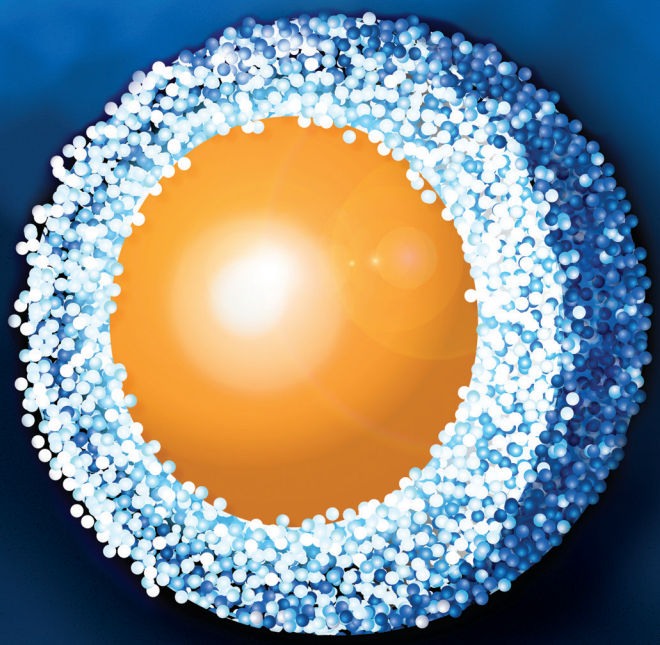
avantor™
delivered by VWR™

VWR.COM

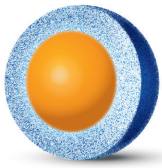


HALO®

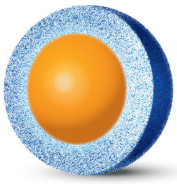
DISCOVER THE ADVANTAGES OF HALO® AND HALO® BIOCLASS FUSED-CORE® COLUMNS

HALO®

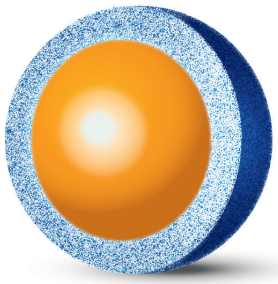
SMALL MOLECULE



2 micron particle

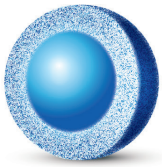


2.7 micron particle

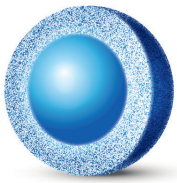


5 micron particle

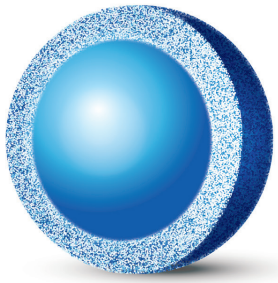
BIOCLASS



2 micron particle

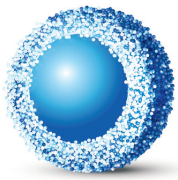


2.7 micron particle

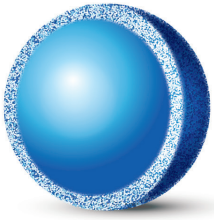


5 micron particle

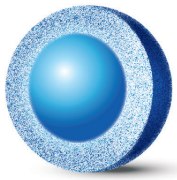
PEPTIDE



2.7 micron particle



3.4 micron particle



2.7 micron particle

PROTEIN

GLYCAN

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

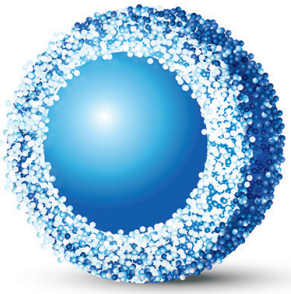
approximately 20 yrs — Smaller totally porous particles develop rapidly and dominate HPLC column market. However, demand for speed and resolution create need for particle innovation that delivers higher performance at lower pressures.

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.

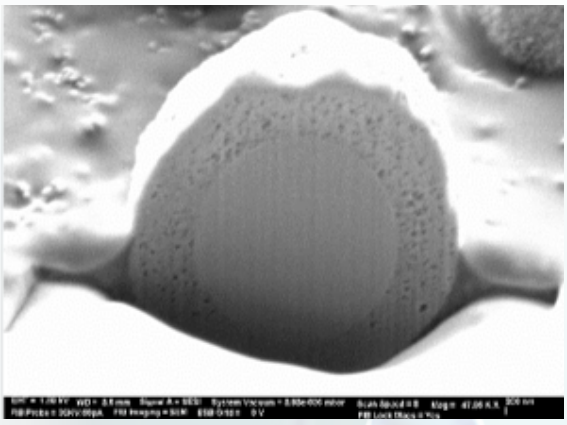


Figure B. SEM image of a focused-ion beam cleaved HALO® 1000 Å 2.7 µm silica particle. This "cut-away" view shows the solid core and shell with large pores allowing unrestricted access of macromolecules to the bonded phase.

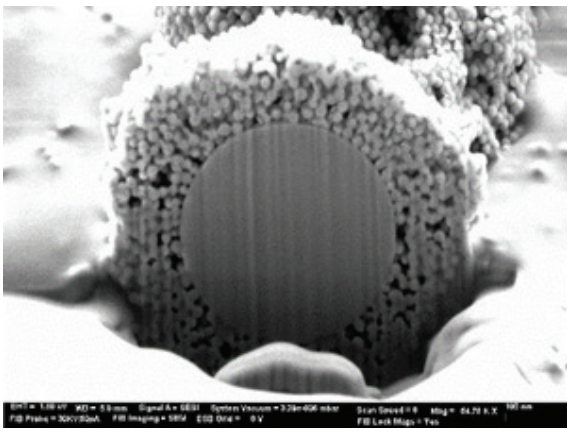
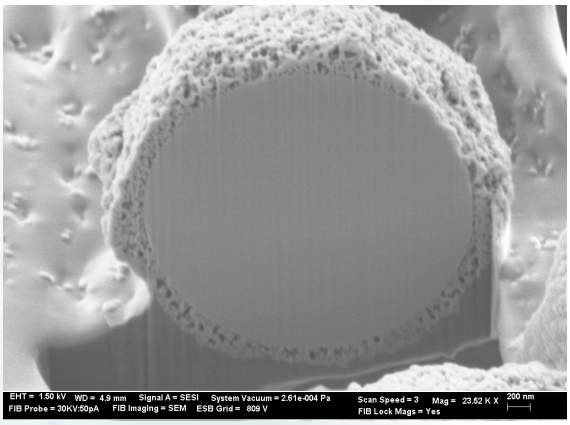


Figure C. SEM image of a focused-ion-beam-cleaved HALO® Protein 3.4 µm silica particle. This "cut-away" view shows the solid core with its very thin 0.2 µm outer porous layer.



Understanding SPP Performance (Figure D)

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

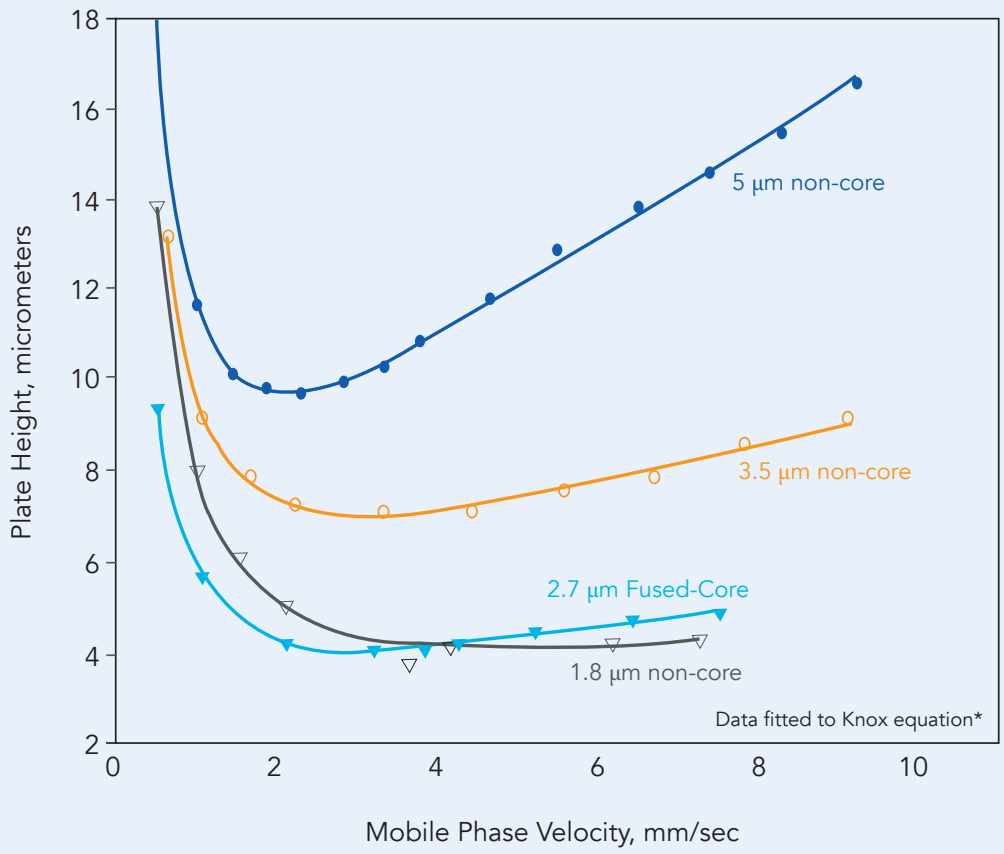
- Reduction in eddy diffusion
  - 40% smaller van Deemter "A term" due to more uniform analyte flow paths through the column bed
- Much lower longitudinal broadening, flat van Deemter plot and higher optimum linear velocity (flow rate)

  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

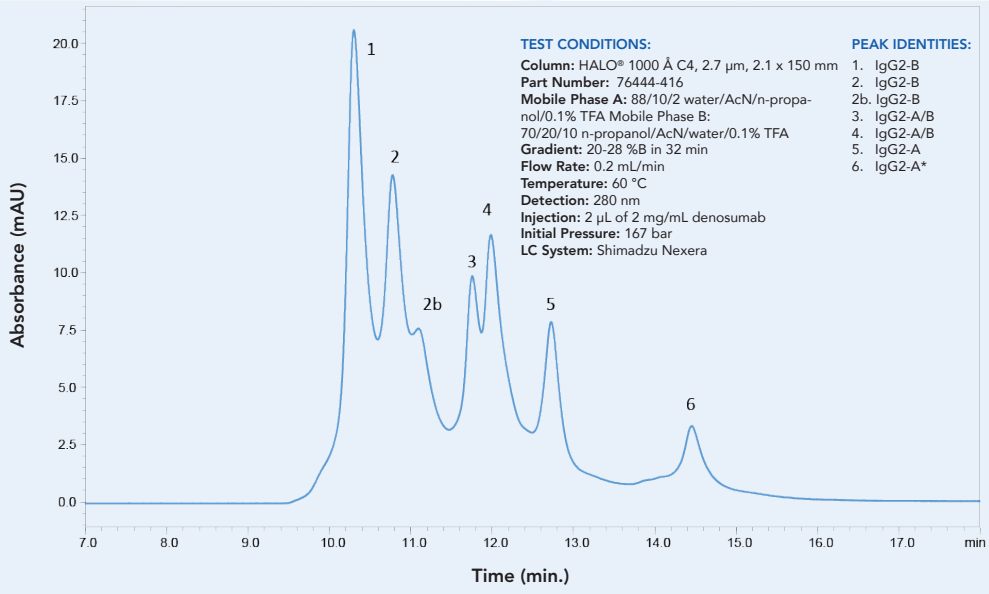
- Less plugging, longer usable column lifetime and greater uptime due to larger porosity frits (vs. sub-2 µm totally porous (non-core) columns)
  - 2 µm frits for HALO® 2.7 µm and 5 µm columns
  - 1 µm frits for HALO® 2 µm columns vs. 0.2–0.5 µm frits for sub-2 µm non-core columns
- Excellent column-to-column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

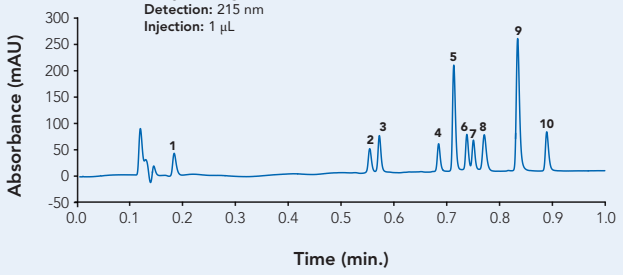
Figure F. Separation of a 10 peptide mixture is accomplished in less than one minute using a HALO® Peptide ES-C18 column on a delay-volume minimized and optimized Agilent 1200 system.

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

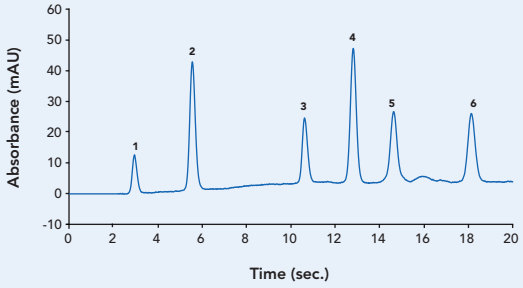
Figure G.
Many researchers have found HALO® 5 µm columns in 2.1 mm ID to be very useful for high-throughput, ballistic separations by LC and LC-MS.

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

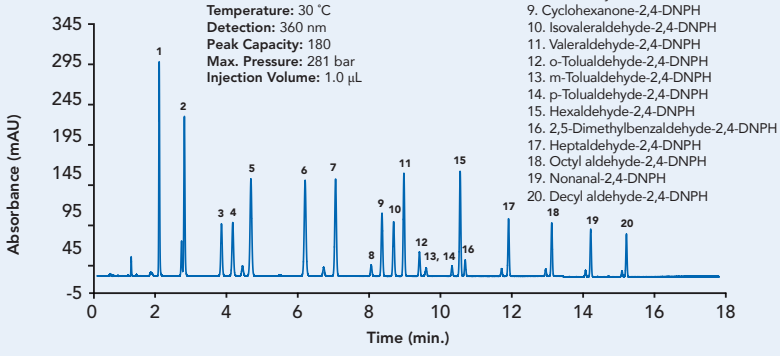
Figure H. Environmental samples can be quite complex as demonstrated by this gradient separation of dinitrophenylhydrazone (DNPH) carbonyl compound derivatives using a HALO® 90 Å C18, 2 µm, 2.1 x 150 mm column.

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:

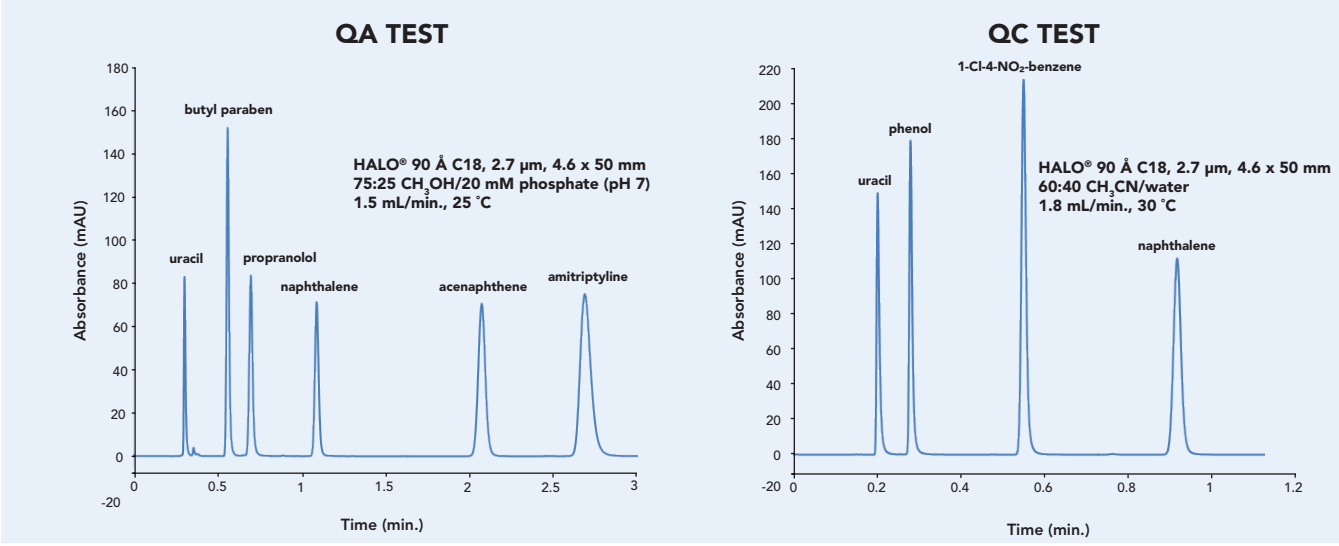
1. Formaldehyde-2,4-DNPH
2. Acetaldehyde-2,4-DNPH
3. Acetone-2,4-DNPH
4. Acrolein-2,4-DNPH
5. Propionaldehyde-2,4-DNPH
6. Crotonaldehyde-2,4-DNPH
7. Butyraldehyde-2,4-DNPH
8. Benzaldehyde-2,4-DNPH
9. Cyclohexanone-2,4-DNPH
10. Isovaleraldehyde-2,4-DNPH
11. Valeraldehyde-2,4-DNPH
12. o-Tolualdehyde-2,4-DNPH
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

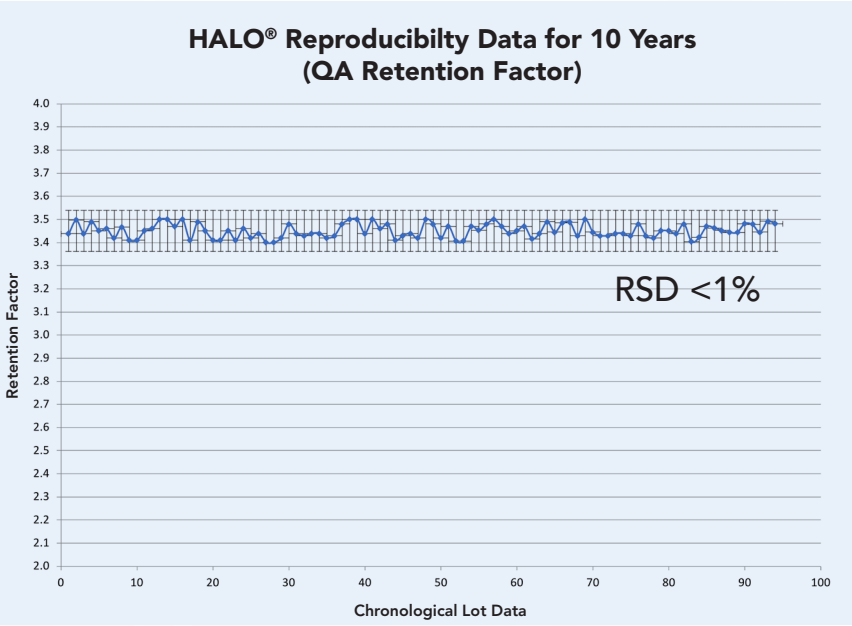
As the originators of Fused-Core® particles, Advanced Materials Technology incorporates the most knowledge in the industry to bring high-quality, innovative products to our customers. Our principal scientists have over 150 years of combined experience in liquid chromatography, particle synthesis and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

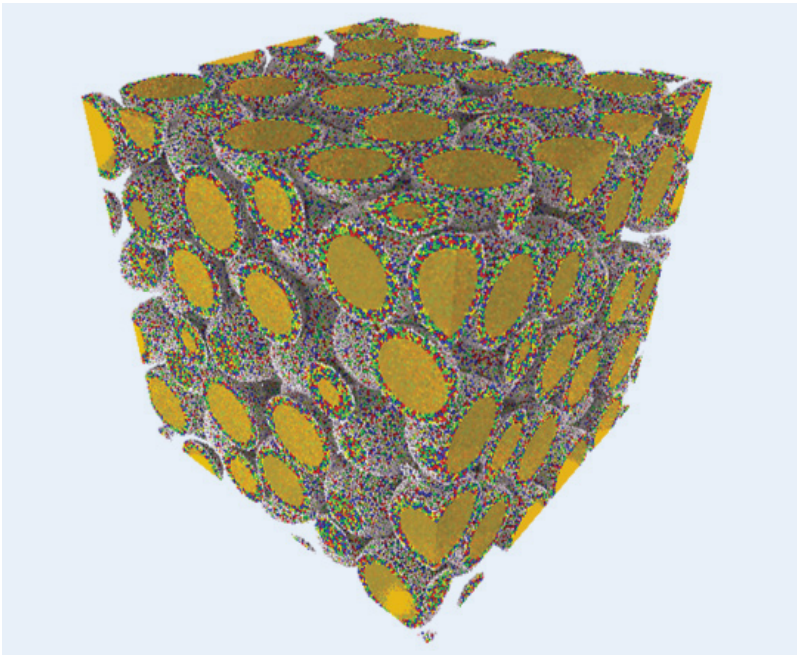
Molecule Size	Pore Size (Å)	Application	Particle Sizes (µm)	Column Family
SMALL (<5000 Da)	90	Small Molecules	2, 2.7, 5	HALO®
SMALL (< 20 kDa*)	90	Glycan	2.7	HALO® BIOCLASS
MEDIUM (100 Da < MW < 15 kDa)	160	Peptide	2, 2.7, 5	
LARGE (2 kDa < MW < 500 kDa)	400	Protein	3.4	
LARGE (> 50 kDa)	1000		2.7	

* 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

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

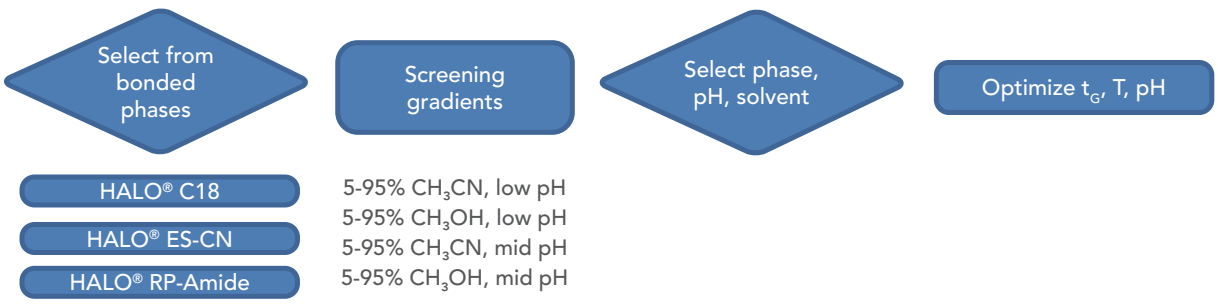
Moreover, column phase selectivity is one of the four most powerful and useful parameters for adjusting HPLC separation selectivity (see Table B). For ionizable analytes, mobile phase pH is, by far, the most effective parameter. However, column stationary phase is comparable to organic modifier choice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

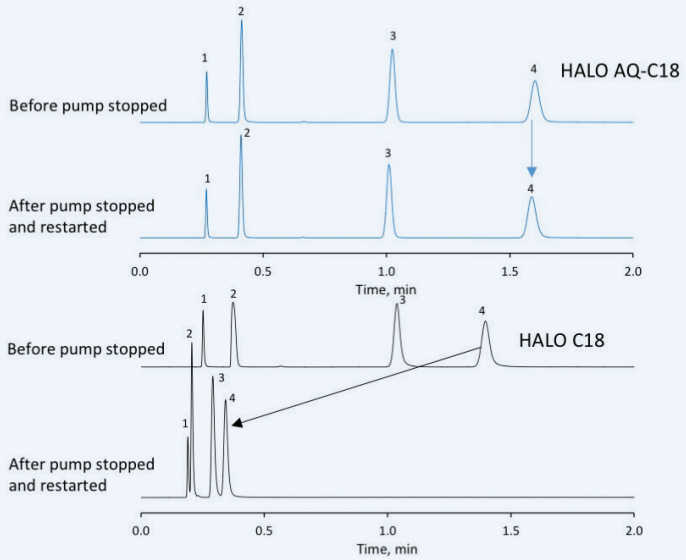
HPLC Parameter	Effectiveness for Changing Selectivity
Mobile phase pH (ionizable analytes only)	Most Effective
Organic modifier choice	
Percent organic modifier or gradient steepness	
Column stationary phase	
Column temperature	
Buffer choice	
Buffer concentration	Least Effective

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	2.7	90	7.0	135	2/60 °C	9/40 °C	Yes
PFP	L43	2 2.7 5	90	5.3 5.5 3.9	120 135 90	2/60 °C	8/40 °C	Yes
ES-CN	L10	2 2.7 5	90	3.4 3.5 2.5	120 135 90	1/80 °C	8/40 °C	Yes
RP-Amide	L60	2 2.7 5	90	7.3 8.2 5.1	120 135 90	2/60 °C	9/40 °C	Yes
HILIC	L3	2 2.7 5	90	Unbonded	120 135 90	1/60 °C	8/40 °C	N.A.
Penta-HILIC	L95	2 2.7 5	90	2.8 3.2 2.1	120 135 90	2/60 °C	9/40 °C	No

HALO® COLUMNS FOR SMALL MOLECULE SEPARATIONS

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

Bonded Phase	Features and Benefits	Target Analytes	Best Applications
C18 (dimethyloctadecylsilane)	• 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®

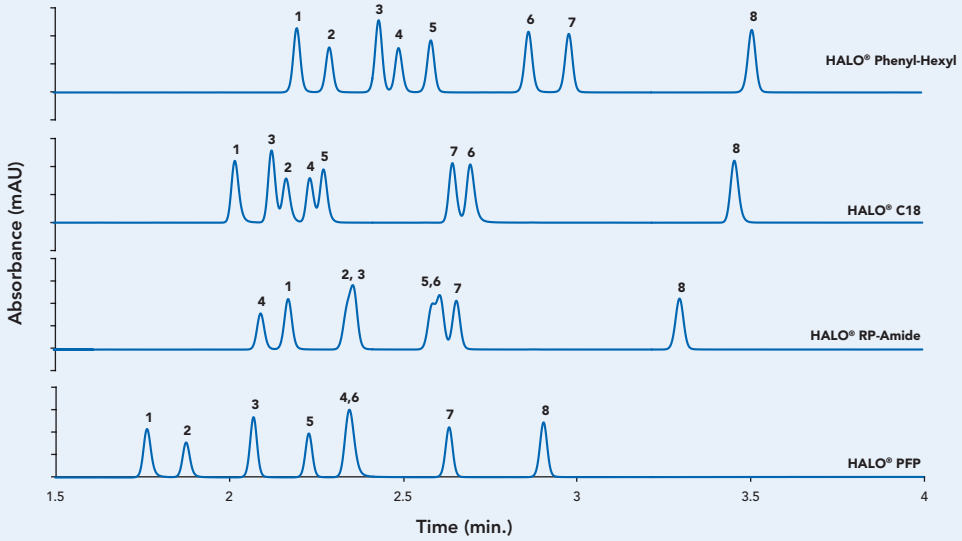
To illustrate the selectivity differences among the various HALO® RPLC phases, the following examples are provided.

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.

TEST CONDITIONS:
Column: HALO® 90 Å Phenyl-Hexyl, C18, RP-Amide, PFP, 2.7 µm, 4.6 x 50 mm
Part Numbers: 76444-970, 76444-964, 76444-972, 76444-976
Mobile Phase A: 25 mM Ammonium Acetate
Mobile Phase B: Acetonitrile
Gradient: 34 - 63% B in 3.5 min.
Flow Rate: 1.5 mL/min;
Temperature: 35 °C
Detection: UV 254 nm
Injection volume: 1 µL
Pressure: 200 bar

PEAK IDENTITIES:
1. Oxazepam
2. Lorazepam
3. Nitrazepam
4. Alprazolam
5. Clonazepam
6. Temazepam
7. Flunitrazepam
8. Diazepam



AROMATIC AND NITROAROMATIC COMPOUNDS

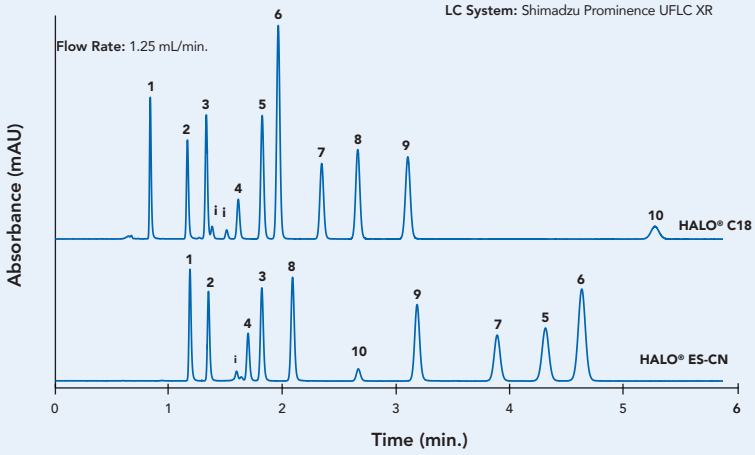
Figure O.
HALO® C18 and HALO® ES-CN columns may be used as orthogonal confirmatory columns for explosives analysis.

TEST CONDITIONS:
Column: HALO® 90 Å C18 and ES-CN, 2.7 µm, 4.6 x 100 mm
Part Numbers: C18= 76444-964, ES-CN=76444-966
Mobile Phase: 40/60-A/B for C18, 50/50-A/B for ES-CN
A= water
B= methanol

Pressure: About 300 bar
Temperature: 30 °C
Detection: UV 254 mn, VWD
Injection Volume: 1.0 µL
Sample Solvent: water/methanol 1:1 (v/v)
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

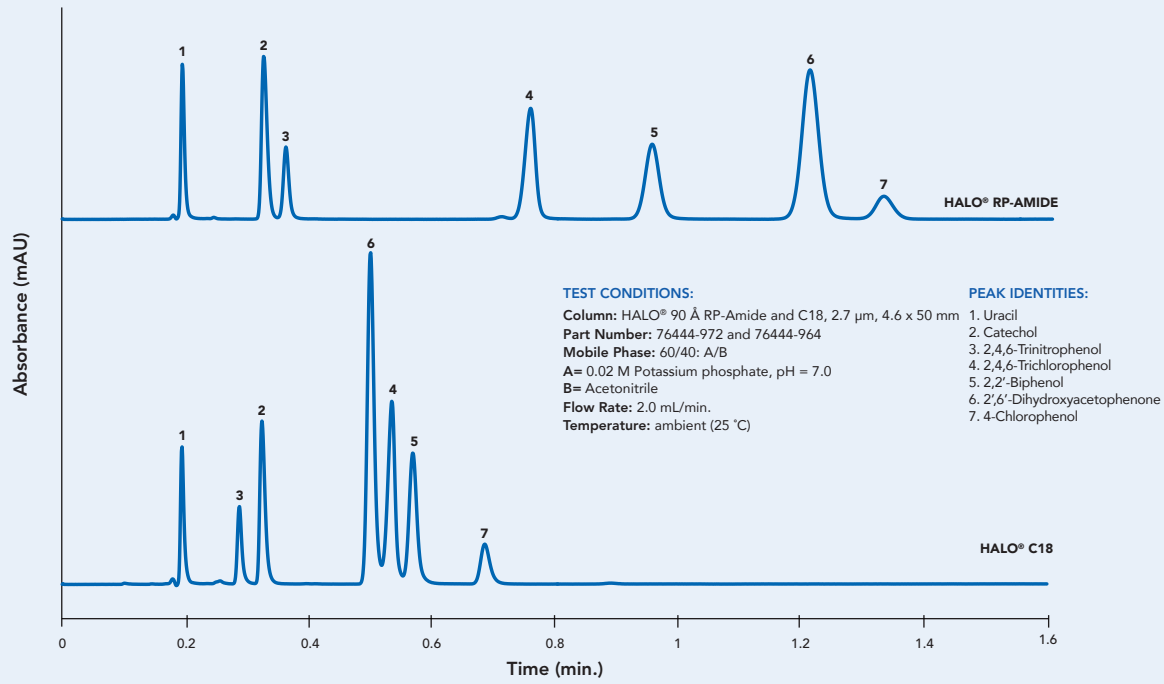
PEAK IDENTITIES:
1. Resorcinol
2. Benzyl alcohol
3. Phenylacetonitrile
4. 1-Indanol
5. 3,4-DNT
6. 2,3-DNT
7. 2,4-DNT
8. Anisole
9. 1-Chloro-4-nitrobenzene
10. Toluene

DNT = dinitrotoluene
i = impurity



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.



TEST CONDITIONS:

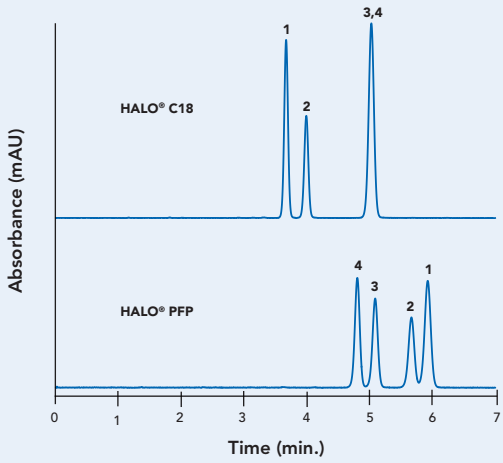
Column: HALO® 90 Å RP-Amide and C18, 2.7 µm, 4.6 x 50 mm
Part Number: 76444-972 and 76444-964
Mobile Phase: 60/40: A/B
A= 0.02 M Potassium phosphate, pH = 7.0
B= Acetonitrile
Flow Rate: 2.0 mL/min.
Temperature: ambient (25 ˚C)

PEAK IDENTITIES:

1. Uracil
2. Catechol
3. 2,4,6-Trinitrophenol
4. 2,4,6-Trichlorophenol
5. 2,2'-Biphenol
6. 2',6'-Dihydroxyacetophenone
7. 4-Chlorophenol

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.



TEST CONDITIONS:

Column: HALO® 90 Å C18, 2.7 µm, 4.6 x 100 mm
HALO® 90 Å PFP, 2.7 µm, 4.6 x 100 mm
Part Number: C18: 76445-004
PFP: 76445-014
Mobile Phase: 50/50: water/methanol
Flow Rate: 1.0 mL/min.
Pressure: about 230 bar
Temperature: 35 ˚C
Detection: UV 240 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 80% methanol in water
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

PEAK IDENTITIES:

1. Prednisone
2. Cortisone
3. Prednisolone
4. Hydrocortisone

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.

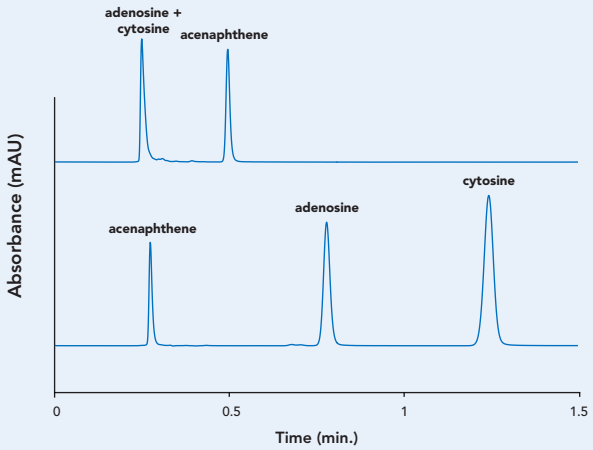
Some Typical Analytes for HILIC Separations

- 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.

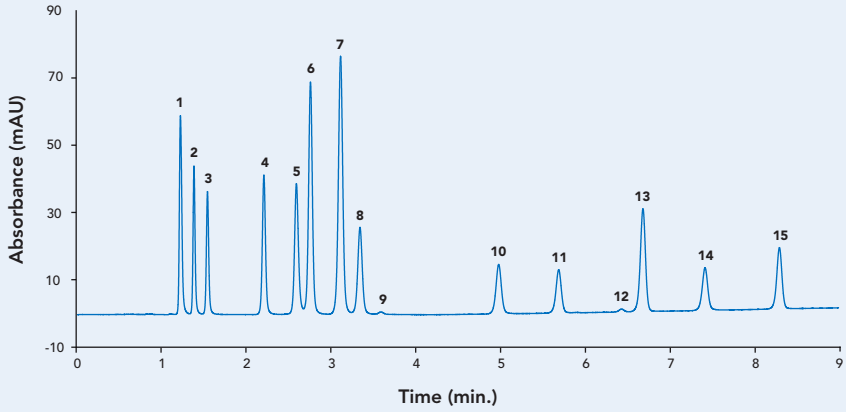


TEST CONDITIONS:
Column: HALO® 90 Å C18, 2.7 µm, 4.6 x 50 mm
Part Number: 76444-964
Mobile Phase A: 90/10 ACN/0.1 M Ammonium Formate
Flow Rate: 1.8 mL/min.
pH: 3.0

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:

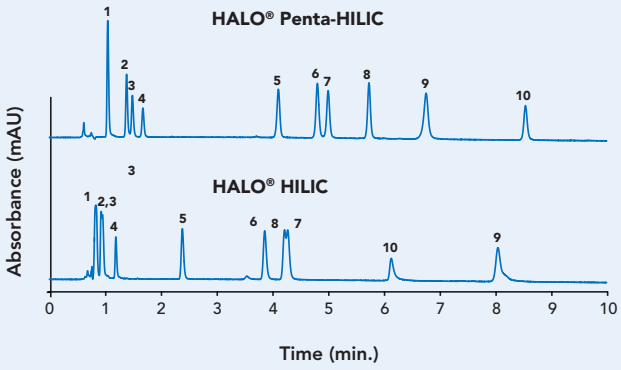
Column: HALO® 90 Å Penta-HILIC, 2.7 µm, 4.6 x 100 mm
Part Number:76443-510
Mobile Phase A: 10 mM Ammonium formate in 85% Acetonitrile/15% water
Mobile Phase B: 10mM Ammonium Formate in 93% Acetonitrile/7% water
Gradient: Time %B
0-3 min. 94
3-9 min. 94-0
Flow Rate: 1.5 mL/min.
Temperature: 35 °C
Detection: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

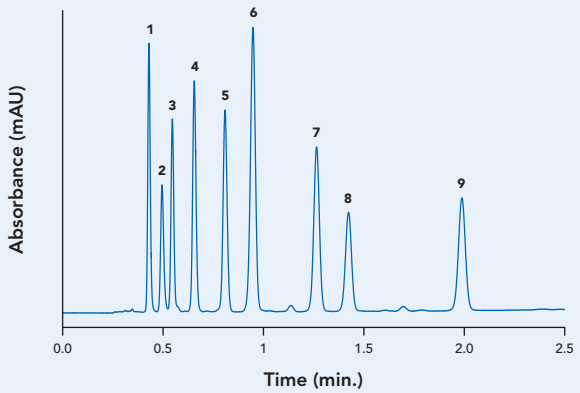
Column: HALO® 90 Å Penta-HILIC and HILIC, 2.7 µm, 2.1 x 50 mm
Part Number: Penta-HILIC: 76443-450
HILIC: 76444-762
Mobile Phase: A= 95/5 ACN/$H_2$0 with 5 mM NH_4formate, pH=3.0 (adj.)
Mobile Phase: B= 50/50 ACN/$H_2$0 with 5 mM NH_4formate, pH=3.0 (adj.)
Gradient: Penta-HILIC: 85-65% B in 10 min.
HILIC: 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 CEPHALO®SPORINS 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 cepHALO®sporins 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

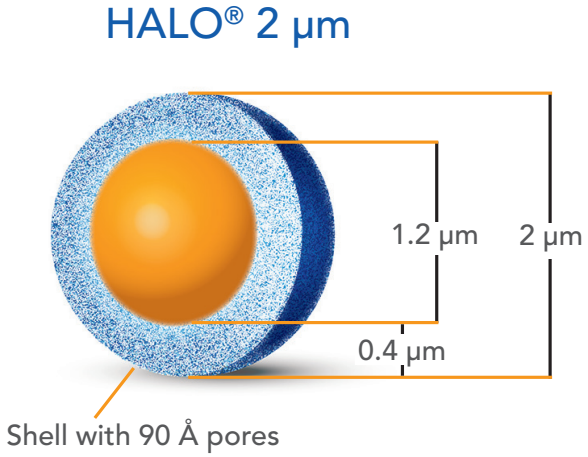
HALO®

HALO® 90 Å 2 µm (UHPLC)

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

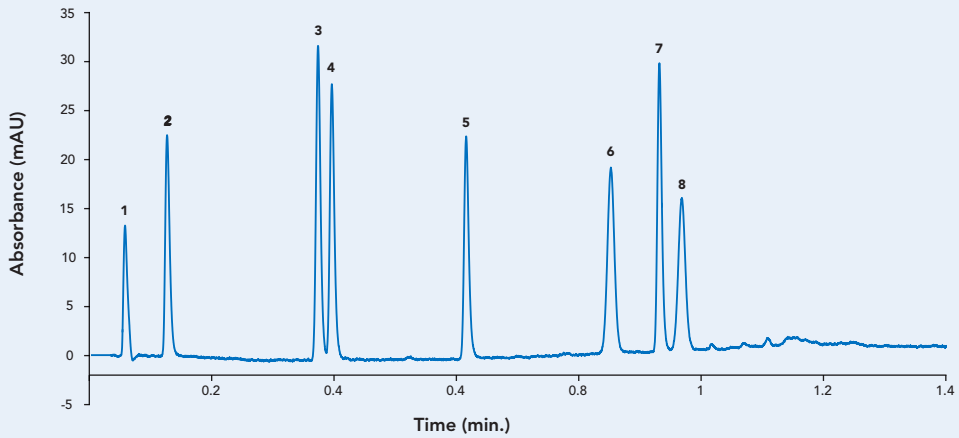
- Use when the highest efficiency is needed
- Excellent for fast method development and column/ condition screening
- Best performance obtained with instrumentation having extracolumn volume (IBW< 10 µL)
- Ruggedness for R&D
- 1 µm inlet frit
- Pressure limit, 1000 bar/14,500 psi

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



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:

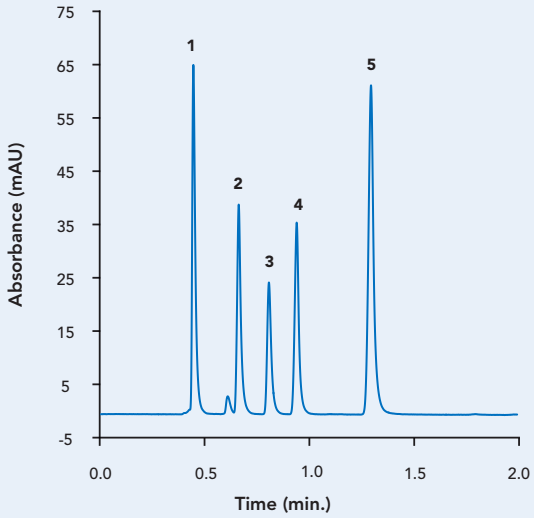
Column: HALO® 90 Å C18, 2 µm, 2.1 x 30 mm
Part Number: 76443-838
Mobile Phase A: 20 mM Formic acid
Mobile Phase B: 50/50 Acetonitrile/Methanol
Gradient: Time %B
0-0.06 20
0.06-1.06 20-75
Flow Rate: 1.1 mL/min.
Temperature: 45 °C
Detection: 254 nm
Injection Volume: 0.2 µL
Maximum Pressure: 430 bar

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:

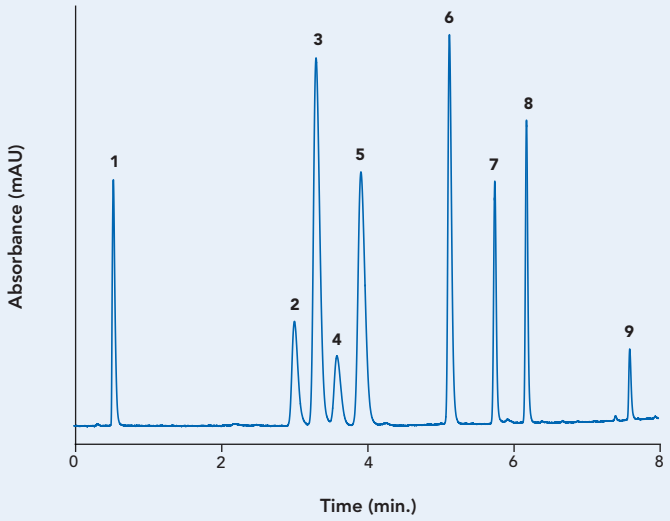
Column: HALO® 90 Å Penta-HILIC, 2 µm, 2.1 x 100 mm
Part Number: 76443-902
Isocratic: 92/8: ACN/water with 5mM Ammonium Formate buffer, pH 3
Flow Rate: 0.5 mL/min.
Temperature: 30 °C
Detection: UV 245 nm, photodiode array detector
Injection Volume: 1.0 µL
Sample Solvent: 90/10 ACN/0.1 M ammonium formate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

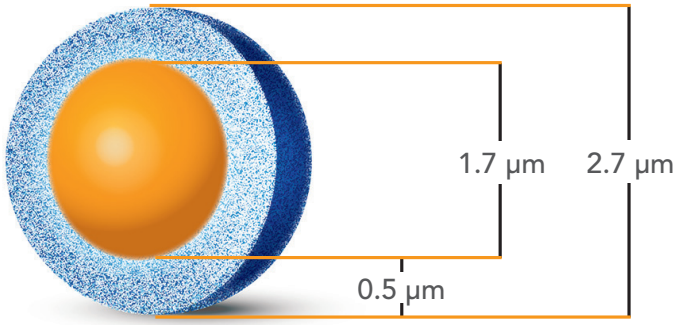
HALO® 90 Å 2.7 µm (UHPLC AND HPLC)

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

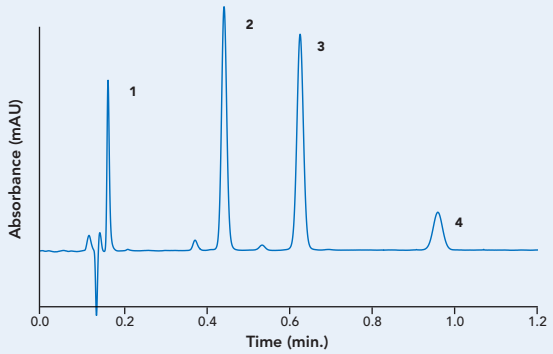
* HALO® C30 160 Å, 2.7 µm

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:

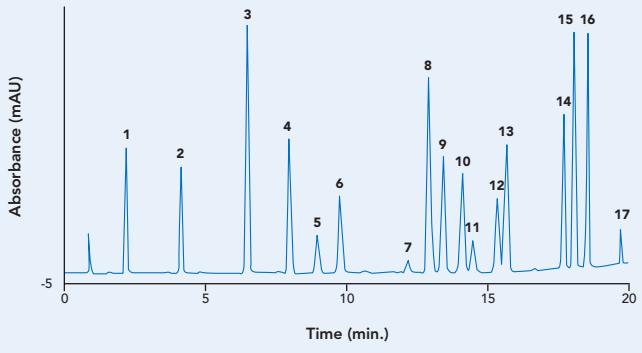
Column: HALO® 90 Å Phenyl-Hexyl, 2.7 µm, 4.6 x 50 mm
Part Number: 76444-970
Mobile Phase: 43/57: A/B
A: 0.02 M formic acid in water
B: Acetonitrile
Flow Rate: 2.5 mL/min.
Pressure: 228 Bar
Temperature: 26 °C
Detection: UV 240 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 80/20 methanol/water (20 mM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

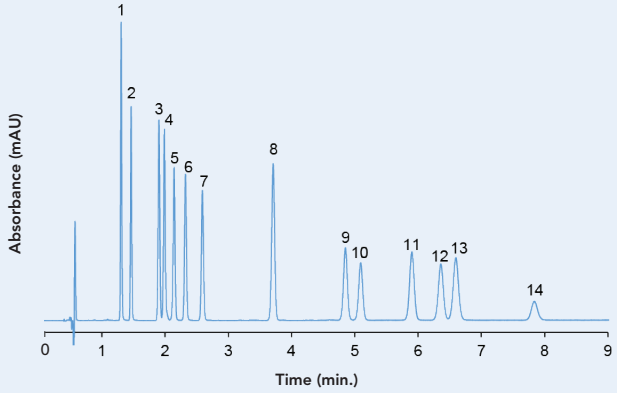
Flow Rate: 1.5 mL/min.
Temperature: 43 °C
Detection: UV 220 nm, VWD
Injection Volume: 40 µL
Sample Solvent: 50/50: Water/methanol
Response Time: 0.02 sec.
Data rate: 25 Hz
Pressure: 366 bar to start, max. 405 bar
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

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.

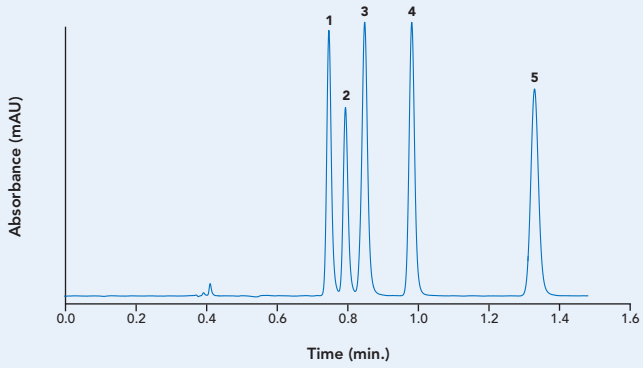


TEST CONDITIONS:
Column: HALO® 90 Å C18, 2.7 µm, 3.0 x 150 mm
Part Number: 76444-892
Mobile Phase: 25/75 A/B
A: Water/0.1% formic acid
B: Acetonitrile/0.085% formic acid
Flow Rate: 1.0 mL/min
Pressure: 350 bar
Temperature: 30 °C
Detection: UV 220 nm, PDA
Injection: 0.6 µL
Sample Solvent: 75/25 methanol/ 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.

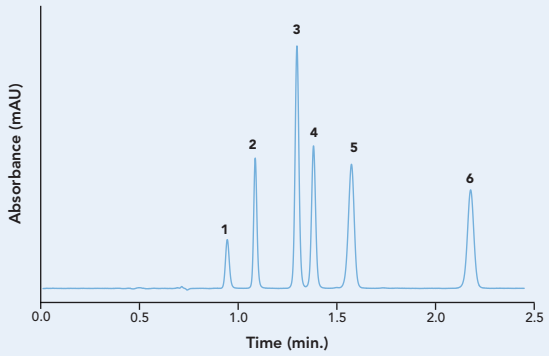


TEST CONDITIONS:
Column: HALO® 90 Å Penta HILIC, 2.7 µm, 4.6 x 100 mm
Part Number: 76443-510
Mobile Phase: 7/93: A/B
A: 0.1 M Ammonium formate, pH=3.5 (adj.)
B: Acetonitrile
Flow Rate: 2.5 mL/min.
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 10/90: Water/acetonitrile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

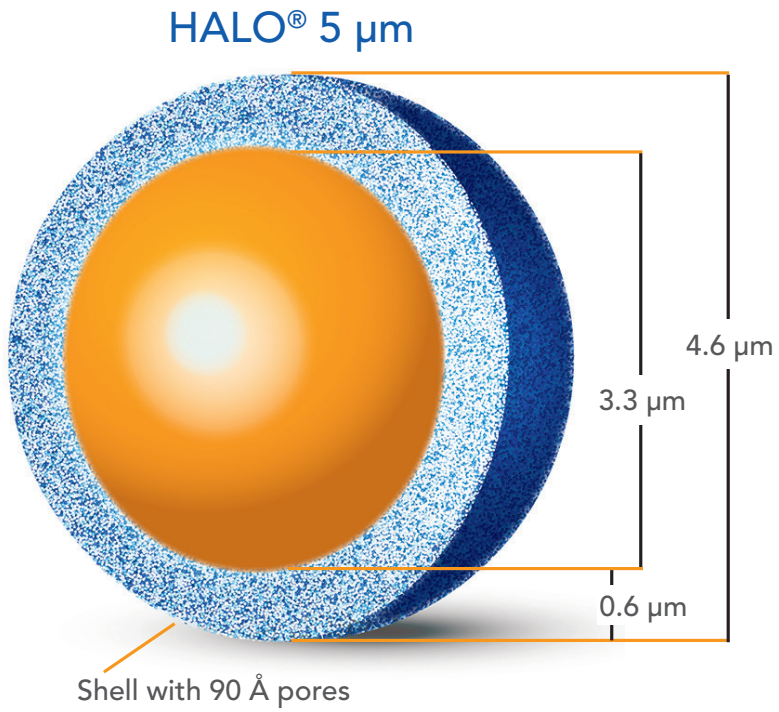
HALO®

HALO® 90 Å 5 µm (HPLC)

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

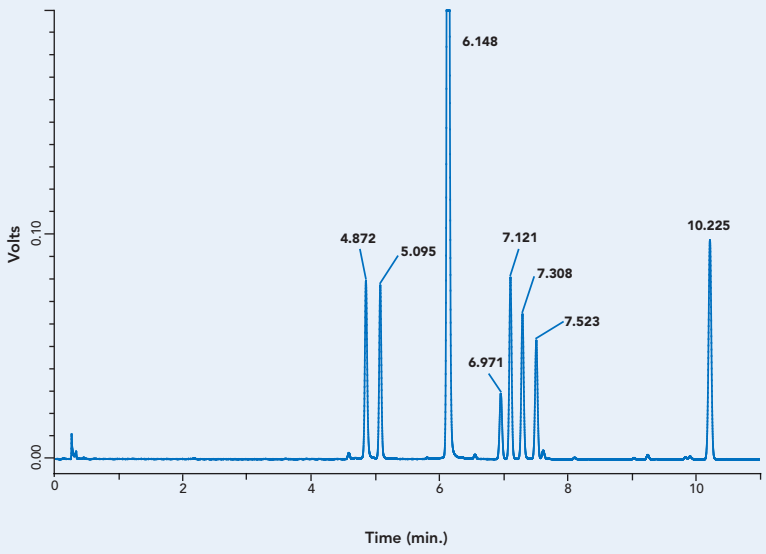
Ideal for:

- 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.



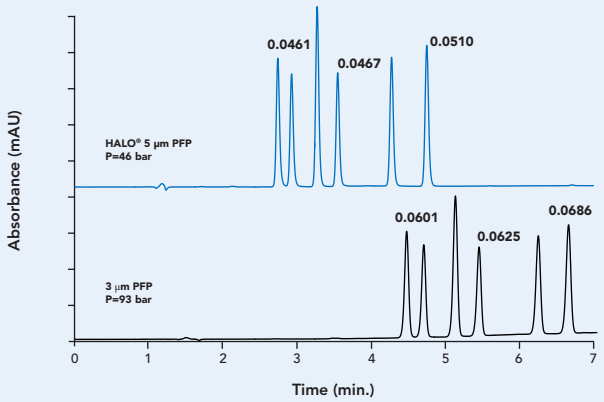
SAMPLE:
Mixture of 8 flavonoids, 1 µL in MeOH

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

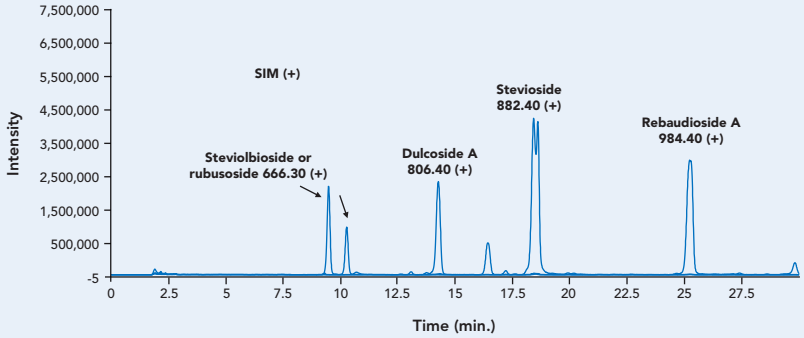
PEAK IDENTITIES:
1. Oxazepam
2. Lorazepam
3. Nitrazepam
4. Clonazeparm
5. Flunitrazepam
6. Diazepam

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

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

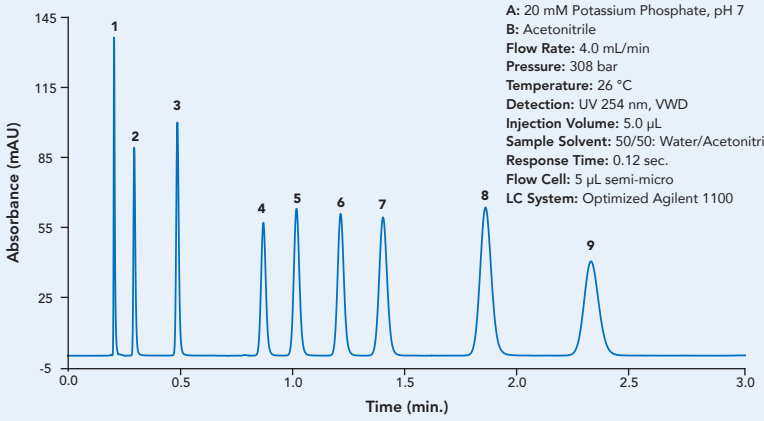
Figure FF.
LC-MS analysis of stevia glycosides from a Stevia natural sweetener extract is easily accomplished using the HALO® 5 µm Penta-HILIC column due to its unique bonded phase containing five OH groups.

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

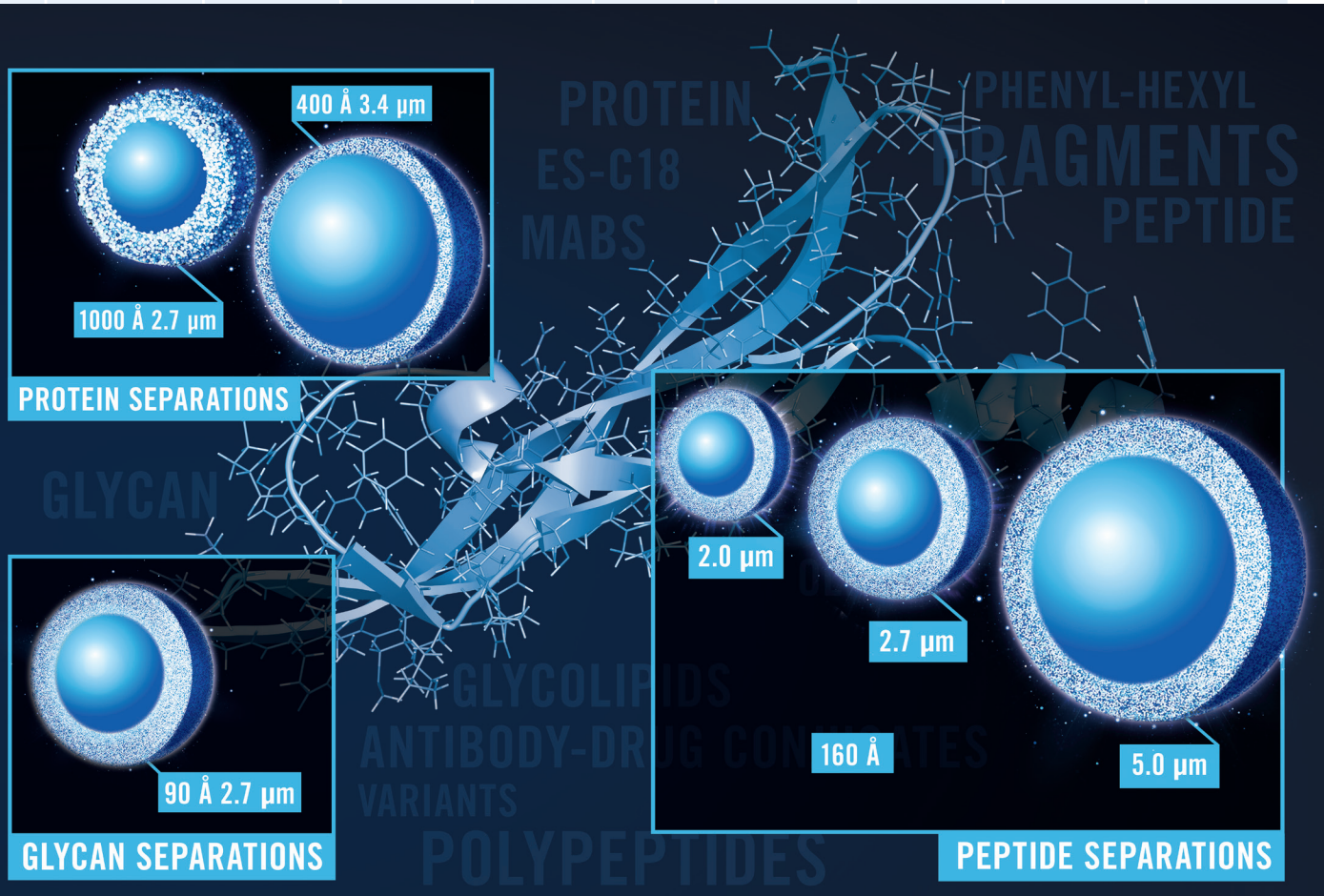


Table G. HALO® BioClass Features & Benefits				
	Bonded Phase	Features and Benefits	Target Analytes	Best Applications
1000 Å Protein	C4 (dimethylbutylsilane)	• Outstanding high temperature stability at low pH • Unrestricted access to bonded phase • Exceptional mass transfer kinetics • Compatible with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

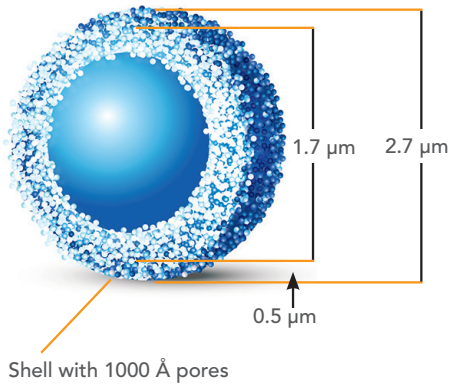
- Benefits of HALO® protein solutions include:
  - Provides narrower peaks and better recoveries for large biomolecules (vs. smaller pore sizes and non-core particles)
  - Allows HALO® Protein columns to be used with both UHPLC and HPLC instrumentation for fast bioseparations at moderate back pressures

- C4, Diphenyl and sterically-protected ES-C18 phases
  - Excellent high temperature stability (up to 90 ˚C) for improved peak shape and recovery

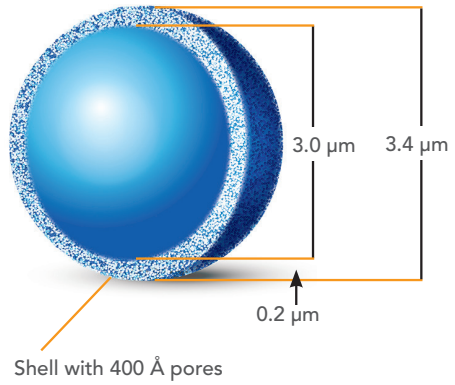
- 2 µm inlet frit

- Pressure limit, 600 bar/9000 psi

HALO® 1000 Å 2.7 µm

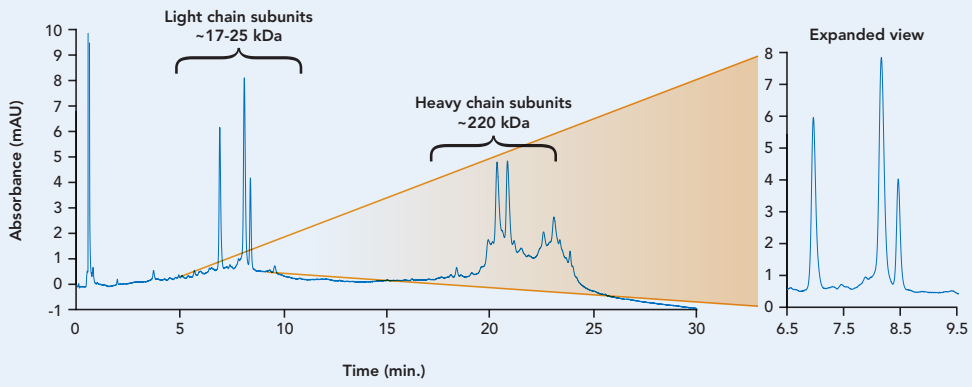


HALO® 400 Å 3.4 µm



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

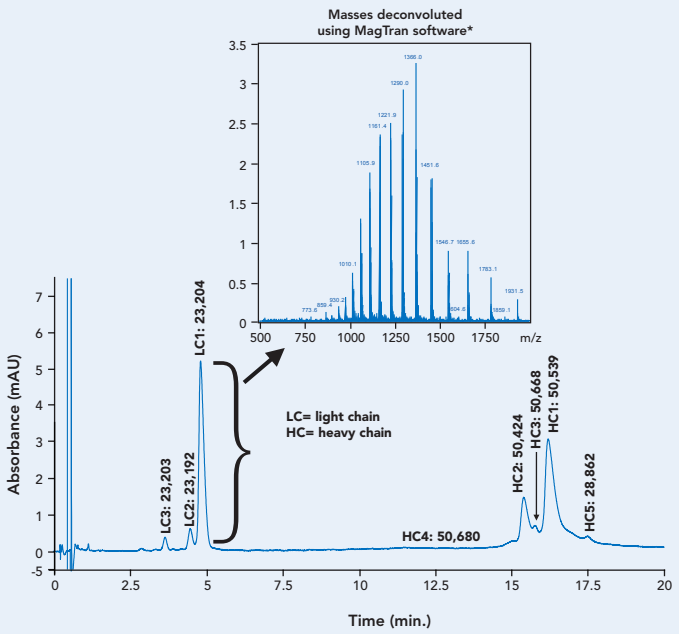
Figure HH. High resolution separation of light and heavy chains of a denatured contractile protein (whole myosin from purified rabbit skeletal muscle) using HALO® 400 Å Protein C4 at 80 ˚C.



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

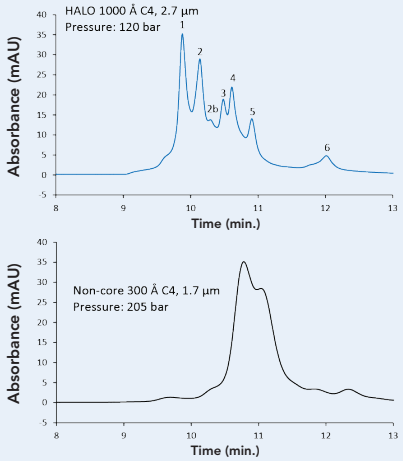
Figure II. Very high resolution is obtained between variants of light and heavy chains of a reduced and alkylated mono-clonal antibody (IgG1) sample using a HALO® 400 Å Protein C4 column.



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.



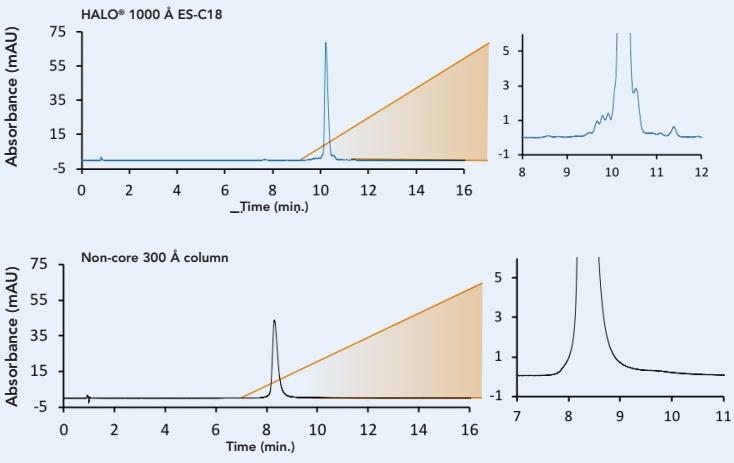
TEST CONDITIONS:
Column: HALO® 1000 Å C4, 2.7 µm, 2.1 x 150 mm
Part Number: 76444-416
Mobile Phase A: 88/10/2 water/AcN/n-propanol/0.1% DFA
Mobile Phase B: 70/20/10 n-propanol/AcN/water/0.1% DFA
Gradient: 14-24 %B in 20 min
Flow Rate: 0.2 mL/min
Temperature: 80 °C
Detection: UV 280 nm, PDA
Injection: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

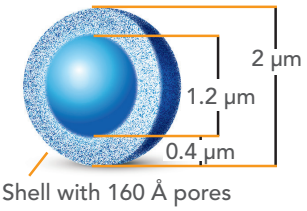
See page 35 for full list of HALO® Protein part numbers.

HALO® BIOCLASS

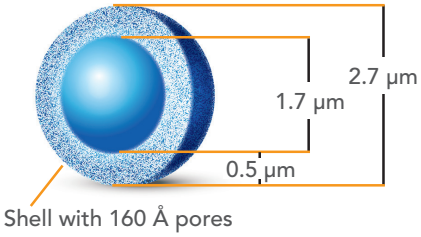
PEPTIDE SOLUTIONS

- 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

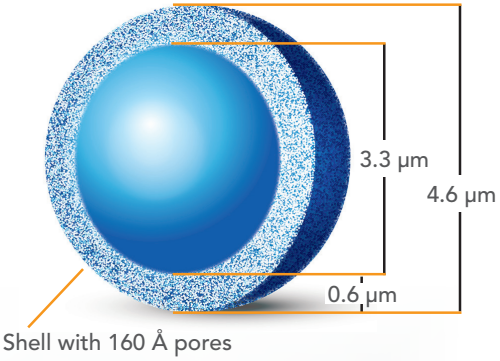
HALO® 2 µm Peptide



HALO® 2.7 µm Peptide

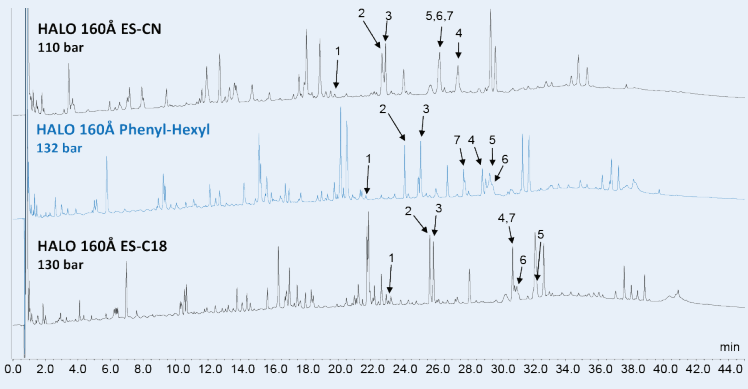


HALO® 5 µm Peptide



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:

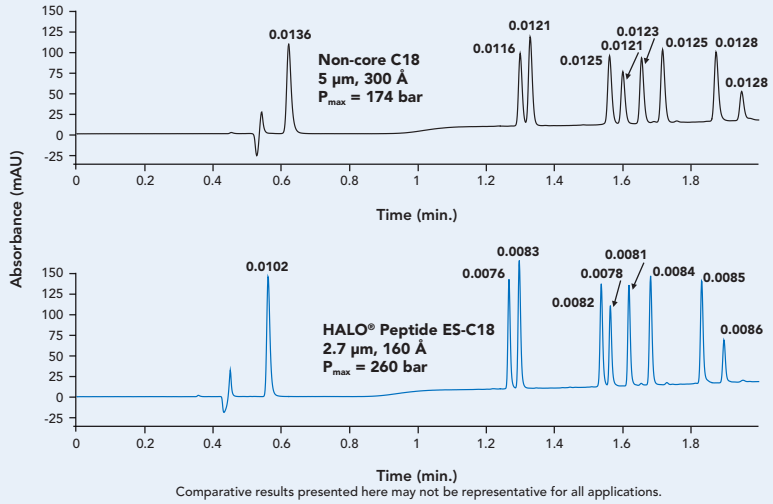
Columns: HALO® 160Å ES-CN, 2.7 µm, 2.1 x 100 mm
Part Number: 76444-274
HALO® 160Å Phenyl-Hexyl, 2.7 µm, 2.1 x 100 mm
Part Number: 76444-148
HALO® 160Å ES-C18, 2.7 µm, 2.1 x 100 mm
Part Number: 76444-272
Mobile Phase:
A = water + 10 mM difluoroacetic acid (DFA)
B = ACN + 10 mM difluoroacetic acid
Flow 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

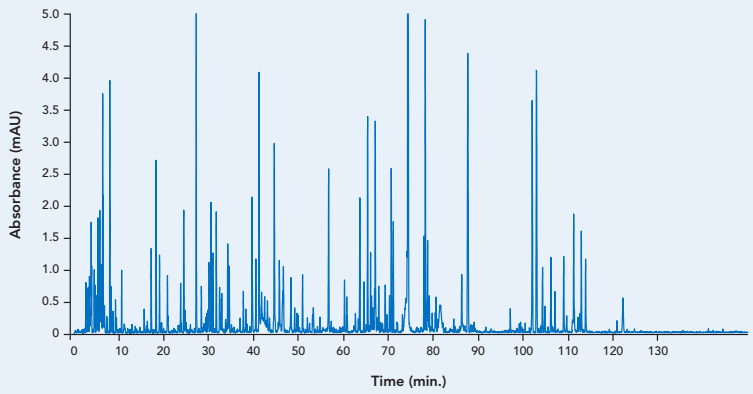
PEAK IDENTITIES:

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.

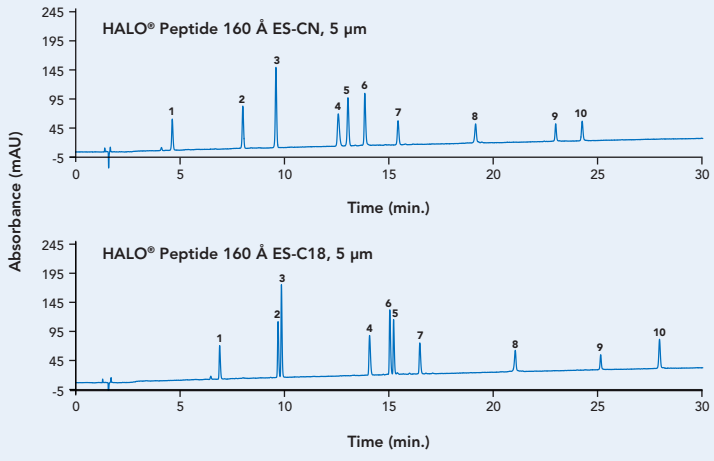


TEST CONDITIONS:

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

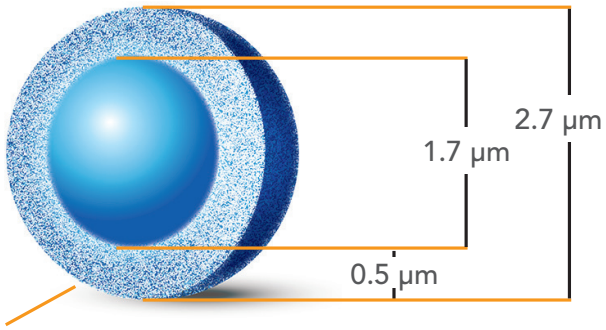
See page 36 for full list of HALO® Peptide part numbers.

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

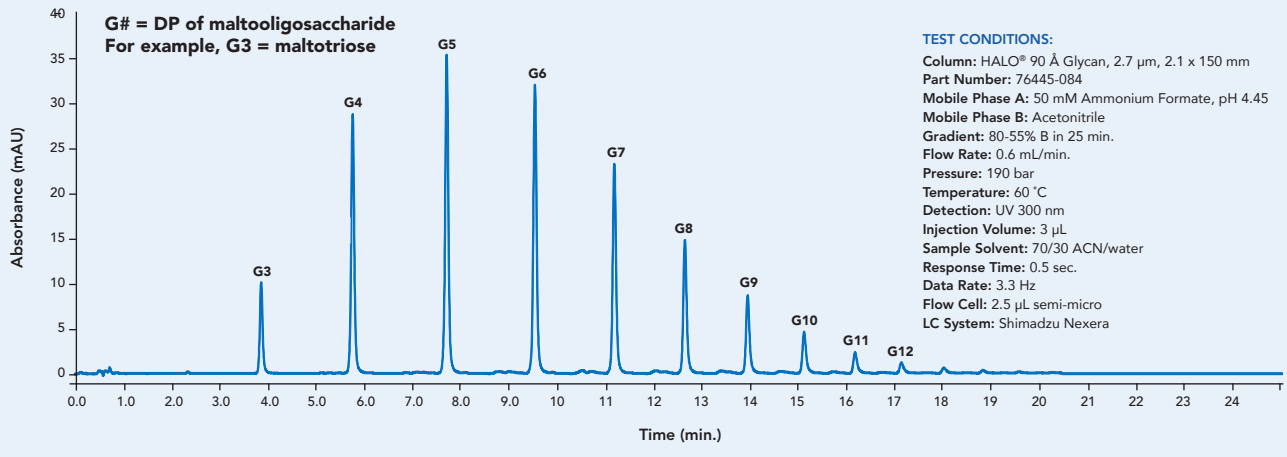
HALO® Glycan



Shell with 90 Å pores

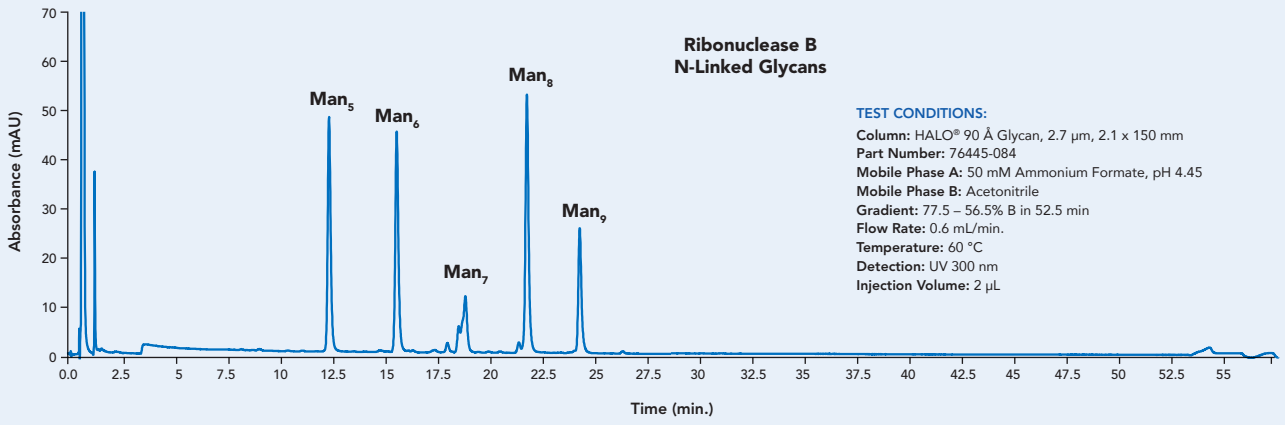
QA ANALYSIS OF HALO® GLYCAN

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.



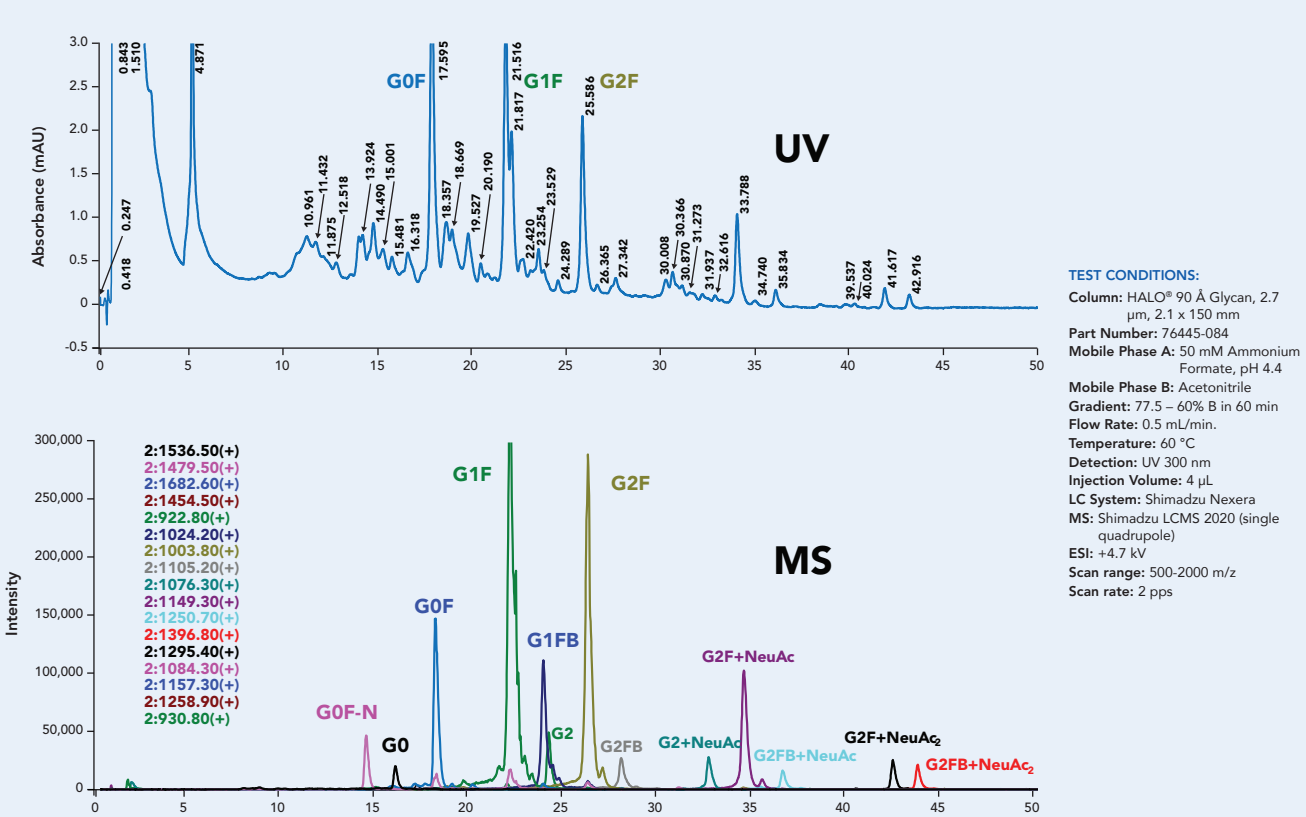
SEPARATION OF N-LINKED GLYCANS FROM RIBONUCLEASE B

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.

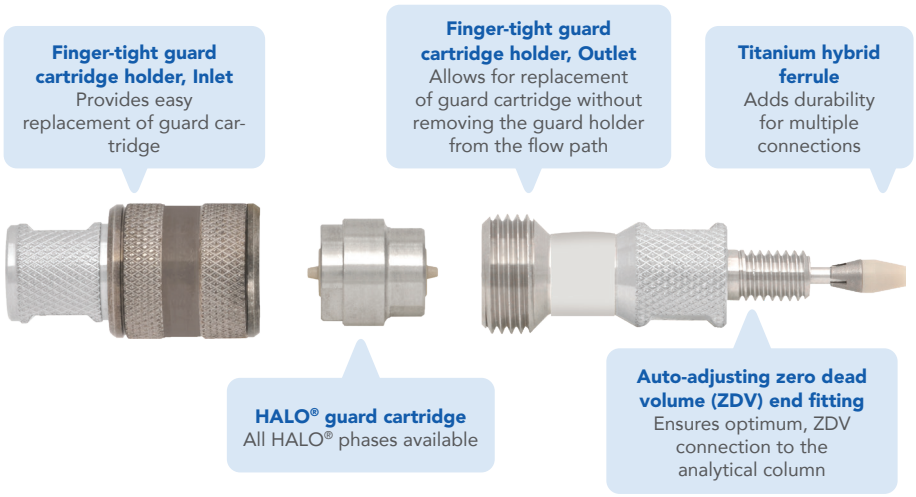


See page 35 for full list of HALO® Glycan part numbers.

HALO® UHPLC AND HPLC GUARD COLUMNS

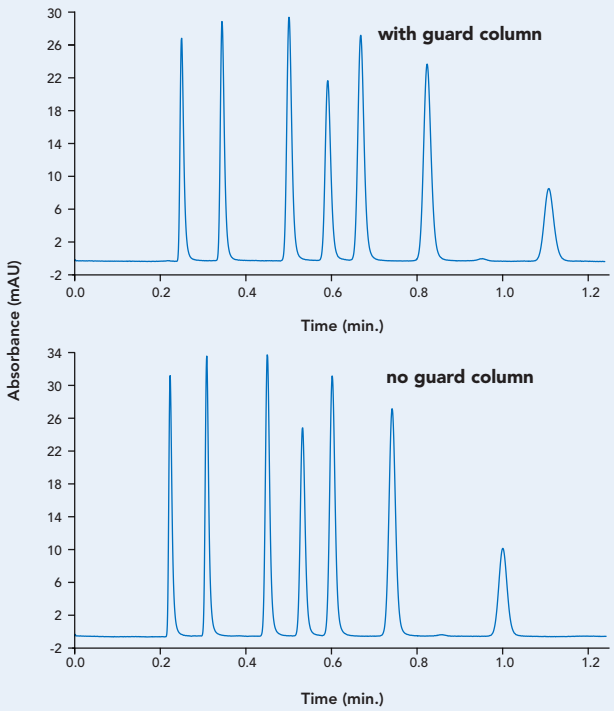
- Collect strongly retained compounds from the sample and minimizes column fouling
- Ultra-low dispersion, easy to use, operate at pressures up to 1000 bar
- Finger-tight, direct-connect units that auto-adjust to any column with a 10–32 inlet port
- Easily replace guard cartridge without removing guard 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	Phenyl-Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 20	76443-818	76443-834	76443-828	76443-824	76443-826	76443-830	76443-820	76443-822	76443-816
2.1 x 30	76443-838	76443-854	76443-848	76443-844	76443-846	76443-850	76443-840	76443-842	76443-836
2.1 x 50	76443-858	76443-874	76443-868	76443-864	76443-866	76443-870	76443-860	76443-862	76443-856
2.1 x 75	76443-878	76443-894	76443-888	76443-884	76443-886	76443-890	76443-880	76443-882	76443-876
2.1 x 100	76443-898	76443-914	76443-908	76443-904	76443-906	76443-910	76443-900	76443-902	76443-896
2.1 x 150	76443-918	76443-934	76443-928	76443-924	76443-926	76443-930	76443-920	76443-922	76443-916
3.0 x 20	76443-958	76443-974	76443-968	76443-964	76443-966	76443-970	76443-960	76443-962	76443-956
3.0 x 30	76443-978	76443-994	76443-988	76443-984	76443-986	76443-990	76443-980	76443-982	76443-976
3.0 x 50	76443-998	76444-014	76444-008	76444-004	76444-006	76444-010	76444-000	76444-002	76443-996
3.0 x 75	76444-018	76444-034	76444-028	76444-024	76444-026	76444-030	76444-020	76444-022	76444-016
3.0 x 100	76444-038	76444-054	76444-048	76444-044	76444-046	76444-050	76444-040	76444-042	76444-036
3.0 x 150	76444-058	76444-074	76444-068	76444-064	76444-066	76444-070	76444-060	76444-062	76444-056
2 µm, 90 Å Guard Columns, 3-Pack									
Dimensions ID x Length (in mm)	C18	AQ-C18	C8	Phenyl-Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 5	76443-318	76443-334	76443-328	76443-324	76443-326	76443-330	76443-320	76443-322	76443-316
3.0 x 5	76443-338	76443-354	76443-348	76443-344	76443-346	76443-350	76443-340	76443-342	76443-336
Guard Column Holder	76445-284								

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	76446-376	76446-392	76446-386	76443-684	76446-382	76446-390	76446-384	76446-388	76446-378	76446-380	76446-374
0.075 x 100	76446-396	76446-412	76446-406	76443-688	76446-402	76446-410	76446-404	76446-408	76446-398	76446-400	76446-394
0.075 x 150	76446-416	76446-432	76446-426	76443-692	76446-422	76446-430	76446-424	76446-428	76446-418	76446-420	76446-414
0.1 x 50	76446-316	76446-332	76446-326	76443-672	76446-322	76446-330	76446-324	76446-328	76446-318	76446-320	76446-314
0.1 x 100	76446-336	76446-352	76446-346	76443-676	76446-342	76446-350	76446-344	76446-348	76446-338	76446-340	76446-334
0.1 x 150	76446-356	76446-372	76446-366	76443-680	76446-362	76446-370	76446-364	76446-368	76446-358	76446-360	76446-354
0.2 x 50	76446-256	76446-272	76446-266	76443-660	76446-262	76446-270	76446-264	76446-268	76446-258	76446-260	76446-254
0.2 x 100	76446-276	76446-292	76446-286	76443-664	76446-282	76446-290	76446-284	76446-288	76446-278	76446-280	76446-274
0.2 x 150	76446-296	76446-312	76446-306	76443-668	76446-302	76446-310	76446-304	76446-308	76446-298	76446-300	76446-294
0.3 x 50	76446-196	76446-212	76446-206	76443-648	76446-202	76446-210	76446-204	76446-208	76446-198	76446-200	76446-194
0.3 x 100	76446-216	76446-232	76446-226	76443-652	76446-222	76446-230	76446-224	76446-228	76446-218	76446-220	76446-214
0.3 x 150	76446-236	76446-252	76446-246	76443-656	76446-242	76446-250	76446-244	76446-248	76446-238	76446-240	76446-234
0.5 x 50	76446-136	76446-152	76446-146	76443-636	76446-142	76446-150	76446-144	76446-148	76446-138	76446-140	76446-134
0.5 x 100	76446-156	76446-172	76446-166	76443-640	76446-162	76446-170	76446-164	76446-168	76446-158	76446-160	76446-154
0.5 x 150	76446-176	76446-192	76446-186	76443-644	76446-182	76446-190	76446-184	76446-188	76446-178	76446-180	76446-174
1.0 x 30	76444-576	76444-592	76444-586	76444-112	76444-582	76444-590	76444-584	76444-588	76444-578	76444-580	76444-574
1.0 x 50	76444-596	76444-612	76444-606	76444-116	76444-602	76444-610	76444-604	76444-608	76444-598	76444-600	76444-594
1.0 x 75	76444-616	76444-632	76444-626	76444-120	76444-622	76444-630	76444-624	76444-628	76444-618	76444-620	76444-614
1.0 x 100	76444-636	76444-648	76444-642	76444-124	76443-420	76444-646	76444-640	76444-644	76444-638	76443-418	76444-634
1.0 x 150	76444-652	76444-664	76444-658	76444-128	76443-424	76444-662	76444-656	76444-660	76444-654	76443-422	76444-650
2.1 x 20	76444-668	76444-684	76444-678	76444-134	76444-674	76444-682	76444-676	76444-680	76444-670	76444-672	76444-666
2.1 x 30	76444-688	76444-704	76444-698	76444-138	76444-694	76444-702	76444-696	76444-700	76444-690	76444-692	76444-686
2.1 x 50	76444-708	76444-724	76444-718	76444-142	76444-714	76444-722	76444-716	76444-720	76444-710	76444-712	76444-706
2.1 x 75	76444-728	76444-744	76444-738	76444-146	76444-734	76444-742	76444-736	76444-740	76444-730	76444-732	76444-726
2.1 x 100	76444-748	76444-760	76444-754	76444-150	76443-448	76444-758	76444-752	76444-756	76444-750	76443-446	76444-746
2.1 x 150	76444-764	76444-776	76444-770	76444-154	76443-452	76444-774	76444-768	76444-772	76444-766	76443-450	76444-762
2.1 x 250	76444-780	76444-792	76444-786	76444-158	76443-456	76444-790	76444-784	76444-788	76444-782	76443-454	76444-778
3.0 x 20	76444-796	76444-812	76444-806	76444-162	76444-802	76444-810	76444-804	76444-808	76444-798	76444-800	76444-794
3.0 x 30	76444-816	76444-832	76444-826	76444-166	76444-822	76444-830	76444-824	76444-828	76444-818	76444-820	76444-814
3.0 x 50	76444-836	76444-852	76444-846	76444-170	76444-842	76444-850	76444-844	76444-848	76444-838	76444-840	76444-834
3.0 x 75	76444-856	76444-872	76444-866	76444-174	76444-862	76444-870	76444-864	76444-868	76444-858	76444-860	76444-854
3.0 x 100	76444-876	76444-888	76444-882	76444-178	76443-480	76444-886	76444-880	76444-884	76444-878	76443-478	76444-874
3.0 x 150	76444-892	76444-904	76444-898	76444-182	76443-484	76444-902	76444-896	76444-900	76444-894	76443-482	76444-890
3.0 x 250	76444-908	76444-920	76444-914	76444-186	76443-488	76444-918	76444-912	76444-916	76444-910	76443-486	76444-906
4.6 x 20	76444-924	76444-940	76444-934	76444-190	76444-930	76444-938	76444-932	76444-936	76444-926	76444-928	76444-922
4.6 x 30	76444-944	76444-960	76444-954	76444-194	76444-950	76444-958	76444-952	76444-956	76444-946	76444-948	76444-942
4.6 x 50	76444-964	76444-980	76444-974	76444-198	76444-970	76444-978	76444-972	76444-976	76444-966	76444-968	76444-962
4.6 x 75	76444-984	76445-000	76444-994	76444-202	76444-990	76444-998	76444-992	76444-996	76444-986	76444-988	76444-982
4.6 x 100	76445-004	76445-018	76445-012	76444-206	76445-008	76445-016	76445-010	76445-014	76445-006	76443-510	76445-002
4.6 x 150	76445-022	76445-038	76445-032	76444-210	76445-028	76445-036	76445-030	76445-034	76445-024	76445-026	76445-020
4.6 x 250	76445-042	76445-058	76445-052	76444-214	76445-048	76445-056	76445-050	76445-054	76445-044	76445-046	76445-040
10.0 x 50	76444-512	76444-524	76444-518	76444-096	76443-404	76444-522	76444-516	76444-520	76444-514	76443-402	76444-510
10.0 x 75	76444-528	76444-540	76444-534	76444-100	76443-408	76444-538	76444-532	76444-536	76444-530	76443-406	76444-526
10.0 x 100	76444-544	76444-556	76444-550	76444-104	76443-412	76444-554	76444-548	76444-552	76444-546	76443-410	76444-542
10.0 x 150	76444-560	76444-572	76444-566	76444-108	76443-416	76444-570	76444-564	76444-568	76444-562	76443-414	76444-558

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	76443-428	76443-444	76443-438	76443-358	76443-434	76443-442	76443-436	76443-440	76443-430	76443-432	76443-426
3.0 x 5	76443-460	76443-476	76443-470	76443-362	76443-466	76443-474	76443-468	76443-472	76443-462	76443-464	76443-458
4.6 x 5	76443-492	76443-508	76443-502	76443-366	76443-498	76443-506	76443-500	76443-504	76443-494	76443-496	76443-490

Guard Column Holder 76445-284

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	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
0.075 x 50	76446-676	76446-692	76446-686	76446-682	76446-684	76446-688	76446-678	76446-680	76446-674
0.075 x 100	76446-696	76446-712	76446-706	76446-702	76446-704	76446-708	76446-698	76446-700	76446-694
0.075 x 150	76446-716	76446-732	76446-726	76446-722	76446-724	76446-728	76446-718	76446-720	76446-714
0.1 x 50	76446-616	76446-632	76446-626	76446-622	76446-624	76446-628	76446-618	76446-620	76446-614
0.1 x 100	76446-636	76446-652	76446-646	76446-642	76446-644	76446-648	76446-638	76446-640	76446-634
0.1 x 150	76446-656	76446-672	76446-666	76446-662	76446-664	76446-668	76446-658	76446-660	76446-654
0.2 x 50	76446-556	76446-572	76446-566	76446-562	76446-564	76446-568	76446-558	76446-560	76446-554
0.2 x 100	76446-576	76446-592	76446-586	76446-582	76446-584	76446-588	76446-578	76446-580	76446-574
0.2 x 150	76446-596	76446-612	76446-606	76446-602	76446-604	76446-608	76446-598	76446-600	76446-594
0.3 x 50	76446-496	76446-512	76446-506	76446-502	76446-504	76446-508	76446-498	76446-500	76446-494
0.3 x 100	76446-516	76446-532	76446-526	76446-522	76446-524	76446-528	76446-518	76446-520	76446-514
0.3 x 150	76446-536	76446-552	76446-546	76446-542	76446-544	76446-548	76446-538	76446-540	76446-534
0.5 x 50	76446-436	76446-452	76446-446	76446-442	76446-444	76446-448	76446-438	76446-440	76446-434
0.5 x 100	76446-456	76446-472	76446-466	76446-462	76446-464	76446-468	76446-458	76446-460	76446-454
0.5 x 150	76446-476	76446-492	76446-486	76446-482	76446-484	76446-488	76446-478	76446-480	76446-474
1.0 x 30	76445-506	76445-522	76445-516	76445-512	76445-514	76445-518	76445-508	76445-510	76445-504
1.0 x 50	76445-526	76445-542	76445-536	76445-532	76445-534	76445-538	76445-528	76445-530	76445-524
1.0 x 75	76445-546	76445-562	76445-556	76445-552	76445-554	76445-558	76445-548	76445-550	76445-544
1.0 x 100	76445-566	76445-582	76445-576	76445-572	76445-574	76445-578	76445-568	76445-570	76445-564
1.0 x 150	76445-586	76445-602	76445-596	76445-592	76445-594	76445-598	76445-588	76445-590	76445-584
2.1 x 20	76445-626	76445-642	76445-636	76445-632	76445-634	76445-638	76445-628	76445-630	76445-624
2.1 x 30	76445-646	76445-662	76445-656	76445-652	76445-654	76445-658	76445-648	76445-650	76445-644
2.1 x 50	76445-666	76445-682	76445-676	76445-672	76445-674	76445-678	76445-668	76445-670	76445-664
2.1 x 75	76445-686	76445-702	76445-696	76445-692	76445-694	76445-698	76445-688	76445-690	76445-684
2.1 x 100	76445-706	76445-722	76445-716	76445-712	76445-714	76445-718	76445-708	76445-710	76445-704
2.1 x 150	76445-726	76445-742	76445-736	76445-732	76445-734	76445-738	76445-728	76445-730	76445-724
2.1 x 250	76445-746	76445-762	76445-756	76445-752	76445-754	76445-758	76445-748	76445-750	76445-744
3.0 x 20	76445-766	76445-782	76445-776	76445-772	76445-774	76445-778	76445-768	76445-770	76445-764
3.0 x 30	76445-786	76445-802	76445-796	76445-792	76445-794	76445-798	76445-788	76445-790	76445-784
3.0 x 50	76445-806	76445-822	76445-816	76445-812	76445-814	76445-818	76445-808	76445-810	76445-804
3.0 x 75	76445-826	76445-842	76445-836	76445-832	76445-834	76445-838	76445-828	76445-830	76445-824
3.0 x 100	76445-846	76445-862	76445-856	76445-852	76445-854	76445-858	76445-848	76445-850	76445-844
3.0 x 150	76445-866	76445-882	76445-876	76445-872	76445-874	76445-878	76445-868	76445-870	76445-864
3.0 x 250	76445-886	76445-902	76445-896	76445-892	76445-894	76445-898	76445-888	76445-890	76445-884
4.6 x 20	76445-906	76445-922	76445-916	76445-912	76445-914	76445-918	76445-908	76445-910	76445-904
4.6 x 30	76445-926	76445-942	76445-936	76445-932	76445-934	76445-938	76445-928	76445-930	76445-924
4.6 x 50	76445-946	76445-962	76445-956	76445-952	76445-954	76445-958	76445-948	76445-950	76445-944
4.6 x 75	76445-966	76445-982	76445-976	76445-972	76445-974	76445-978	76445-968	76445-970	76445-964
4.6 x 100	76445-986	76446-002	76445-996	76445-992	76445-994	76445-998	76445-988	76445-990	76445-984
4.6 x 150	76446-006	76446-022	76446-016	76446-012	76446-014	76446-018	76446-008	76446-010	76446-004
4.6 x 250	76446-026	76446-042	76446-036	76446-032	76446-034	76446-038	76446-028	76446-030	76446-024
10.0 x 50	76445-406	76445-422	76445-416	76445-412	76445-414	76445-418	76445-408	76445-410	76445-404
10.0 x 75	76445-426	76445-442	76445-436	76445-432	76445-434	76445-438	76445-428	76445-430	76445-424
10.0 x 100	76445-446	76445-462	76445-456	76445-452	76445-454	76445-458	76445-448	76445-450	76445-444
10.0 x 150	76445-466	76445-482	76445-476	76445-472	76445-474	76445-478	76445-468	76445-470	76445-464
10.0 x 250	76445-486	76445-502	76445-496	76445-492	76445-494	76445-498	76445-488	76445-490	76445-484

5 µm, 90Å Guard Columns, 3-Pack

Dimensions ID x Length (in mm)	C18	AQ-C18	C8	Phenyl-Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 5	76443-552	76443-568	76443-562	76443-558	76443-560	76443-564	76443-554	76443-556	76443-550
3.0 x 5	76443-572	76443-588	76443-582	76443-578	76443-580	76443-584	76443-574	76443-576	76443-570
4.6 x 5	76443-592	76443-608	76443-602	76443-598	76443-600	76443-604	76443-594	76443-596	76443-590

Guard Column Holder 76445-284

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	C4	ES-C18	Diphenyl
0.075 x 50	76445-274	76445-272	76446-118	76446-116	76446-120
0.075 x 100	76445-278	76445-276	76446-124	76446-122	76446-126
0.075 x 150	76445-282	76445-280	76446-130	76446-128	76446-132
0.1 x 50	76445-262	76445-260	76446-100	76446-098	76446-102
0.1 x 100	76445-266	76445-264	76446-106	76446-104	76446-108
0.1 x 150	76445-270	76445-268	76446-112	76446-110	76446-114
0.2 x 50	76445-250	76445-248	76446-082	76446-080	76446-084
0.2 x 100	76445-254	76445-252	76446-088	76446-086	76446-090
0.2 x 150	76445-258	76445-256	76446-094	76446-092	76446-096
0.3 x 50	76445-238	76445-236	76446-064	76446-062	76446-066
0.3 x 100	76445-242	76445-240	76446-070	76446-068	76446-072
0.3 x 150	76445-246	76445-244	76446-076	76446-074	76446-078
0.5 x 50	76445-226	76445-224	76446-046	76446-044	76446-048
0.5 x 100	76445-230	76445-228	76446-052	76446-050	76446-054
0.5 x 150	76445-234	76445-232	76446-058	76446-056	76446-060
1.0 x 30	76445-110	76445-108			
1.0 x 50	76445-114	76445-112	76444-362	76444-360	76444-364
1.0 x 75	76445-118	76445-116	76444-368	76444-366	76444-370
1.0 x 100	76445-122	76445-120	76444-374	76444-372	76444-376
1.0 x 150	76445-126	76445-124	76444-380	76444-378	76444-382
2.1 x 20	76445-130	76445-128	76444-386	76444-384	76444-388
2.1 x 30	76445-134	76445-132	76444-392	76444-390	76444-394
2.1 x 50	76445-138	76445-136	76444-398	76444-396	76444-400
2.1 x 75	76445-144	76445-142	76444-404	76444-402	76444-406
2.1 x 100	76445-148	76445-146	76444-410	76444-408	76444-412
2.1 x 150	76445-154	76445-152	76444-416	76444-414	76444-418
2.1 x 250	76445-160	76445-158	76444-422	76444-420	76444-424
3.0 x 20	76445-164	76445-162	76444-428	76444-426	76444-430
3.0 x 30	76445-168	76445-166	76444-434	76444-432	76444-436
3.0 x 50	76445-172	76445-170	76444-440	76444-438	76444-442
3.0 x 75	76445-176	76445-174	76444-446	76444-444	76444-448
3.0 x 100	76445-180	76445-178	76444-452	76444-450	76444-454
3.0 x 150	76445-184	76445-182	76444-458	76444-456	76444-460
3.0 x 250	76445-188	76445-186	76444-464	76444-462	76444-466
4.6 x 20	76445-192	76445-190	76444-470	76444-468	76444-472
4.6 x 30	76445-196	76445-194	76444-476	76444-474	76444-478
4.6 x 50	76445-200	76445-198	76444-482	76444-480	76444-484
4.6 x 75	76445-206	76445-204	76444-488	76444-486	76444-490
4.6 x 100	76445-210	76445-208	76444-494	76444-492	76444-496
4.6 x 150	76445-216	76445-214	76444-500	76444-498	76444-502
4.6 x 250	76445-222	76445-220	76444-506	76444-504	76444-508
10.0 x 50	76445-094	76445-092	76444-342	76444-340	76444-344
10.0 x 75	76445-098	76445-096	76444-348	76444-346	76444-350
10.0 x 100	76445-102	76445-100	76444-354	76444-352	76443-380
10.0 x 150	76445-106	76445-104	76444-358	76444-356	76443-382

Guard Columns, 3-Pack					
Dimensions ID x Length (in mm)	C4	ES-C18	C4	ES-C18	Diphenyl
2.1 x 5	76443-524	76443-522	76443-386	76443-384	76443-388
3.0 x 5	76443-530	76443-528	76443-392	76443-390	76443-394
4.6 x 5	76443-534	76443-532	76443-398	76443-396	76443-400
Guard Column Holder	76445-284				

HALO® 90 Å GLYCAN COLUMNS

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

Dimensions ID x Length (in mm)	HALO® Glycan
2.1 x 50	76445-080
2.1 x 100	76445-082
2.1 x 150	76445-084
4.6 x 50	76445-086
4.6 x 100	76445-088
4.6 x 150	76445-090

Guard Columns, 3-Pack	
Dimensions ID x Length (in mm)	HALO® Glycan
2.1 x 5	76443-518
4.6 x 5	76443-520
Guard Column Holder	76445-284

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	
Dimensions ID x Length (in mm)	ES-C18	ES-C18	ES-CN	ES-C18	ES-CN
0.075 x 50		76443-744	76443-746	76443-804	76443-806
0.075 x 100		76443-748	76443-750	76443-808	76443-810
0.075 x 150		76443-752	76443-754	76443-812	76443-814
0.1 x 50		76443-732	76443-734	76443-792	76443-794
0.1 x 100		76443-736	76443-738	76443-796	76443-798
0.1 x 150		76443-740	76443-742	76443-800	76443-802
0.2 x 50		76443-720	76443-722	76443-780	76443-782
0.2 x 100		76443-724	76443-726	76443-784	76443-786
0.2 x 150		76443-728	76443-730	76443-788	76443-790
0.3 x 50		76443-708	76443-710	76443-768	76443-770
0.3 x 100		76443-712	76443-714	76443-772	76443-774
0.3 x 150		76443-716	76443-718	76443-776	76443-778
0.5 x 50		76443-696	76443-698	76443-756	76443-758
0.5 x 100		76443-700	76443-702	76443-760	76443-762
0.5 x 150		76443-704	76443-706	76443-764	76443-766
1.0 x 30		76444-232			76445-306
1.0 x 50		76444-236	76444-238	76445-308	76445-310
1.0 x 75		76444-240	76444-242	76445-312	76445-314
1.0 x 100		76444-244	76444-246	76445-316	76445-318
1.0 x 150		76444-248	76444-250	76445-320	76445-322
2.1 x 20	76443-610	76444-256	76444-258	76445-326	76445-328
2.1 x 30	76443-612	76444-260	76444-262	76445-330	76445-332
2.1 x 50	76443-614	76444-264	76444-266	76445-334	76445-336
2.1 x 75	76443-616	76444-268	76444-270	76445-338	76445-340
2.1 x 100	76443-618	76444-272	76444-274	76445-342	76445-344
2.1 x 150	76443-620	76444-276	76444-278	76445-346	76445-348
2.1 x 250	76443-622	76444-280	76444-282	76445-350	76446-748
3.0 x 20		76444-284	76444-286	76445-352	76445-354
3.0 x 30	76443-624	76444-288	76444-290	76445-356	76445-358
3.0 x 50	76443-626	76444-292	76444-294	76445-360	76445-362
3.0 x 75	76443-628	76444-296	76444-298	76445-364	76445-366
3.0 x 100	76443-630	76444-300	76444-302	76445-368	76445-370
3.0 x 150	76443-632	76444-304	76444-306	76445-372	76445-374
3.0 x 250	76443-634	76444-308	76444-310	76445-376	76446-750
4.6 x 20		76444-312	76444-314	76445-378	76445-380
4.6 x 30		76444-316	76444-318	76445-382	76445-384
4.6 x 50		76444-320	76444-322	76445-386	76445-388
4.6 x 75		76444-324	76444-326	76445-390	76445-392
4.6 x 100		76444-328	76444-330	76445-394	76445-396
4.6 x 150		76444-332	76444-334	76445-398	76445-400
4.6 x 250		76444-336	76444-338	76445-402	76446-752
10.0 x 50		76444-216	76444-218	76445-286	76445-288
10.0 x 75		76444-220	76444-222	76445-290	76445-292
10.0 x 100		76444-224	76444-226	76445-294	76445-296
10.0 x 150		76444-228	76444-230	76445-298	76445-300
10.0 x 250				76445-302	76446-744
Guard Columns, 3-pack					
Dimensions ID x Length (in mm)	ES-C18	ES-C18	ES-CN	ES-C18	ES-CN
2.1 x 5	76443-312	76443-368	76443-370	76443-538	76443-540
3.0 x 5	76443-314	76443-372	76443-374		76443-544
4.6 x 5		76443-376	76443-378		76443-548
Guard Column Holder	76445-284				

HALO®

0421 Lit. No. 010220W

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