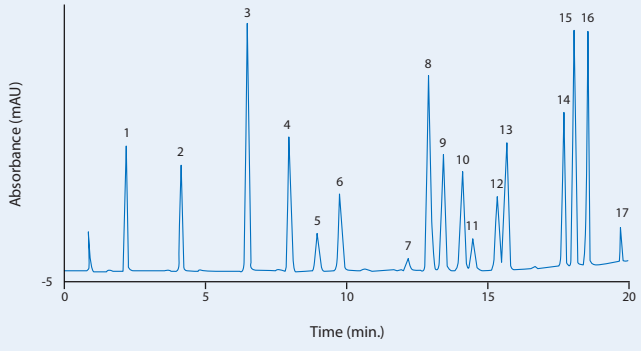
Column: HALO® 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 76444-970
Mobile Phase: 43/57: A/B
A: 0.02 M formic acid in water
B: Acetonitrile
Flow Rate: 2.5 mL/min.
Pressure: 228 Bar
Temperature: 26 °C
Detection: UV 240 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 80/20 methanol/water (20 mM 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 x 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: 76445-022
Mobile Phase A: Water
Mobile Phase B: Methanol
Gradient:

Time	%B
0.0	25
14.0	35
20.0	62

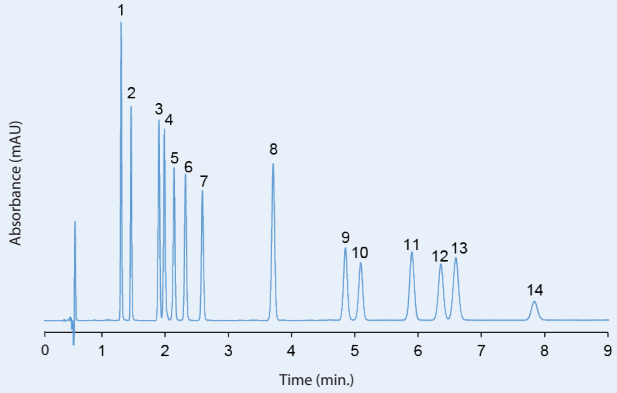
Flow Rate: 1.5 mL/min.
Temperature: 43 °C
Detection: UV 220 nm, VWD
Injection Volume: 40 µL
Sample Solvent: 50/50: Water/methanol
Response Time: 0.02 sec.
Data rate: 25 Hz
Pressure: 366 bar to start, max. 405 bar
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

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
10. 2-Amino-4,6-Dinitrotoluene
11. 4-Amino-2,6-Dinitrotoluene
12. 2,4-Dinitrotoluene
13. 2,6-Dinitrotoluene
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.

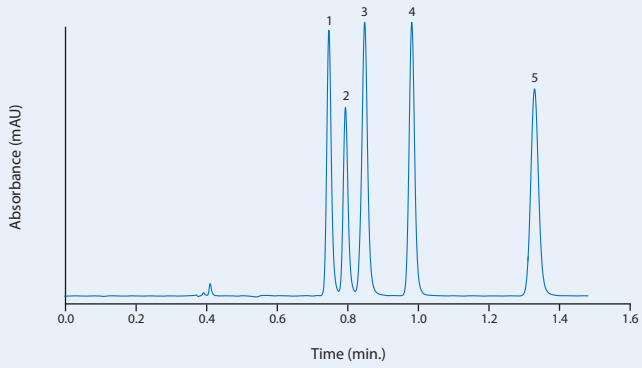


TEST CONDITIONS:
Column: HALO® 90 Å C18, 2.7 µm, 3.0 x 150 mm
Part Number: 76444-892
Mobile Phase: 25/75 A/B
A: Water/0.1% formic acid
B: Acetonitrile/0.085% formic acid
Flow Rate: 1.0 mL/min
Pressure: 350 bar
Temperature: 30 °C
Detection: UV 220 nm, PDA
Injection: 0.6 µL
Sample Solvent: 75/25 methanol/ water
Response Time: 0.025 sec.
Data Rate: 100 Hz
Flow Cell: 1 µL
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 (Δ9-THC)
10. delta-8-Tetrahydrocannabinol (Δ8-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.

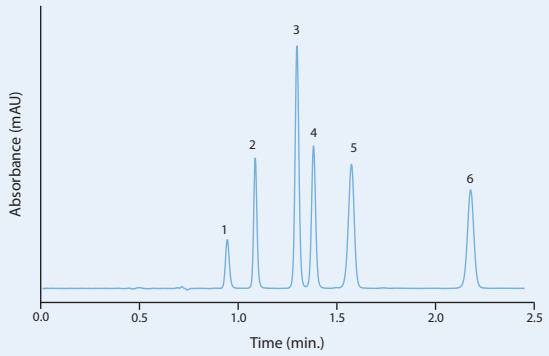


TEST CONDITIONS:
Column: HALO® 90 Å Penta HILIC, 2.7 µm, 4.6 x 100 mm
Part Number: 76443-510
Mobile Phase: 7/93: A/B
A: 0.1 M Ammonium formate, pH=3.5 (adj.)
B: Acetonitrile
Flow Rate: 2.5 mL/min.
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 10/90: Water/acetonitrile
Response Time: 0.02 sec.
Maximum Pressure: 165 Bar
Flow Cell: 2.5 µL 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 µm ES-CN

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



TEST CONDITIONS:
Column: HALO® 90 Å ES-CN, 2.7 µm, 4.6 x 100 mm
Part Number: 76445-006
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, VWD
Injection Volume: 0.5 µL
Sample Solvent: Acetonitrile
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

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

See page 33 for full list of HALO® 2.7 µm part numbers.

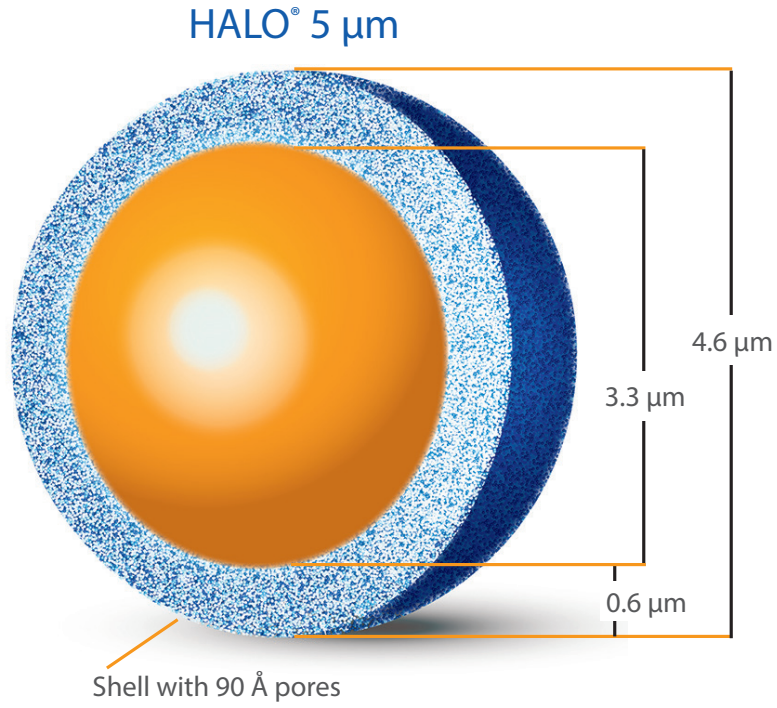
HALO®

HALO® 90 Å 5 µm (HPLC)

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

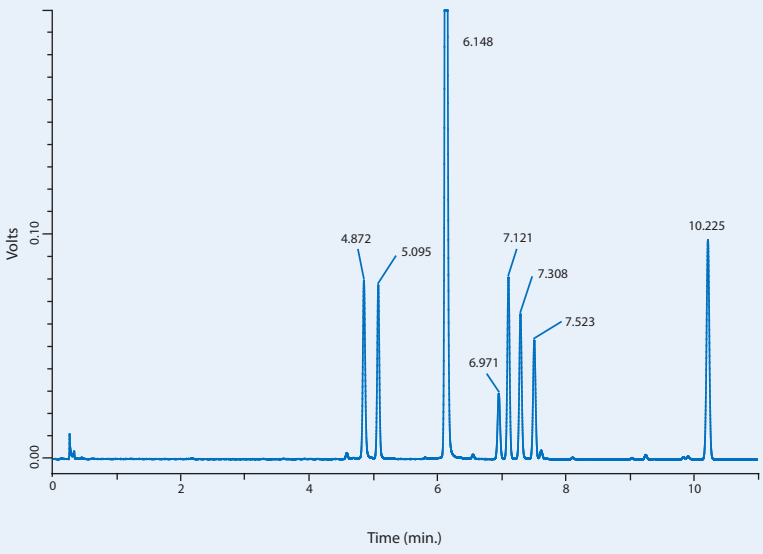
Ideal for:

- 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.



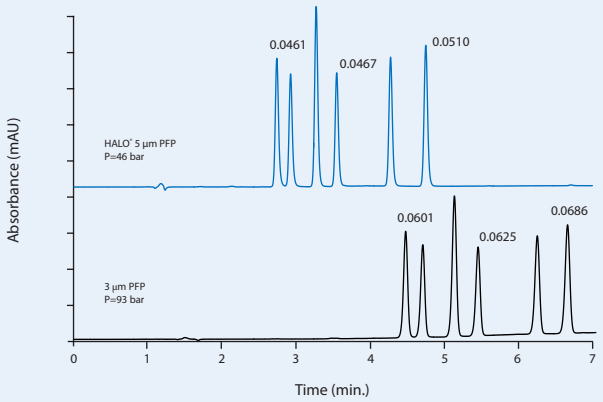
SAMPLE:
Mixture of 8 flavonoids, 1 µL in MeOH

TEST CONDITIONS:
Column: HALO® 90 Å C18, 5 µm, 2.1 x 150 mm
Part Number: 76445-726
Flow Rate: 1.0 mL/min.
Temperature: 40 °C
Gradient: 5% CH_3CN for 0.5 min.
5-60% CH_3CN/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.



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

TEST CONDITIONS:
Column: HALO® 90 Å PFP, 5 µm, 4.6 x 100 mm
Part Number: 76445-998
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

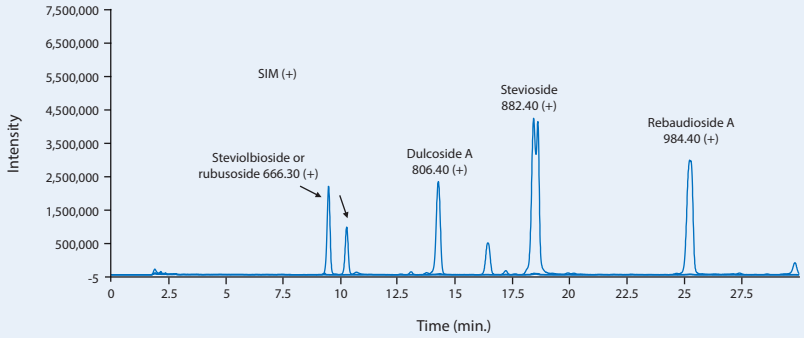
PEAK IDENTITIES:
1. Oxazepam
2. Lorazepam
3. Nitrazepam
4. Clonazeparm
5. Flunitrazepam
6. Diazepam

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

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

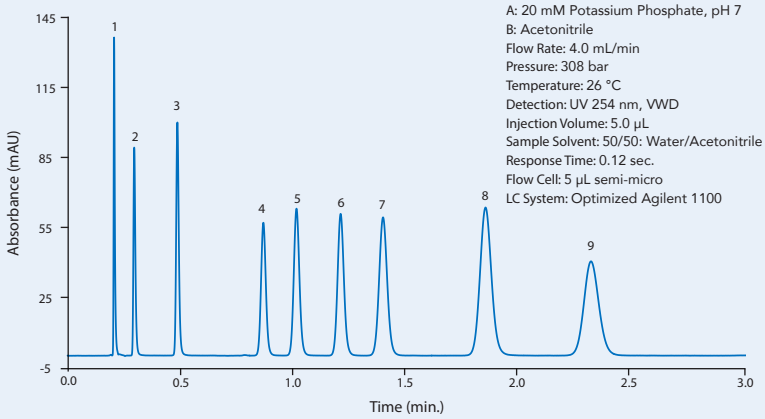
Figure FF.
LC-MS analysis of stevia glycosides from a Stevia natural sweetener extract is easily accomplished using the HALO® 5 µm Penta-HILIC column due to its unique bonded phase containing five OH groups.

TEST CONDITIONS:
Column: HALO® 90 Å Penta-HILIC, 5 µm, 3.0 x 250 mm
Part Number: 76445-890
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: 76445-994
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

See page 34 for full list of HALO® 5 µm part numbers.

HALO® BIOCLASS

Table F. HALO® BioClass Column Specifications

Bonded Phase		USP Designation	Particle Sizes (s) (µm)	Pore Size (Å)	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 2.7 5	160	4.0 4.6 4.0	65 90 60	1/90 °C	8/40 °C	No
	ES-CN	L10	2.7 5	160	2.2 1.5	90 60	1/90 °C	8/40 °C	Yes
	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

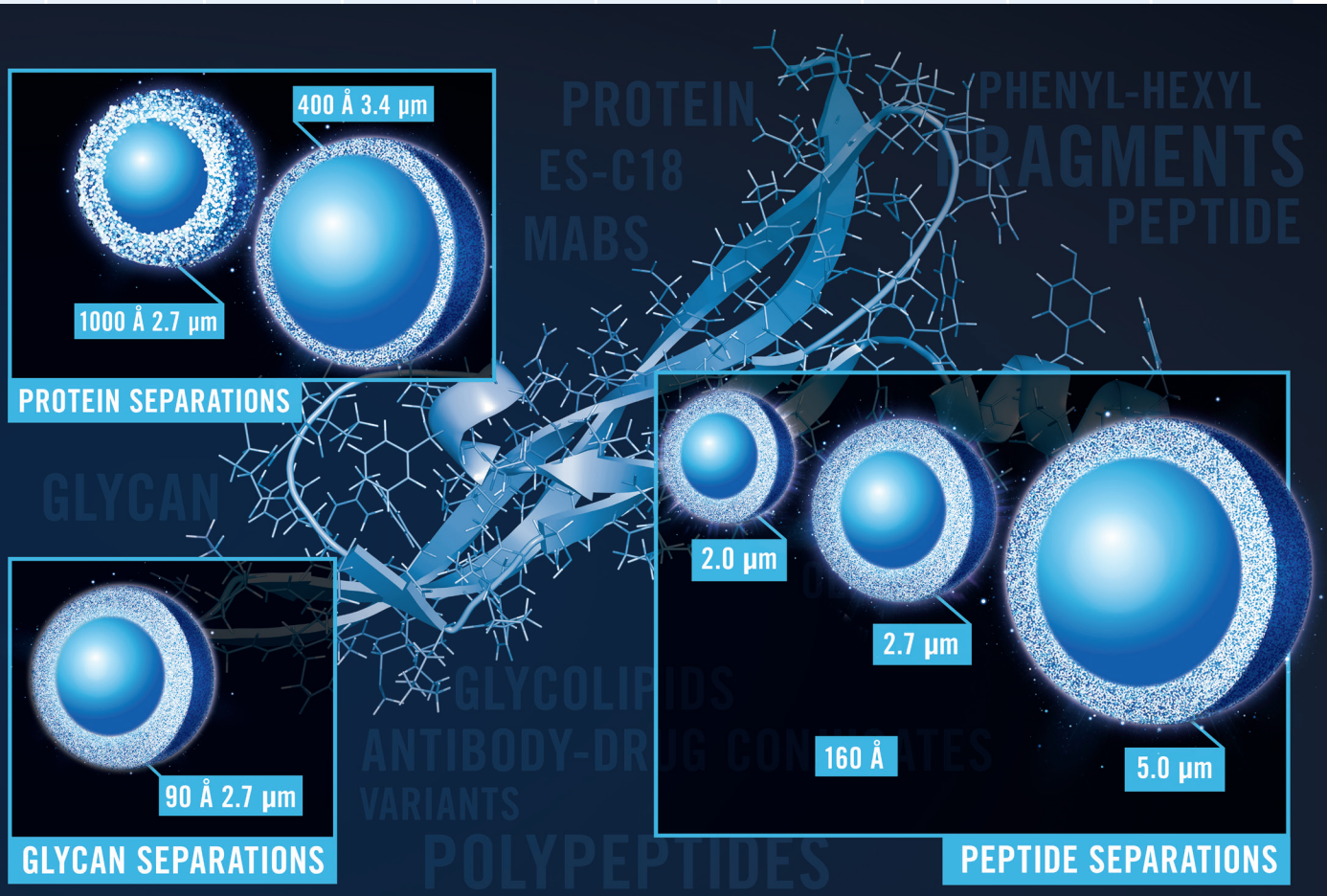


Table G. HALO® BioClass Features & Benefits				
	Bonded Phase	Features and Benefits	Target Analytes	Best Applications
1000 Å Protein	C4 (dimethylbutylsilane)	• Outstanding high temperature stability at low pH • Unrestricted access to bonded phase • Exceptional mass transfer kinetics • Compatible with 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)	• 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)	• 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)	• 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)	• 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)	• 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)	• 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)	• 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	• 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

HALO® BIOCLASS

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.

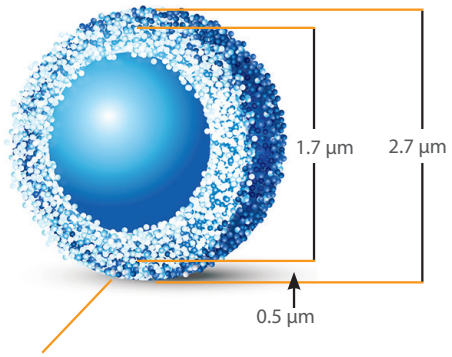
- Benefits of HALO® protein solutions include:
  - Provides narrower peaks and better recoveries for large biomolecules (vs. smaller pore sizes and non-core particles)
  - Allows HALO® Protein columns to be used with both UHPLC and HPLC instrumentation for fast bioseparations at moderate back pressures

- 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

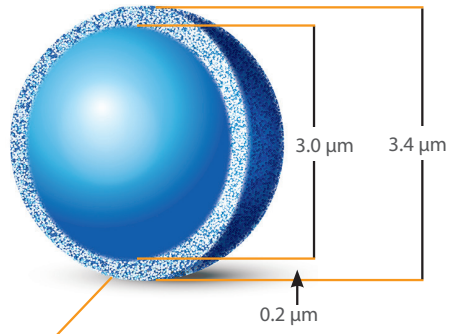
- Pressure limit, 600 bar/9000 psi

HALO® 1000 Å 2.7 µm



Shell with 1000 Å pores

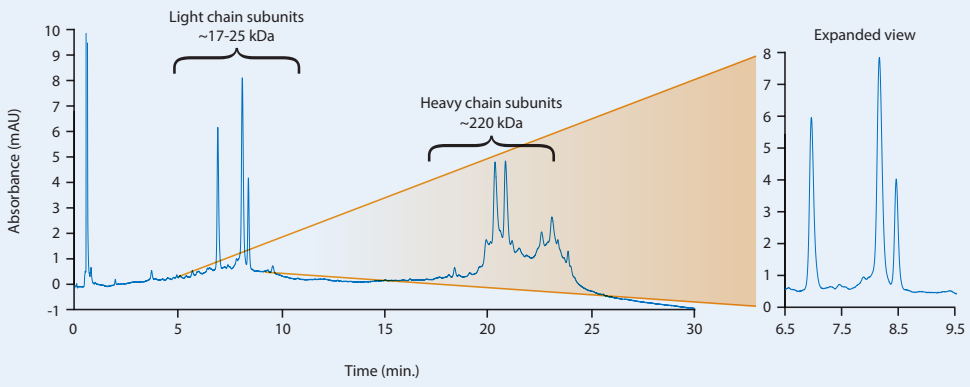
HALO® 400 Å 3.4 µm



Shell with 400 Å pores

LARGE PROTEIN SEPARATION USING HALO® PROTEIN C4 FUSED-CORE COLUMN

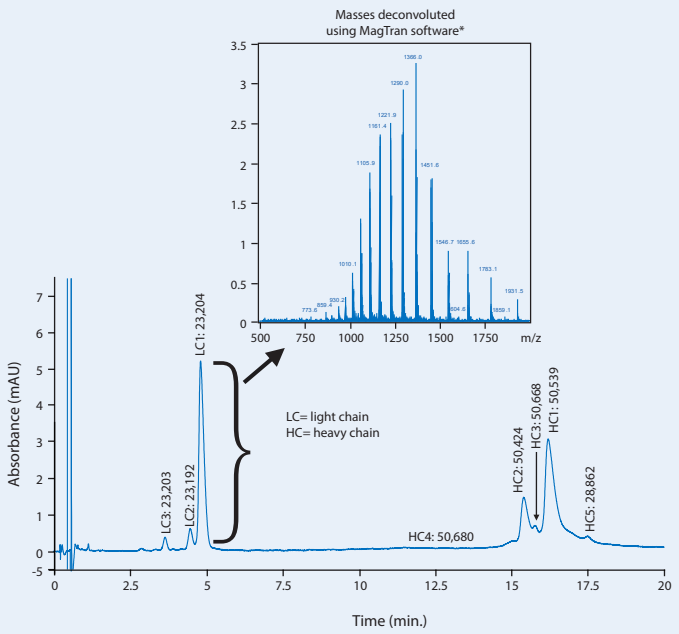
Figure HH. High resolution separation of light and heavy chains of a denatured contractile protein (whole myosin from purified rabbit skeletal muscle) using HALO® 400 Å Protein C4 at 80 ˚C.



TEST CONDITIONS:
Column: HALO® 400 Å C4, 3.4 µm, 2.1 x 100 mm Part Number: 76445-148
Instrument: Agilent 1200 SL
Injection Volume: 1 µL
Sample: denatured myosin
Detection: 215 nm
Flow Rate: 0.45 mL/min.
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.09% TFA
Gradient: 35–55% B in 30 min.
Temperature: 80 ˚C

HIGH RESOLUTION OF LIGHT AND HEAVY CHAIN VARIANTS OF IgG1

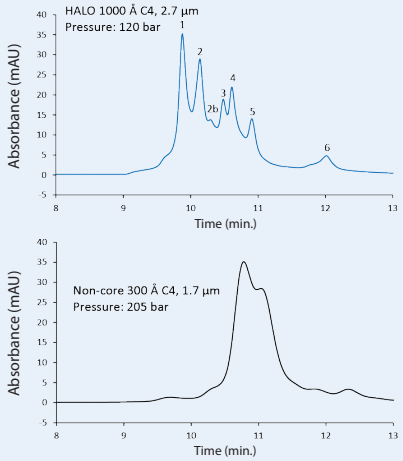
Figure II.
Very high resolution is obtained between variants of light and heavy chains of a reduced and alkylated monoclonal antibody (IgG1) sample using a HALO® 400 Å Protein C4 column.



TEST CONDITIONS:
Column: HALO® 400 Å C4, 3.4 µm, 2.1 x 100 mm
Part Number: 76445-148
Mobile Phase A: 0.5% formic acid with 20 mM Ammonium Formate
Mobile Phase B: 45% ACN/45% IPA/10% A solvent
Gradient: 29–32% B in 20 min.
Temperature: 80 °C
Detection: 280 nm and MS using 2pps scan rate from 500 to 2000 m/z
Injection Volume: 2 µL of 2 µg/µL reduced and alkylated IgG1
Sample Solvent: 0.25% (v/v) formic acid in water
MS Parameters: Positive ion mode, ESI at +4.5 kV, 400 °C heatblock, 225 °C capillary
LC-MS System: Shimadzu Nexera and LCMS-2020 (single quadrupole MS)

INCREASED RESOLUTION OF IGG2 OVER TOTALLY POROUS COLUMN

Figure JJ.
The larger pores of the HALO® 1000 Å C4 column allow improved access to the stationary phase and increased resolution for IgG2 isoforms compared to the smaller 300 Å pores of the non-core C4 column.



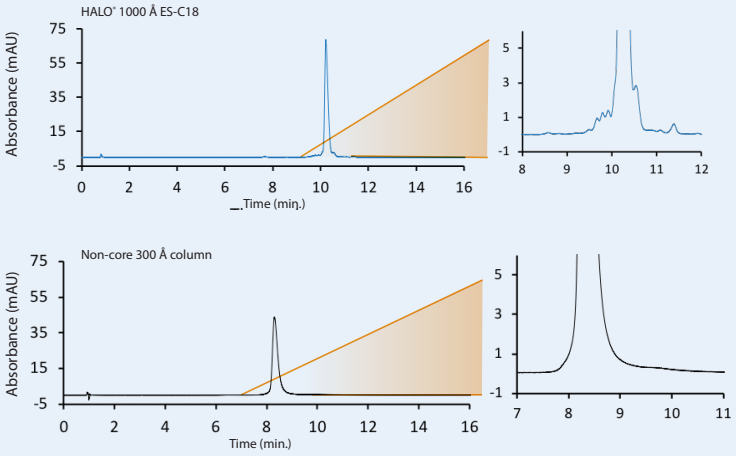
TEST CONDITIONS:
Column: HALO® 1000 Å C4, 2.7 µm, 2.1 x 150 mm
Part Number: 76444-416
Mobile Phase A: 88/10/2 water/AcN/n-propanol/0.1% DFA
Mobile Phase B: 70/20/10 n-propanol/AcN/water/0.1% DFA
Gradient: 14-24 %B in 20 min
Flow Rate: 0.2 mL/min
Temperature: 80 °C
Detection: UV 280 nm, PDA
Injection: 2 µL of 2 mg/mL denosumab in water/0.1% DFA
LC System: Shimadzu Nexera

PEAK IDENTITIES
1. IgG2-B
2. IgG2-B
2b. IgG2-B
3. IgG2-A/B
4. IgG2-A/B
5. IgG2-A
6. IgG2-A*

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

NARROWER PEAK AND MORE RESOLUTION THAN TOTALLY POROUS COLUMN

Figure KK.
HALO® 1000 Å ES-C18 outperforms a non-core column with 300 Å pores. The zoomed-in region of the base of the NISTmAb peak shows more resolution with HALO® 1000 Å ES-C18, as well.



TEST CONDITIONS:
Column: HALO® 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 76444-414
Mobile Phase A: Water/0.1% TFA
Mobile Phase B: Acetonitrile/0.1% TFA
Gradient: 36-44 %B in 16 min
Flow Rate: 0.4 mL/min
Temperature: 60 °C
Detection: UV 280 nm, PDA
Injection: 2 µl of 2 mg/mL NISTmAb
Flow Cell: 1 µL
LC System: Shimadzu Nexera

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

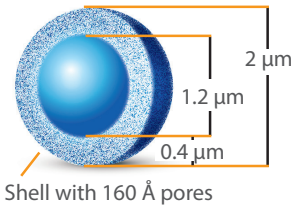
See page 35 for full list of HALO® Protein part numbers.

HALO® BIOCLASS

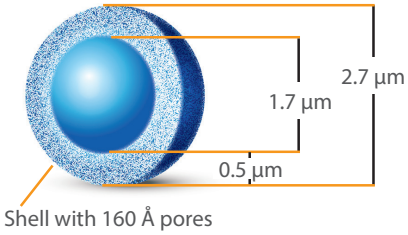
PEPTIDE SOLUTIONS

- 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

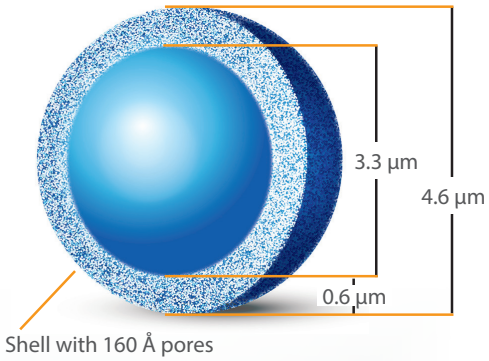
HALO® 2 µm Peptide



HALO® 2.7 µm Peptide

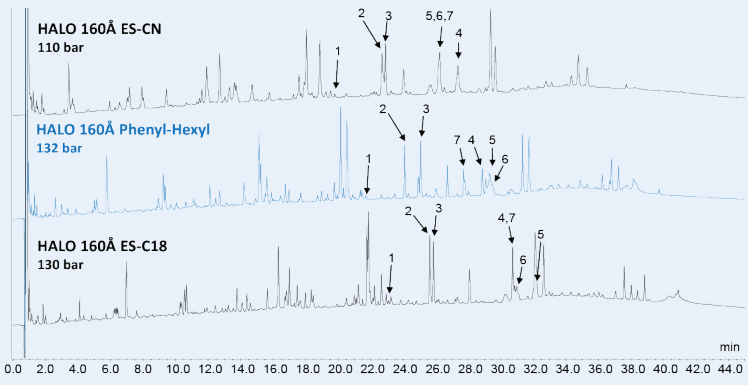


HALO® 5 µm Peptide



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:

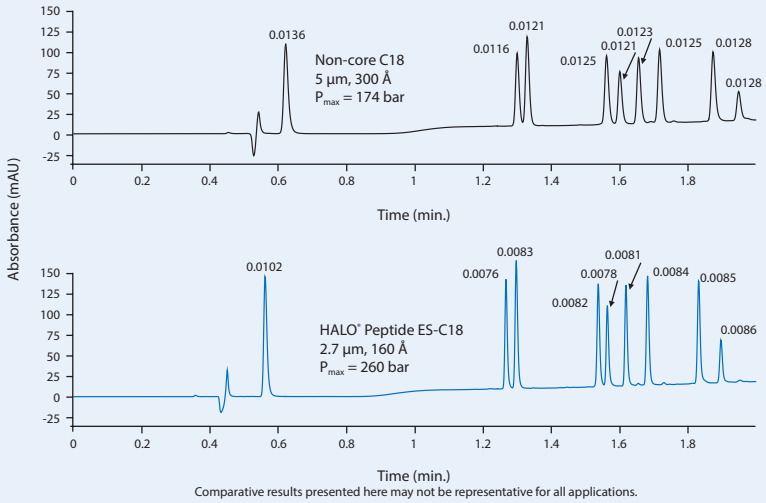
Columns: HALO® 160Å ES-CN, 2.7 µm, 2.1 x 100 mm
Part Number: 76444-274
HALO® 160Å Phenyl-Hexyl, 2.7 µm, 2.1 x 100 mm
Part Number: 76444-148
HALO® 160Å ES-C18, 2.7 µm, 2.1 x 100 mm
Part Number: 76444-272
Mobile Phase:
A = water + 10 mM difluoroacetic acid (DFA)
B = ACN + 10 mM difluoroacetic acid
Flow 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. FTISADTSKNTAYLQMNSLR (754 m/z)
2. LScAASGFNIKDTYIHWVR (747 m/z)
3. GFYPSDIAVEWESNGQPENNYK (849 m/z)
4. LLIYSASFLYSGVPSR (592 m/z)
5. SGTASVVcLLNNFYPR (899 m/z)
6. ScDKTHTcPPcPAPELLGGPSVFLFPPKPK (834 m/z)
7. VVSVLTVLHQDWLNGKEYK (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.



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

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: 76444-328
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

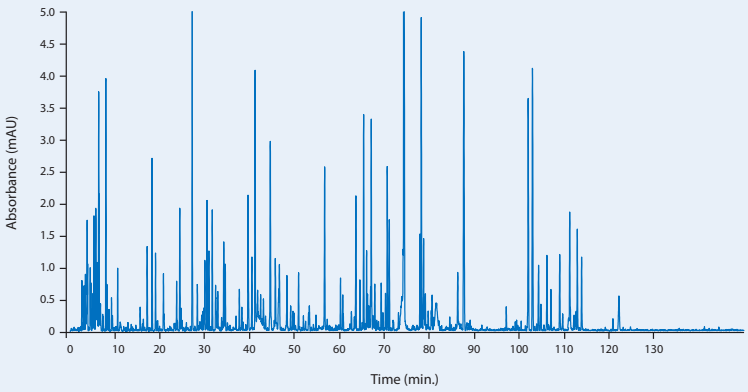
PEAK IDENTITIES:

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.

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.

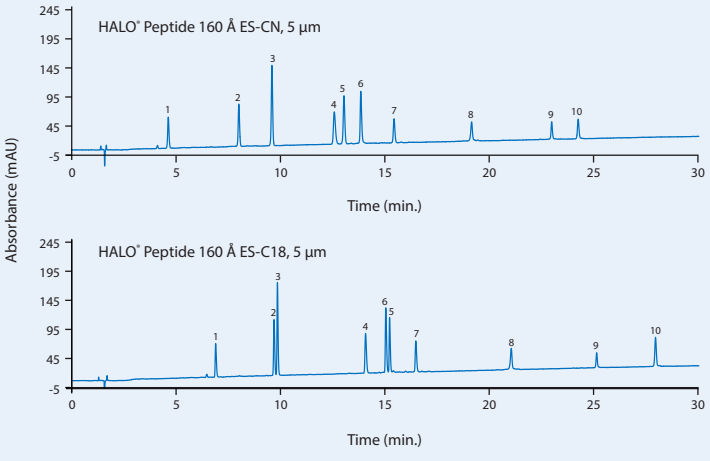


TEST CONDITIONS:

Columns: HALO® 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm (3)
Part Number: 3 of 76444-276
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: 76445-400
ES-C18: 76445-398
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. ß–endorphin
9. Sauvagine
10. Mellitin

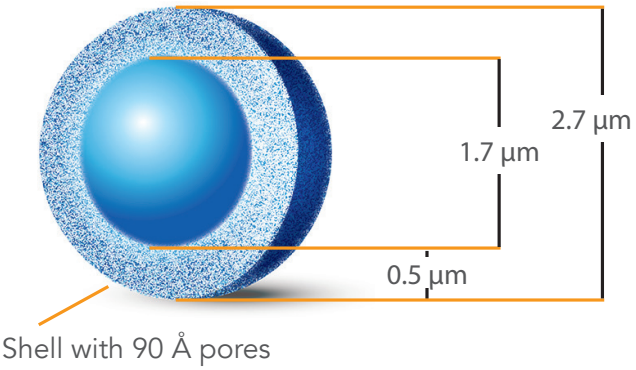
See page 36 for full list of HALO® Peptide part numbers.

HALO® BIOCLASS

GLYCAN SOLUTIONS

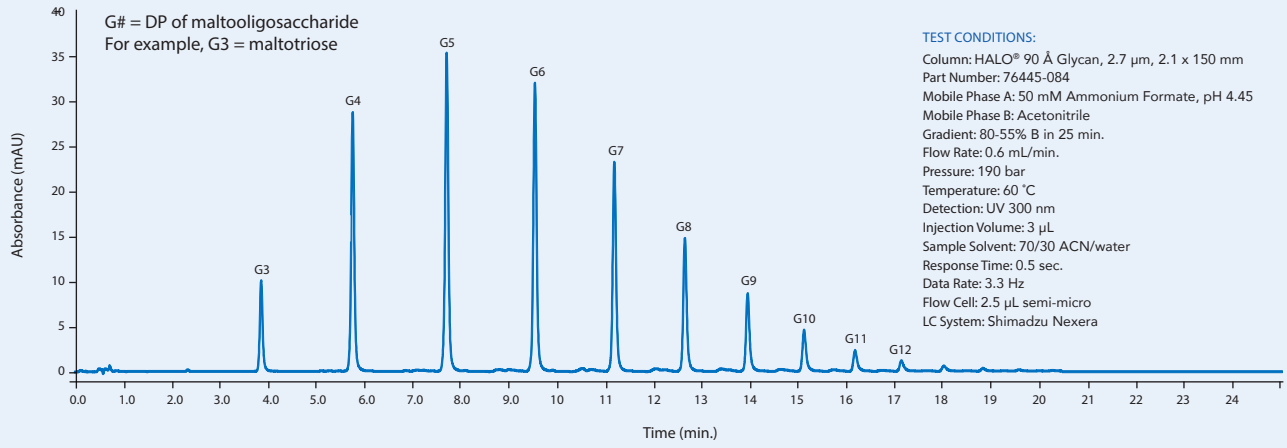
- 90 Å pore size
- Incorporates a highly polar ligand that contains 5 hydroxyl groups tethered to 2.7 µm Fused-Core® silica particles via novel, proprietary linkage chemistry
- Ideal for hydrophilic interaction liquid chromatography (HILIC) separations of oligosaccharides, and particularly, of released 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

HALO® Glycan



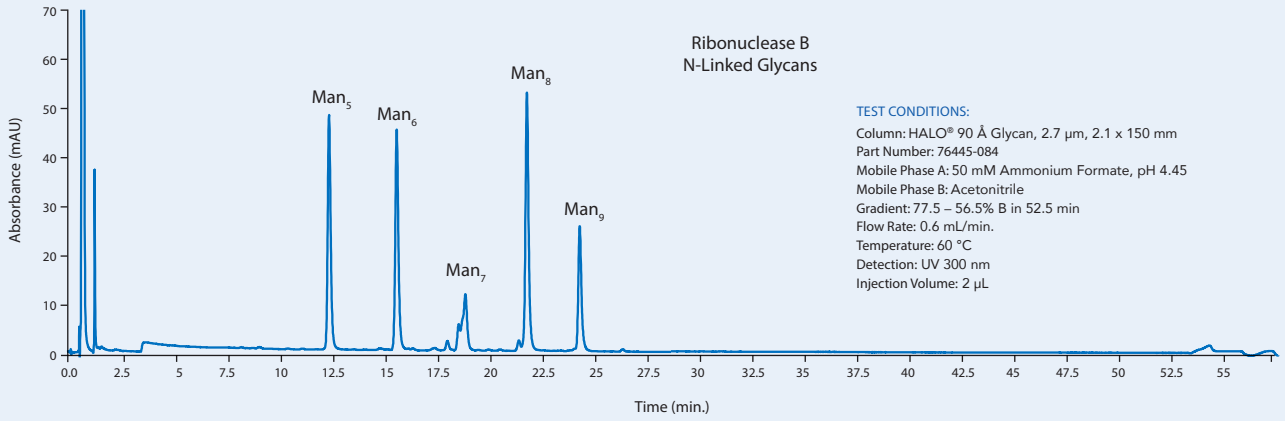
QA ANALYSIS OF HALO® GLYCAN

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.



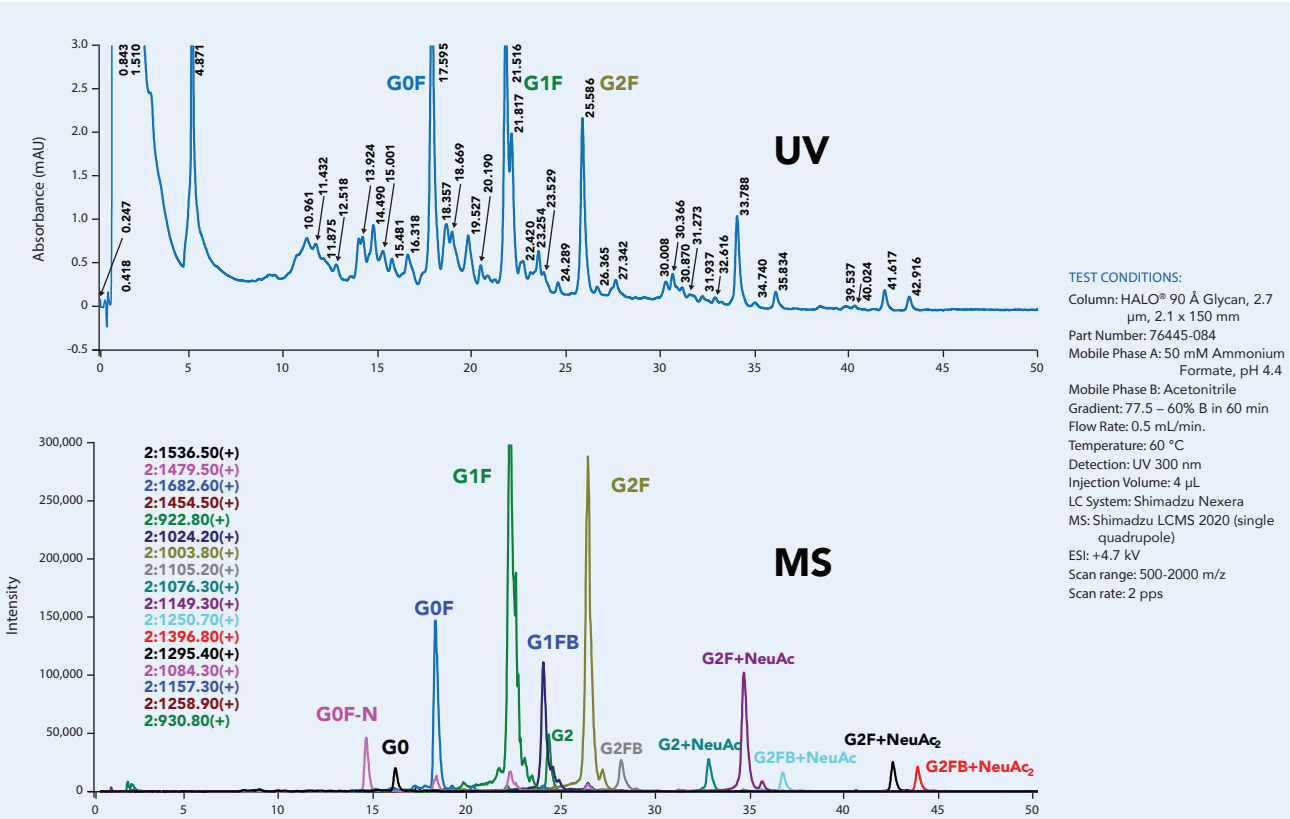
SEPARATION OF N-LINKED GLYCANS FROM RIBONUCLEASE B

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.

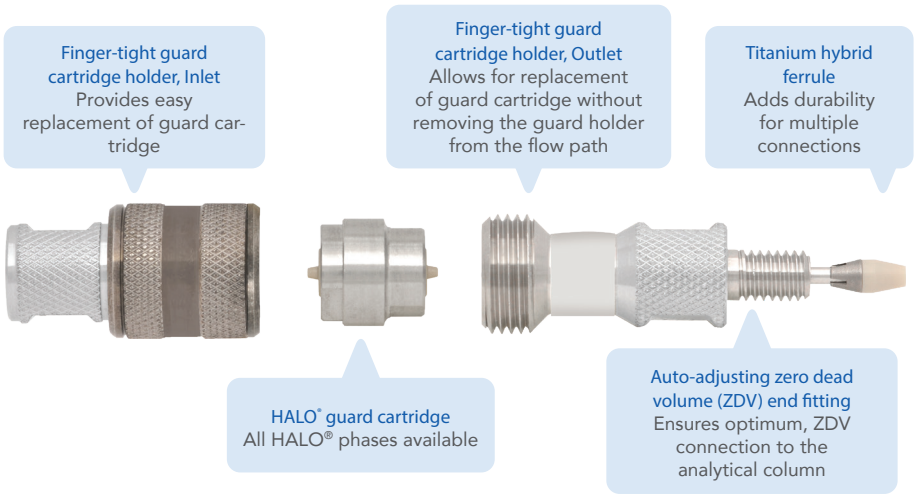


See page 35 for full list of HALO® Glycan part numbers.

HALO® UHPLC AND HPLC GUARD COLUMNS

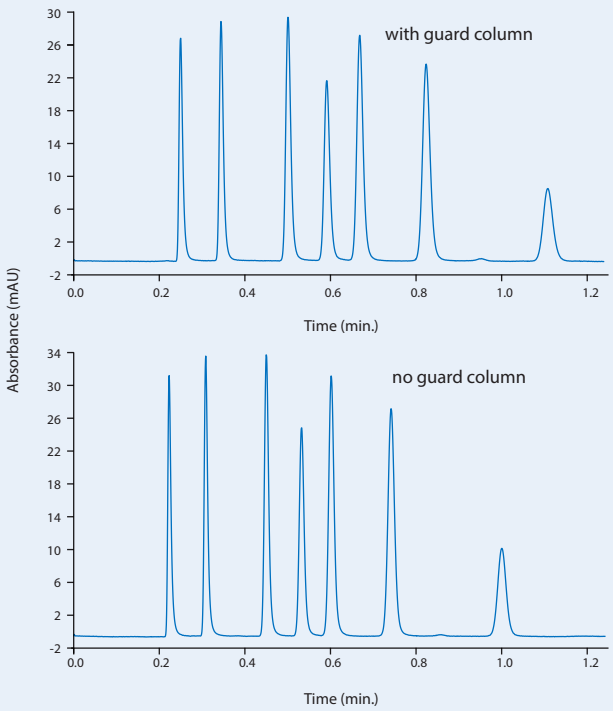
- Collect strongly retained compounds from the sample and minimizes column fouling
- Ultra-low dispersion, easy to use, operate at pressures up to 1000 bar
- Finger-tight, direct-connect units that auto-adjust to any column with a 10–32 inlet port
- Easily replace guard cartridge without removing guard 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 SS.
HALO® guard columns provide optimum protection for your HALO® HPLC and UHPLCcolumn 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.

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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.

Dimensions ID x Length (in mm)	C18	AQ-C18	C8	Biphenyl	Phenyl-Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 20	91812-202	91812-222	91812-208	91812-211	91812-206	91812-207	91812-209	91812-204	91812-205	91812-201
2.1 x 30	91812-302	91812-322	91812-308	91812-311	91812-306	91812-307	91812-309	91812-304	91812-305	91812-301
2.1 x 50	91812-402	91812-422	91812-408	91812-411	91812-406	91812-407	91812-409	91812-404	91812-405	91812-401
2.1 x 75	91812-502	91812-522	91812-508	91812-511	91812-506	91812-507	91812-509	91812-504	91812-505	91812-501
2.1 x 100	91812-602	91812-622	91812-608	91812-611	91812-606	91812-607	91812-609	91812-604	91812-605	91812-601
2.1 x 150	91812-702	91812-722	91812-708	91812-711	91812-706	91812-707	91812-709	91812-704	91812-705	91812-701
2.1 x 250	91812-902	91812-922	91812-908	91812-911	91812-906	91812-907	91812-909	91812-904	91812-905	91812-901
3.0 x 20	91813-202	91813-222	91813-208	91813-211	91813-206	91813-207	91813-209	91813-204	91813-205	91813-201
3.0 x 30	91813-302	91813-322	91813-308	91813-311	91813-306	91813-307	91813-309	91813-304	91813-305	91813-301
3.0 x 50	91813-402	91813-422	91813-408	91813-411	91813-406	91813-407	91813-409	91813-404	91813-405	91813-401
3.0 x 75	91813-502	91813-522	91813-508	91813-511	91813-506	91813-507	91813-509	91813-504	91813-505	91813-501
3.0 x 100	91813-602	91813-622	91813-608	91813-611	91813-606	91813-607	91813-609	91813-604	91813-605	91813-601
3.0 x 150	91813-702	91813-722	91813-708	91813-711	91813-706	91813-707	91813-709	91813-704	91813-705	91813-701
3.0 x 250	91813-902	91813-922	91813-908	91813-911	91813-906	91813-907	91813-909	91813-904	91813-905	91813-901

Dimensions ID x Length (in mm)	C18	AQ-C18	C8	Biphenyl	Phenyl-Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 5	91812-102	91812-122	91812-108	91812-111	91812-106	91812-107	91812-109	91812-104	91812-105	91812-101
3.0 x 5	91813-102	91813-122	91813-108	91813-111	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.

Dimensions ID x Length (in mm)	C18	AQ-C18	C8	Phenyl-Hexyl	Biphenyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
0.075 x 50	98519-402	98519-422	98519-408	98519-406	98519-411	98519-407	98519-409	98519-404	98519-405	98519-401
0.075 x 100	98519-602	98519-622	98519-608	98519-606	98519-611	98519-607	98519-609	98519-604	98519-605	98519-601
0.075 x 150	98519-702	98519-722	98519-708	98519-706	98519-711	98519-707	98519-709	98519-704	98519-705	98519-701
0.1 x 50	98518-402	98518-422	98518-408	98518-406	98518-411	98518-407	98518-409	98518-404	98518-405	98518-401
0.1 x 100	98518-602	98518-622	98518-608	98518-606	98518-611	98518-607	98518-609	98518-604	98518-605	98518-601
0.1 x 150	98518-702	98518-722	98518-708	98518-706	98518-711	98518-707	98518-709	98518-704	98518-705	98518-701
0.2 x 50	98517-402	98517-422	98517-408	98517-406	98517-411	98517-407	98517-409	98517-404	98517-405	98517-401
0.2 x 100	98517-602	98517-622	98517-608	98517-606	98517-611	98517-607	98517-609	98517-604	98517-605	98517-601
0.2 x 150	98517-702	98517-722	98517-708	98517-706	98517-711	98517-707	98517-709	98517-704	98517-705	98517-701
0.3 x 50	98516-402	98516-422	98516-408	98516-406	98516-411	98516-407	98516-409	98516-404	98516-405	98516-401
0.3 x 100	98516-602	98516-622	98516-608	98516-606	98516-611	98516-607	98516-609	98516-604	98516-605	98516-601
0.3 x 150	98516-702	98516-722	98516-708	98516-706	98516-711	98516-707	98516-709	98516-704	98516-705	98516-701
0.5 x 50	98515-402	98515-422	98515-408	98515-406	98515-411	98515-407	98515-409	98515-404	98515-405	98515-401
0.5 x 100	98515-602	98515-622	98515-608	98515-606	98515-611	98515-607	98515-609	98515-604	98515-605	98515-601
0.5 x 150	98515-702	98515-722	98515-708	98515-706	98515-711	98515-707	98515-709	98515-704	98515-705	98515-701
1.0 x 30	95811-302	95811-322	95811-308	95811-306	95811-311	95811-307	95811-309	95811-304	95811-305	95811-301
1.0 x 50	95811-402	95811-422	95811-408	95811-406	95811-411	95811-407	95811-409	95811-404	95811-405	95811-401
1.0 x 75	95811-502	95811-522	95811-508	95811-506	95811-511	95811-507	95811-509	95811-504	95811-505	95811-501
1.0 x 100	95811-602	95811-622	95811-608	95811-606	95811-611	95811-607	95811-609	95811-604	95811-605	95811-601
1.0 x 150	95811-702	95811-722	95811-708	95811-706	95811-711	95811-707	95811-709	95811-704	95811-705	95811-701
1.0 x 250	95811-902	95811-922	95811-908	95811-906	95811-911	95811-907	95811-909	95811-904	95811-905	95811-901
2.1 x 20	95812-202	95812-222	95812-208	95812-206	95812-211	95812-207	95812-209	95812-204	95812-205	95812-201
2.1 x 30	95812-302	95812-322	95812-308	95812-306	95812-311	95812-307	95812-309	95812-304	95812-305	95812-301
2.1 x 50	95812-402	95812-422	95812-408	95812-406	95812-411	95812-407	95812-409	95812-404	95812-405	95812-401
2.1 x 75	95812-502	95812-522	95812-508	95812-506	95812-511	95812-507	95812-509	95812-504	95812-505	95812-501
2.1 x 100	95812-602	95812-622	95812-608	95812-606	95812-611	95812-607	95812-609	95812-604	95812-605	95812-601
2.1 x 150	95812-702	95812-722	95812-708	95812-706	95812-711	95812-707	95812-709	95812-704	95812-705	95812-701
2.1 x 250	95812-902	95812-922	95812-908	95812-906	95812-911	95812-907	95812-909	95812-904	95812-905	95812-901
3.0 x 20	95813-202	95813-222	95813-208	95813-206	95813-211	95813-207	95813-209	95813-204	95813-205	95813-201
3.0 x 30	95813-302	95813-322	95813-308	95813-306	95813-311	95813-307	95813-309	95813-304	95813-305	95813-301
3.0 x 50	95813-402	95813-422	95813-408	95813-406	95813-411	95813-407	95813-409	95813-404	95813-405	95813-401
3.0 x 75	95813-502	95813-522	95813-508	95813-506	95813-511	95813-507	95813-509	95813-504	95813-505	95813-501
3.0 x 100	95813-602	95813-622	95813-608	95813-606	95813-611	95813-607	95813-609	95813-604	95813-605	95813-601
3.0 x 150	95813-702	95813-722	95813-708	95813-706	95813-711	95813-707	95813-709	95813-704	95813-705	95813-701
3.0 x 250	95813-902	95813-922	95813-908	95813-906	95813-911	95813-907	95813-909	95813-904	95813-905	95813-901
4.6 x 20	95814-202	95814-222	95814-208	95814-206	95814-211	95814-207	95814-209	95814-204	95814-205	95814-201
4.6 x 30	95814-302	95814-322	95814-308	95814-306	95814-311	95814-307	95814-309	95814-304	95814-305	95814-301
4.6 x 50	95814-402	95814-422	95814-408	95814-406	95814-411	95814-407	95814-409	95814-404	95814-405	95814-401
4.6 x 75	95814-502	95814-522	95814-508	95814-506	95814-511	95814-507	95814-509	95814-504	95814-505	95814-501
4.6 x 100	95814-602	95814-622	95814-608	95814-606	95814-611	95814-607	95814-609	95814-604	95814-605	95814-601
4.6 x 150	95814-702	95814-722	95814-708	95814-706	95814-711	95814-707	95814-709	95814-704	95814-705	95814-701
4.6 x 250	95814-902	95814-922	95814-908	95814-906	95814-911	95814-907	95814-909	95814-904	95814-905	95814-901
10.0 x 50	95810-402	95810-422	95810-408	95810-406	95810-411	95810-407	95810-409	95810-404	95810-405	95810-401
10.0 x 75	95810-502	95810-522	95810-508	95810-506	95810-511	95810-507	95810-509	95810-504	95810-505	95810-501
10.0 x 100	95810-602	95810-622	95810-608	95810-606	95810-611	95810-607	95810-609	95810-604	95810-605	95810-601
10.0 x 150	95810-702	95810-722	95810-708	95810-706	95810-711	95810-707	95810-709	95810-704	95810-705	95810-701
10.0 x 250	95810-902	95810-922	95810-908	95810-906	95810-911	95810-907	95810-909	95810-904	95810-905	95810-901

5 µm, 90Å Guard Columns, 3-Pack

Dimensions ID x Length (in mm)	C18	AQ-C18	C8	Phenyl-Hexyl	Biphenyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 5	95812-102	95812-122	95812-108	95812-106	95812-111	95812-107	95812-109	95812-104	95812-105	95812-101
3.0 x 5	95813-102	95813-122	95813-108	95813-106	95813-111	95813-107	95813-109	95813-104	95813-105	95813-101
4.6 x 5	95814-102	95814-122	95814-108	95814-106	95814-111	95814-107	95814-109	95814-104	95814-105	95814-101

Guard Column Holder 94900-001

HALO® 1000 Å AND 400 Å PROTEIN COLUMNS

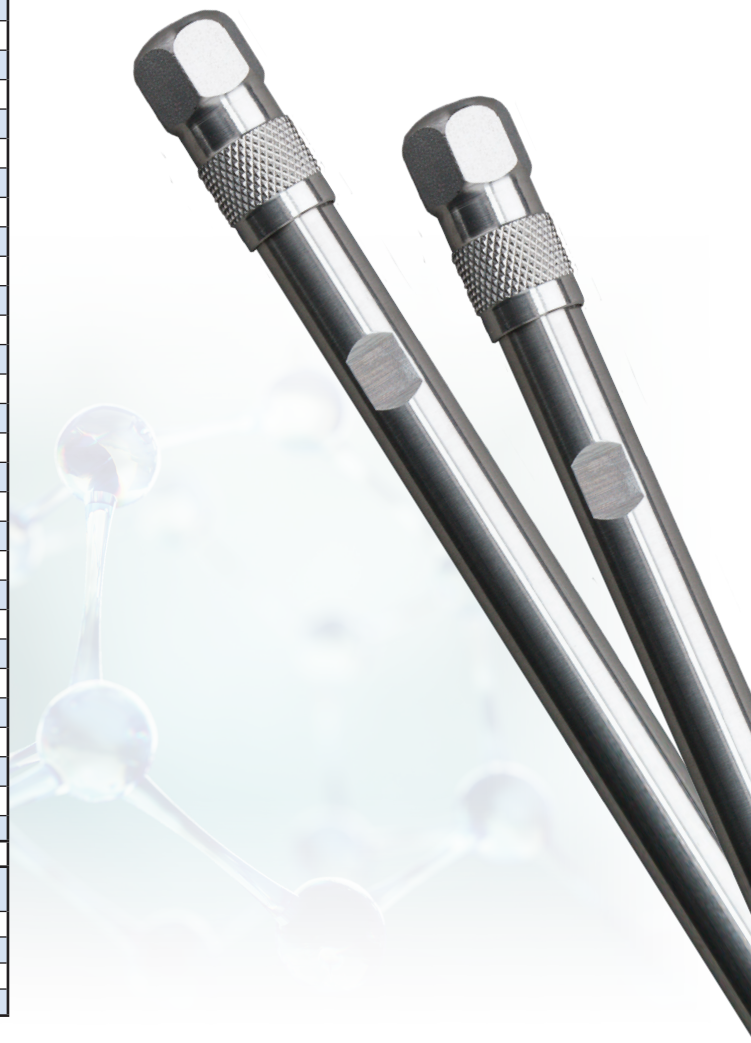
Part numbers for nano, capillary, analytical and semi-preparative HALO® 1000 and 400 Å in 2.7 and 3.4 µm phases are provided below. Guard columns are available in 2.1, 3.0 and 4.6 mm IDs for UHPLC and HPLC applications to provide additional column protection when desired.

	400 Å, 3.4 µm			1000 Å, 2.7 µm		
Dimensions ID x Length (in mm)	C4	ES-C18	Diphenyl	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 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	93412-426	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	93412-626	92712-614	92712-602	92712-626
2.1 x 150	93412-714	93412-702	93412-726	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	93414-426	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	93414-626	92714-614	92714-602	92714-626
4.6 x 150	93414-714	93414-702	93414-726	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	Diphenyl	C4	ES-C18	Diphenyl
2.1 x 5	93412-114	93412-102	93412-126	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	93414-126	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 x 150	99227-705
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
10 x 150	92910-705
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.

	160 Å, 2 µm	160 Å, 2.7 µm			160 Å, 5 µm	
ID x Length (in mm)	ES-C18	ES-C18	ES-CN	Phenyl-Hexyl	ES-C18	ES-CN
0.075 x 50	-	91229-402	91229-404	91219-406	91529-402	91529-404
0.075 x 100	-	91229-602	91229-604	91219-606	91529-602	91529-604
0.075 x 150	-	91229-702	91229-704	91219-706	91529-702	91529-704
0.1 x 50	-	91228-402	91228-404	91218-406	91528-402	91528-404
0.1 x 100	-	91228-602	91228-604	91218-606	91528-602	91528-604
0.1 x 150	-	91228-702	91228-704	91218-706	91528-702	91528-704
0.2 x 50	-	91227-402	91227-404	91217-406	91527-402	91527-404
0.2 x 100	-	91227-602	91227-604	91217-606	91527-602	91527-604
0.2 x 150	-	91227-702	91227-704	91217-706	91527-702	91527-704
0.3 x 50	-	91226-402	91226-404	91216-406	91526-402	91526-404
0.3 x 100	-	91226-602	91226-604	91216-606	91526-602	91526-604
0.3 x 150	-	91226-702	91226-704	91216-706	91526-702	91526-704
0.5 x 50	-	91225-402	91225-404	91215-406	91525-402	91525-404
0.5 x 100	-	91225-602	91225-604	91215-606	91525-602	91525-604
0.5 x 150	-	91225-702	91225-704	91215-706	91525-702	91525-704
1.0 x 30	-	92121-302	92121-304	92111-306	95121-302	95121-304
1.0 x 50	-	92121-402	92121-404	92111-406	95121-402	95121-404
1.0 x 75	-	92121-502	92121-504	92111-506	95121-502	95121-504
1.0 x 100	-	92121-602	92121-604	92111-606	95121-602	95121-604
1.0 x 150	-	92121-702	92121-704	92111-706	95121-702	95121-704
2.1 x 20	91122-202	92122-202	92122-204	92112-206	95122-202	95122-204
2.1 x 30	91122-302	92122-302	92122-304	92112-306	95122-302	95122-304
2.1 x 50	91122-402	92122-402	92122-404	92112-406	95122-402	95122-404
2.1 x 75	91122-502	92122-502	92122-504	92112-506	95122-502	95122-504
2.1 x 100	91122-602	92122-602	92122-604	92112-606	95122-602	95122-604
2.1 x 150	91122-702	92122-702	92122-704	92112-706	95122-702	95122-704
2.1 x 250	91122-902	92122-902	92122-904	92112-906	95122-902	95122-904
3.0 x 20	91123-202	92123-202	92123-204	92113-206	95123-202	95123-204
3.0 x 30	91123-302	92123-302	92123-304	92113-306	95123-302	95123-304
3.0 x 50	91123-402	92123-402	92123-404	92113-406	95123-402	95123-404
3.0 x 75	91123-502	92123-502	92123-504	92113-506	95123-502	95123-504
3.0 x 100	91123-602	92123-602	92123-604	92113-606	95123-602	95123-604
3.0 x 150	91123-702	92123-702	92123-704	92113-706	95123-702	95123-704
3.0 x 250	91123-902	92123-902	92123-904	92113-906	95123-902	95123-904
4.6 x 20	-	92124-202	92124-204	92114-206	95124-202	95124-204
4.6 x 30	-	92124-302	92124-304	92114-306	95124-302	95124-304
4.6 x 50	-	92124-402	92124-404	92114-406	95124-402	95124-404
4.6 x 75	-	92124-502	92124-504	92114-506	95124-502	95124-504
4.6 x 100	-	92124-602	92124-604	92114-606	95124-602	95124-604
4.6 x 150	-	92124-702	92124-704	92114-706	95124-702	95124-704
4.6 x 250	-	92124-902	92124-904	92114-906	95124-902	95124-904
10.0 x 50	-	92120-402	92120-404	92110-406	95120-402	95120-404
10.0 x 75	-	92120-502	92120-504	92110-506	95120-502	95120-504
10.0 x 100	-	92120-602	92120-604	92110-606	95120-602	95120-604
10.0 x 150	-	92120-702	92120-704	92110-706	95120-702	95120-704
10.0 x 250	-	-	-		95120-902	95120-904
10.0 x 250						

5 µm, 90Å Guard Columns, 3-Pack

Dimensions ID x Length (in mm)	C18	AQ-C18	C8	Phenyl-Hexyl	Biphenyl	RP-Amide
2.1 x 5	91122-102	92122-102	92122-104	92112-106	95122-102	95122-104
3.0 x 5	91123-102	92123-102	92123-104	92113-106	95123-102	95123-104
4.6 x 5	-	92124-102	92124-104	92114-106	95124-102	95124-104

Guard Column Holder 94900-001

HALO® GLOBAL DISTRIBUTION NETWORK

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