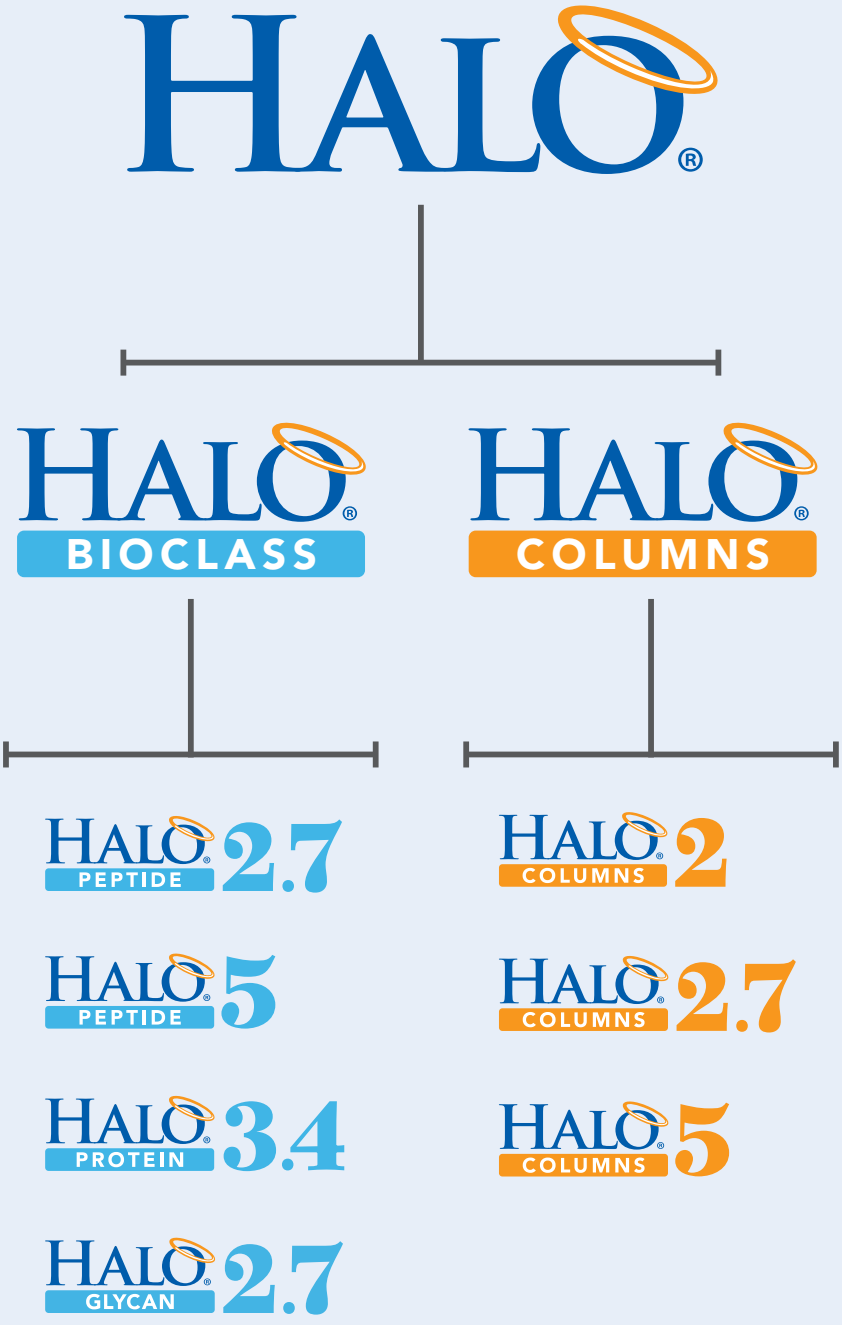
通 微
UNIMICRO

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



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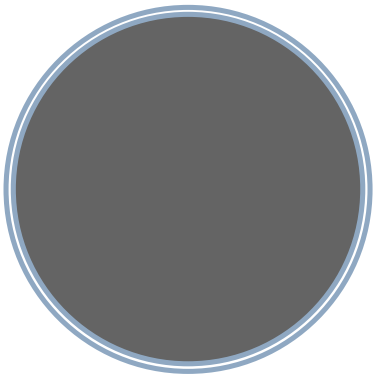
MILESTONES IN THE DEVELOPMENT OF FUSED-CORE PARTICLES

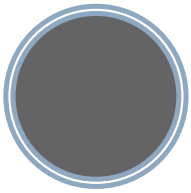
1960 — Superficially porous particles (SPPs) were first proposed by Golay

1967 — Horvath, Preiss, and Lipsky described a nucleotides separation using ~50 µm cores with a thin layer of anion exchange resin; called the particles "pellicular" referring to the thin, skin-like coating



Horvath pellicular

1969 — Kirkland, Zipax® - Silica sol on glass beads that were ~30 µm particle size



Zipax®

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

approximately 20 yrs — Interest in SPPs dwindled due to the low surface areas and lower efficiencies, slower speeds of these column supports compared to non-core particles that could be made in smaller sizes

1992 — Kirkland describes 5 µm SPPs with 300 Å pores

Poroshell® 300

2000 — Agilent introduces 5 µm Poroshell® 300 based on Kirkland's work

2006 — Kirkland, Langlois and DeStefano, working at Advanced Materials Technology, Inc. described HALO® particles with a 1.7 µm core and a 0.5 µm thick shell for a total particle size of 2.7 µm

HALO 2.7

HALO 2

Summary

- Dr. Joseph (Jack) Kirkland has been intimately involved in the development of HPLC packings, including porous and Fused-Core (SPP), throughout his distinguished career
- SPPs were among the first packings developed for HPLC and have become important again after a 20-year hiatus
- Advanced Materials Technology (AMT) was the first company to commercialize Fused-Core particles smaller than 3 µm in 2006
- Columns packed with these 2.7 µm particles have 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
- AMT continues to be a leader in the development and commercialization of novel packing materials for small and large molecules (HALO 2, 2014; BioClass, 2013)

Poroshell 300 is a trademark of Agilent Technologies

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

Some of the early and current explanations for the excellent performance of Fused-Core columns are described below.

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)

Current Understanding of SPP Performance (Figure C)

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 A. Scanning Electron Microscope (SEM) image of HALO 2 particles. The particle size distribution is very narrow due to the separate sizing steps for solid cores and finished Fused-Core silica particles.

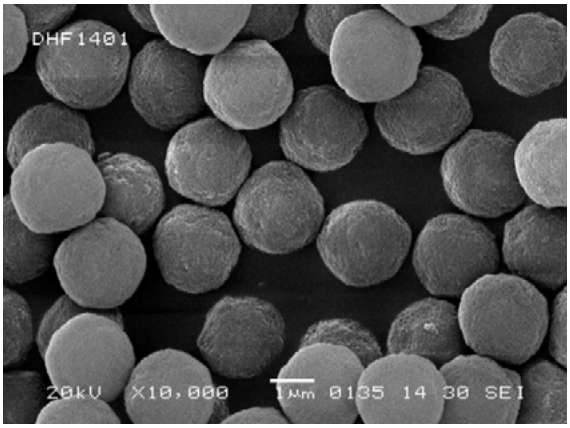


Figure B. SEM image of a focused-ion-beam-cleaved HALO Protein 3.4 µm silica particle. This "cut-away" view shows the solid core with its very thin 0.2 µm outer porous layer.

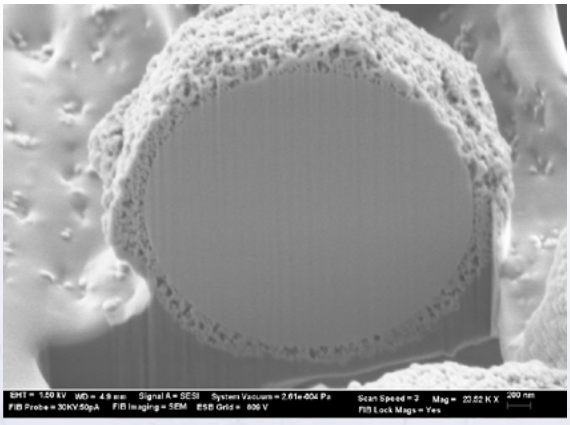
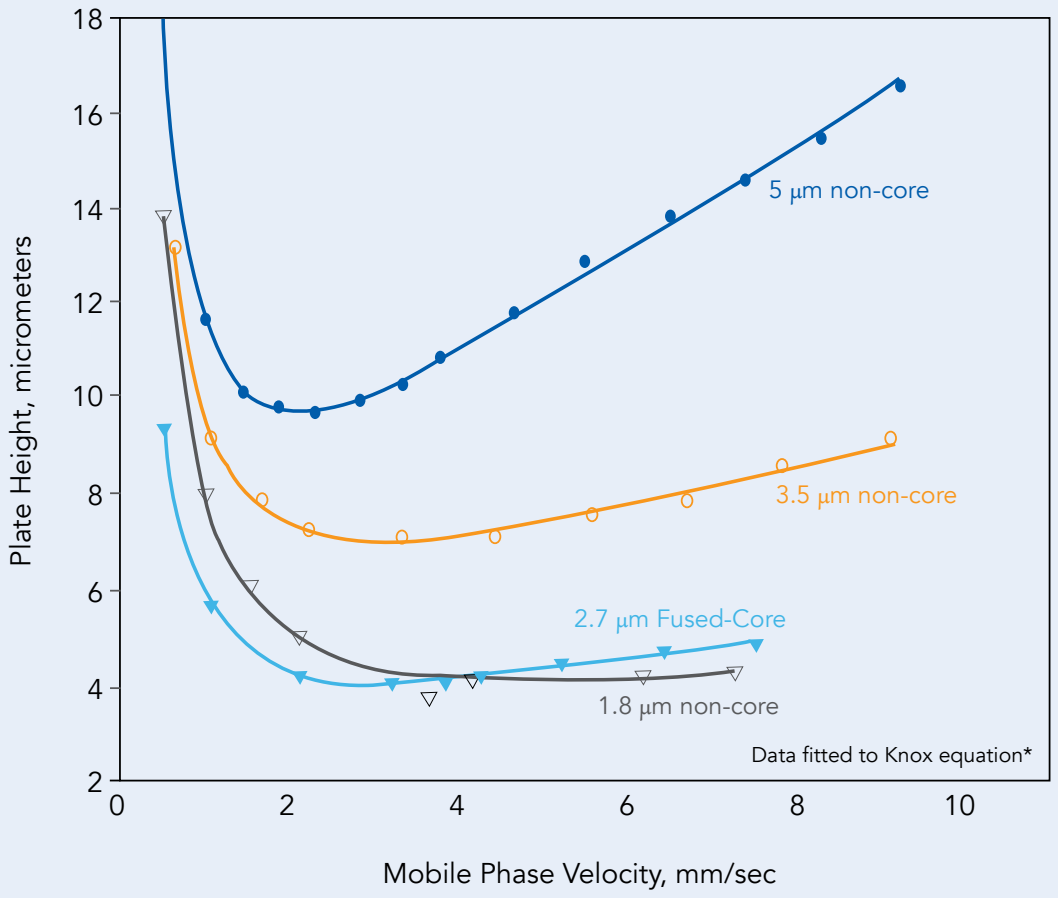


Figure C. van Deemter Plot of Plate Height vs. Linear Velocity (flow rate)

Effect of Particle Size and Type
Columns: 50 x 4.6 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 D and E)

- 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 C, page 3) allow higher flow rates with minimal column efficiency loss

High Resolution Separations (Figure F)

- 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 and HALO 5
  - 1 µm frits for HALO 2 vs. 0.2–0.5 µm frits for sub-2 µm non-core columns
- Excellent column-to-column and lot-to-lot reproducibility (Page 6)

PERFORMANCE VS. NON-CORE COLUMNS

HALO 2

- Superior efficiency compared to many popular sub-2 µm non-core columns
- ~ 20% lower back pressure than most commercially available sub-2 µm non-core columns

HALO 2.7

- Comparable resolution and peak capacity to sub-2 µm non-core columns at half of the back pressure under the same conditions, or…
- Two-fold higher throughput at twice the flow rate vs. sub-2 µm non-core columns at similar pressures

HALO 5

- Provides comparable efficiency and resolution to 3 µm non-core columns for HPLC separations at 50% lower back pressure

HALO BioClass

- Comparable to or better than performance of sub-2 µm non-core columns for bioseparations at 1/3 to 1/2 of the back pressure
- Outperforms legacy non-core columns in terms of peak shape, peak capacity and recovery

ULTRAFAST PEPTIDE SEPARATION

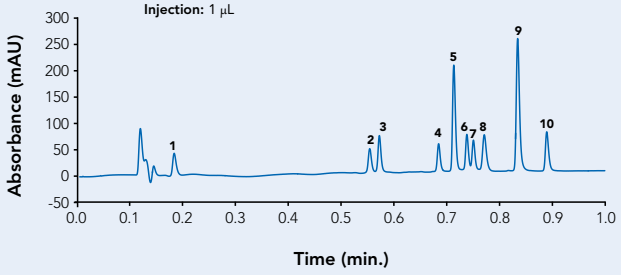
Figure D. Separation of a 10-peptide mixture is accomplished in less than one minute using a 2.1 x 50 mm, HALO 2.7 Peptide ES-C18 column using a delay-volume-minimized and-optimized Agilent 1200 system.

TEST CONDITIONS:

Column: 2.1 x 50 mm, HALO Peptide ES-C18
Part Number: 92122-402
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

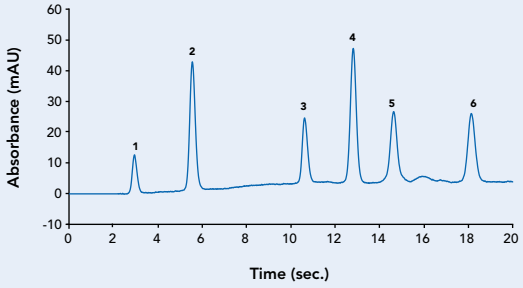
Figure E.
Many researchers have found HALO 5 columns in 2.1 mm ID to be very useful for high-throughput, ballistic separations by LC and LC-MS.

TEST CONDITIONS:

Column: 2.1 x 20 mm, HALO 5 C18
Part Number: 95812-202
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

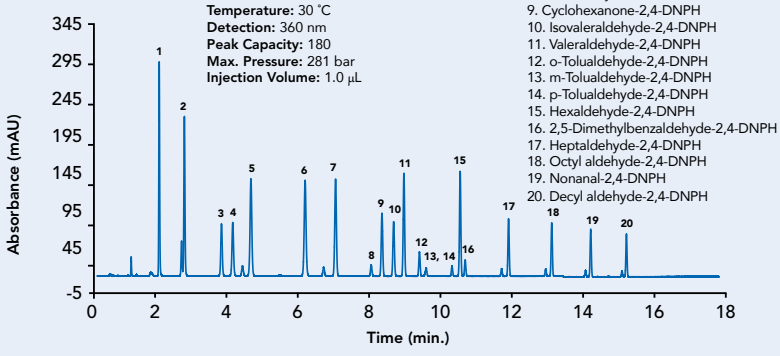
Figure F. Environmental samples can be quite complex as demonstrated by this gradient separation dinitrophenylhydrazone (DNPH) carbonyl compound derivatives using a 2.1 x 150 mm, HALO 2 C18 column.

TEST CONDITIONS:

Column: 2.1 x 150 mm, HALO 2 C18, 2 µm, 90 Å
Part Number: 91812-702
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:

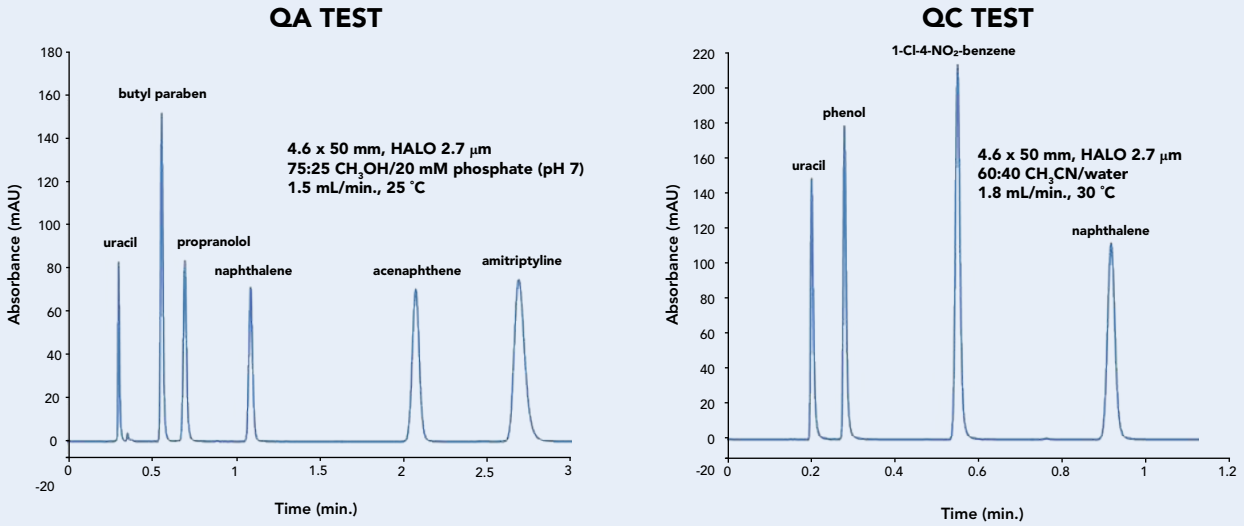
1. Formaldehyde-2,4-DNPH
2. Acetaldehyde-2,4-DNPH
3. Acetone-2,4-DNPH
4. Acrolein-2,4-DNPH
5. Propionaldehyde-2,4-DNPH
6. Crotonaldehyde-2,4-DNPH
7. Butyraldehyde-2,4-DNPH
8. Benzaldehyde-2,4-DNPH
9. Cyclohexanone-2,4-DNPH
10. Isovaleraldehyde-2,4-DNPH
11. Valeraldehyde-2,4-DNPH
12. o-Tolualdehyde-2,4-DNPH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

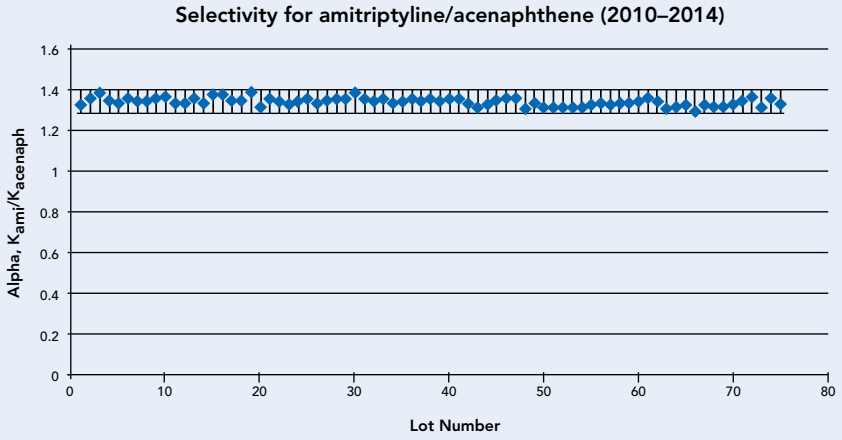
Figure G. Consistent reproducible performance from column to column and lot to lot is ensured because of well-designed processes and practices in the manufacture of HALO Fused-Core particles, HALO phases and HALO columns. Representative chromatograms of QA and QC tests are shown below, along with a historical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

Advanced Materials Technology incorporates the know-how from its **principal scientists, each with over 40 years** of experience in liquid chromatography, particle synthesis and column manufacturing to bring high quality, innovative products to our customers.



REPRODUCIBLE PERFORMANCE OVER TIME

Figure H. The selectivity value, alpha ($k_{ami}/k_{acenaph}$) is plotted versus lot number for the amitriptyline/acenaphthene pair of individual HALO C18 lots from 2010 to 2014. The vertical error bars show the bounds for two standard deviations about the mean value of 1.35. The reproducible selectivity is indicative of processes that are well-controlled, which can produce reliable, high-quality columns.



HALO COLUMNS FOR SMALL AND LARGE MOLECULES:
SELECTING THE APPROPRIATE PORE SIZE

How to Choose the Right Pore Size?

- Match the column pore size according to 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 (Page 8)
  - When an analyte is too large for the pores, restricted diffusion can occur, which can lead to peak broadening and reduced retention (Figure I)
- For macrocyclic antibiotics and biomolecules such as peptides and proteins, use larger pore sizes such as 160 Å HALO Peptide and 400 Å HALO Protein columns (Page 22)

Table A. Guidance for Pore Size Selection

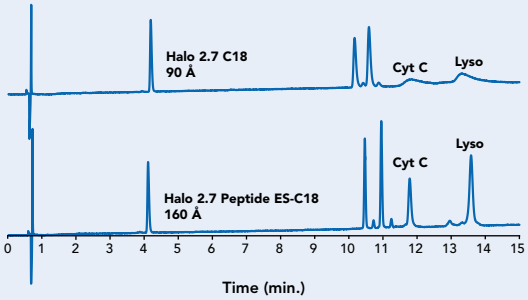
MW Range	Pore Size (Å)	HALO Column Family	Products
< 5000 Da	**90**	**HALO 90Å (small molecules)**	**HALO 2 HALO 2.7 HALO 5**
< 20 kDa*	90	HALO BioClass	HALO Glycan
100 Da < MW < 15 kDa	160	HALO BioClass	HALO 2.7 Peptide HALO 5 Peptide
2 kDa < MW < 500 kDa	400	HALO BioClass	HALO Protein

* for glycans, glycopeptides and glycoproteins

RESTRICTED DIFFUSION FOR HIGHER MW POLYPEPTIDES ON HALO 90Å

Figure I. In this example, broad peaks are observed in the upper chromatogram for bovine and human insulin, cytochrome C and lysozyme (MWs > ~5700 Da) chromatographed using a HALO 90 Ångstrom column.This broadening is due to restricted diffusion using the smaller pore column. However, those same four peaks are much narrower and taller in the lower chromatogram using the 160 Ångstrom HALO Peptide ES-C18 column.

Peptide or Polypeptide	PW $_{0.5}$ HALO 90Å	PW $_{0.5}$ HALO 160Å	MW (kDa)
Leucine enkephalin	0.0516	0.0516	0.56
Bovine insulin	0.0819	0.0457	5.73
Human insulin	0.0861	0.0491	5.81
Cytochrome C	0.5411	0.0837	12.4
Lysozyme	0.4671	0.1043	14.4



Peptides (in RT order): Leu-Enk, Bovine Insulin, Human Insulin, Cytochrome C, Lysozyme

TEST CONDITIONS:
Column: 4.6 x 100 mm
Part Number: HALO 2.7 C18: 92814-602
HALO 2.7 Peptide ES-C18: 92124-602
Mobile Phase A: 90/10 water/acetonitrile/0.1% TFA
Mobile Phase B: 30/70 water/acetonitrile/0.1% TFA
Gradient: 15% B to 50% B in 15 min.
Flow Rate: 1.5 mL/min.
Temperature: 30 °C
Detection: UV 220nm, VWD
Injection Volume: 5.0 µL
LC System: Agilent 1100

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.

HALO columns are available in different stationary phases to use for method development and to apply 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, RP-Amide, ES-CN) also retain and separate analytes via retention mechanisms different from HALO C18 and HALO C8, 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 J).

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

HALO 2, HALO 2.7 and HALO 5 columns for small molecule analyses are available in the following phases:

Reversed Phase

- C18
- C8
- RP-Amide
- Phenyl-Hexyl
- ES-CN
- PFP

Hydrophilic Interaction (HILIC) and Normal Phase

- HILIC (silica)
- Penta-HILIC

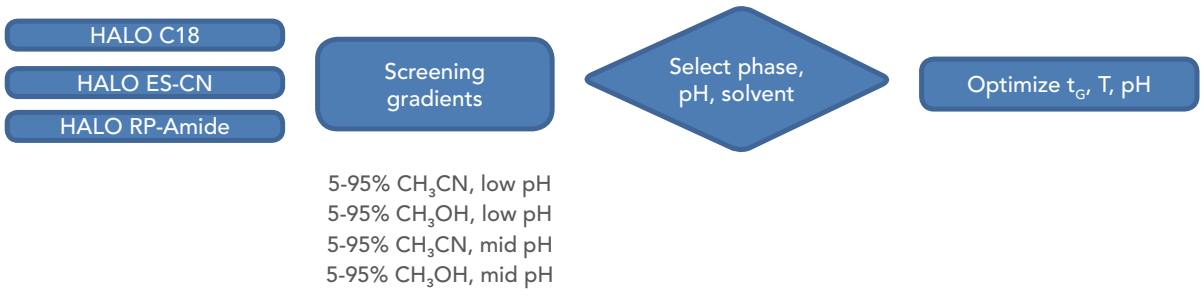
Complete descriptions of the various phases are provided in Table D, along with physical and chemical properties, target analytes and best applications for each phase (Table E).

See pages 14-15 for additional information about and applications for HALO HILIC and Penta-HILIC.

Table C. Relative Reversed-Phase Orthogonality of HALO Phases vs. HALO C18 at Low and Mid pH (Refs. 3-6)

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

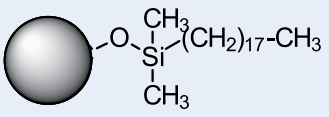
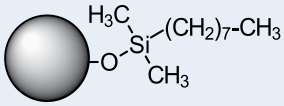
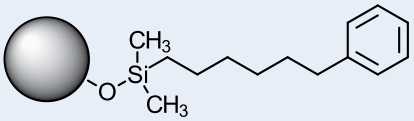
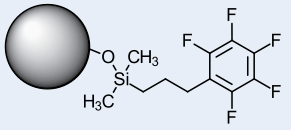
Figure J. Example Strategy for Comprehensive Method Development Using Multiple HALO Stationary Phases and Column/Condition Screening, Followed by Optimization of Gradient Time, Temperature and pH

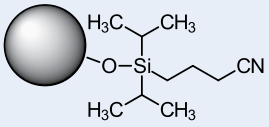
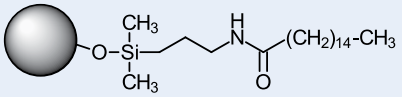
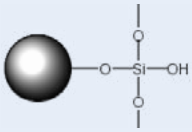
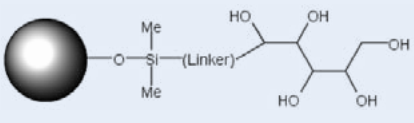


HALO COLUMNS FOR SMALL MOLECULE SEPARATIONS

Table D. HALO Small Molecule Column Specifications

	USP Designation	Particle Size(s) (µm)	Carbon Load (%)	Surface Area (m^2/g)	Low pH Limit	High pH Limit	Max. Temp. Lower pH Limit	Max. Temp. Upper pH Limit	Endcapped
C18	L1	2 2.7 5	7.5 7.7 5.4	120 135 90	2	9	60	40	Yes
C8	L7	2 2.7 5	4.8 5.4 3.7	120 135 90	2	9	60	40	Yes
Phenyl-Hexyl	L11	2 2.7 5	6.3 7.1 5.2	120 135 90	2	9	60	40	Yes
PFP	L43	2 2.7 5	5.3 5.5 3.9	120 135 90	2	9	60	40	Yes
ES-CN	L10	2 2.7 5	3.5 3.5 2.5	120 135 90	1	8	80	40	Yes
RP-Amide	L60	2 2.7 5	7.3 8.2 5.5	120 135 90	2	9	60	40	Yes
HILIC	L3	2 2.7 5	N.A.	120 135 90	1	8	60	40	N.A.
Penta-HILIC	Pending	2 2.7 5	2.8 3.2 2.1	120 135 90	2	9	60	40	No

Phase	Structure
C18	
C8	
Phenyl-Hexyl	
PFP	

Phase	Structure
ES-CN	
RP-Amide	
HILIC	
Penta-HILIC	

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	C18 (octadecyldimethylsilane)	Excellent performance for broad range of analyte polarities	Diverse analytes ranging from polar to non-polar Uncharged acids and bases, uncharged ion pairs	Analytes differing by an aliphatic or aromatic group
C8	C8 (octyldimethylsilane)	Excellent performance for broad range of analyte polarities	Diverse analytes ranging from polar to non-polar Uncharged acids and bases, uncharged ion pairs	Analytes differing by an aliphatic or aromatic group
Phenyl-Hexyl	Phenyl-Hexyl (phenylhexyldimethylsilane)	Complementary selectivity to alkyl phases Enhanced selectivity for aromatic compounds	Electron-poor molecules, aromatic or unsaturated compounds (ketones, nitriles, alkenes)	Aromatic molecules with electron-withdrawing groups (NO_2, COOH, COOR, halogens), heterocycles, benzodiazepines, highly aqueous conditions
PFP	PFP (pentafluorophenylpropylsilane)	Complementary selectivity to alkyl phases Enhanced selectivity for stereoisomers Can be used in RPLC and HILIC modes	Electron-rich compounds, aromatics, unsaturated compounds with double and/or triple bonds	Basic analytes at low pH, stereoisomers, steroids, taxanes, substituted aromatics, highly aqueous conditions
ES-CN	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 Very hydrophobic analytes retained too strongly on C18 phase	Aromatic molecules with electron-withdrawing groups (NO_2, COOH, COOR, halogens), heterocycles, benzodiazepines, highly aqueous conditions
RP-Amide	C16 Amide	Complementary selectivity to alkyl phases Enhanced stability for minimum bleed and long life	Alcohols, Acids, Phenols, Catechins	Acidic and basic analytes, heterocycles, proton donors and acceptors, highly aqueous conditions
HILIC	None (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	Enhanced sensitivity and peak shape for LC-MS, analyses of basic analytes in HILIC mode, normal-phase analysis with non-aqueous mobile phases
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 acids, bases and zwitterions that are not retained or are poorly retained with RPLC

REVERSED-PHASE SEPARATIONS WITH HALO

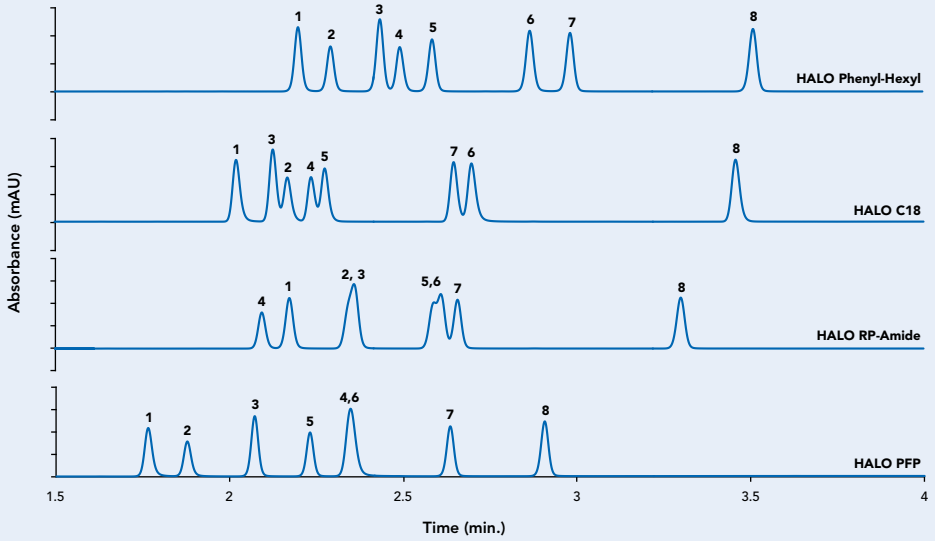
To illustrate the selectivity differences among the various HALO RPLC phases, the following examples are provided.

BENZODIAZEPINES ON HALO FUSED-CORE BONDED PHASES

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

TEST CONDITIONS:
Column: 4.6 x 50 mm
Part Numbers: 92814-406, 92814-402, 92814-407, 92814-409
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

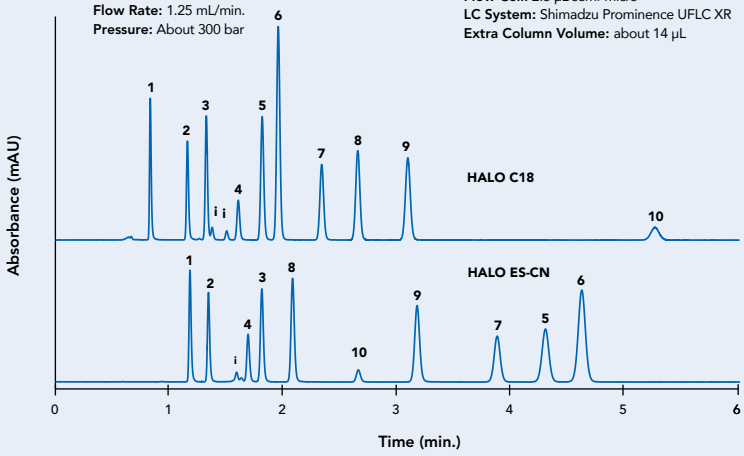
Figure L.
HALO C18 and HALO ES-CN columns may be used as orthogonal confirmatory columns for explosives analysis.

TEST CONDITIONS:
Column: 4.6 x 100 mm, HALO C18, ES-CN
Part Numbers: C18=92814-402, ES-CN=92814-404
Mobile Phase: 40/60-A/B for C18, 50/50-A/B for ES-CN
A= water
B= methanol
Flow Rate: 1.25 mL/min.
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
Extra Column Volume: about 14 µL

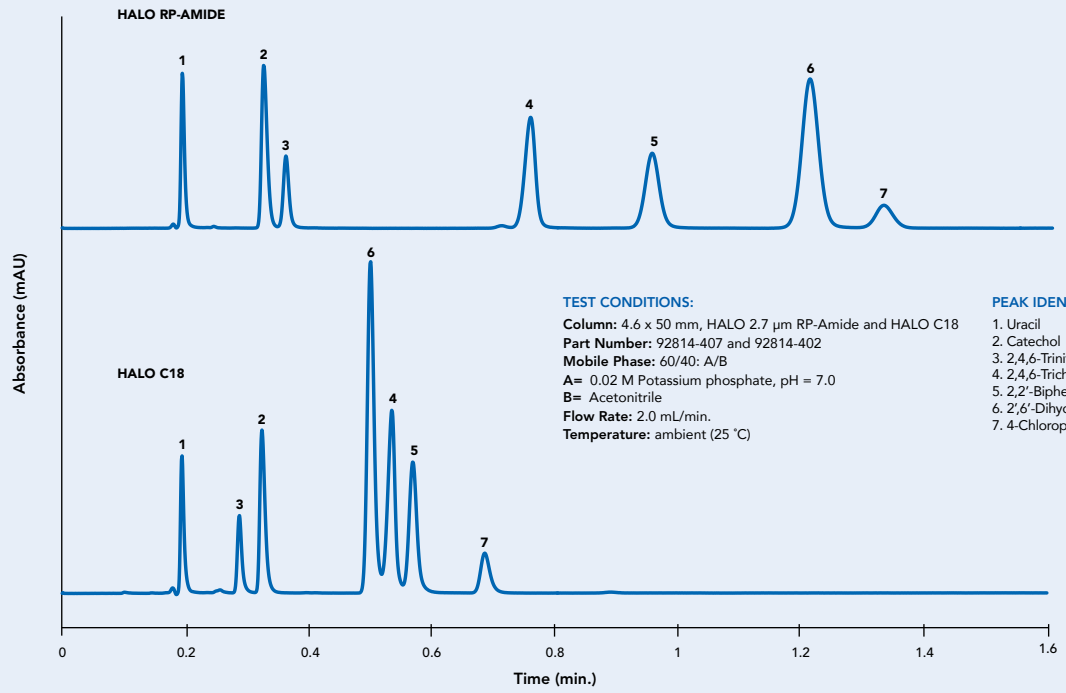
PEAK IDENTITIES:
1. Resorcinol
2. Benzyl alcohol
3. Phenylacetonitrile
4. 1-Indanol
5. 3,4-DNT
6. 2,3-DNT
7. 2,4-DNT
8. Anisole
9. 1-Chloro-4-nitrobenzene
10. Toluene

DNT = dinitrotoluene
i = impurity



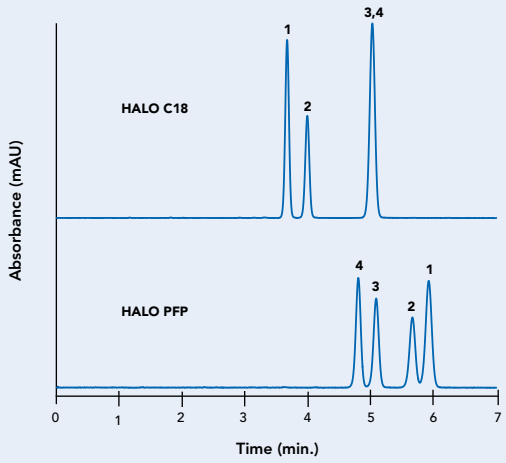
HALO C18 VS. RP-AMIDE FOR PHENOLICS

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



SEPARATION OF STRUCTURALLY SIMILAR STEROIDS ON HALO C18 AND PFP

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



HILIC SEPARATIONS WITH HALO

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

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

HALO columns are currently available in two different phases for HILIC separations (see Tables D and E, pages 10 and 11, for properties, features and benefits):

- 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.

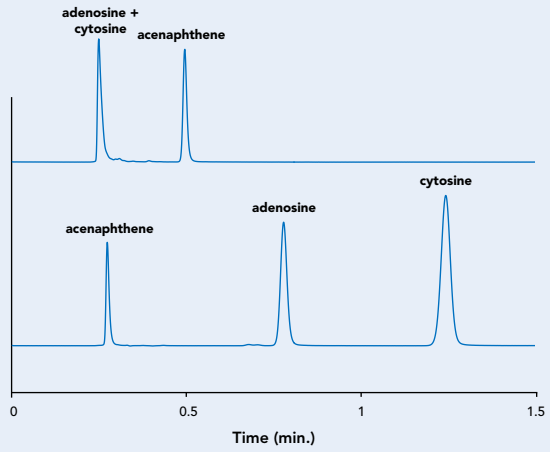
Some Typical Analytes for HILIC Separations

- 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 37.

RETENTION ORDER REVERSAL AND IMPROVED RETENTION WITH HILIC

Figure O.
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.

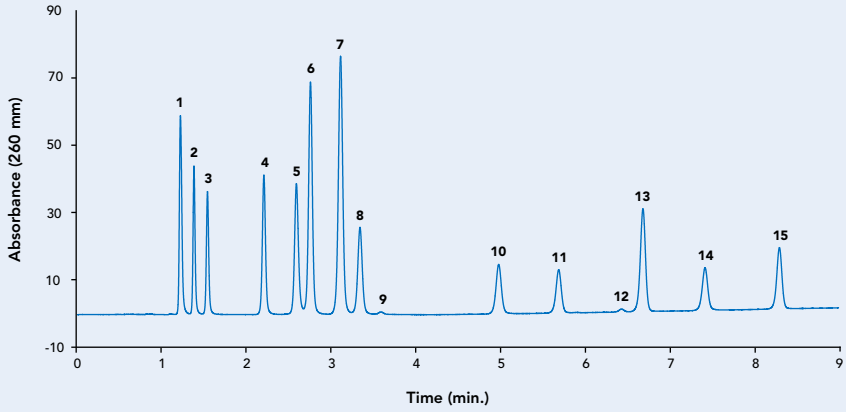


TEST CONDITIONS:
Column: 4.6 x 50 mm
Part Number: 2.7 µm HALO C18: 92814-402
Mobile Phase A: 90/10 ACN/0.1 M Ammonium Formate
Flow Rate: 1.8 mL/min.
pH: 3.0

TEST CONDITIONS:
Column: 4.6 x 50 mm
Part Number: 2.7 µm HALO HILIC: 92814-401
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 P. These 15 nucleosides and nucleobases are separated in under 10 minutes using a HALO 2.7 Penta-HILIC column.



TEST CONDITIONS:

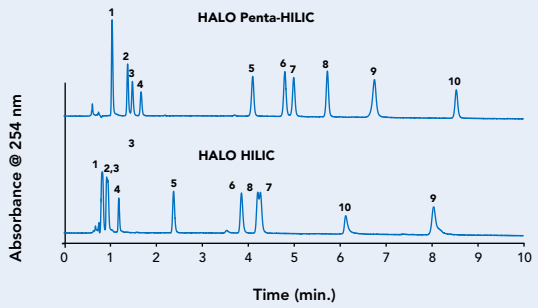
Column: 4.6 x 100 mm, HALO Penta-HILIC
Part Number: 92814-605
Mobile Phase A: 10 mM Ammonium formate in 85% Acetonitrile/15% water
Mobile Phase B: 10mM Ammonium Formate in 93% Acetonitrile/7% water
Gradient: Time %B
0-3 min. 94
3-9 min. 94-0
Flow Rate: 1.5 mL/min.
Temperature: 35 °C
Detection: UV 260 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

CEPHALOSPORINS ON HALO PENTA-HILIC AND HALO HILIC

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



TEST CONDITIONS:

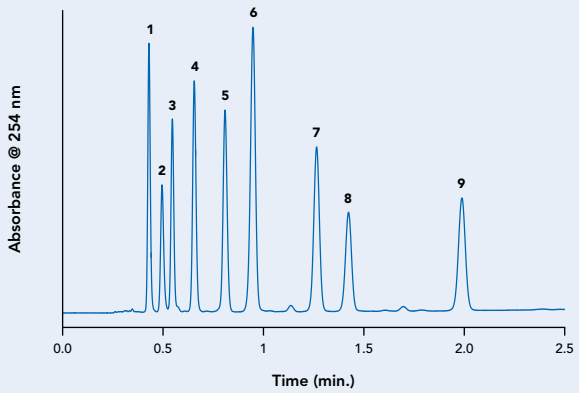
Column: 2.1 x 150 mm
Part Number: HALO Penta-HILIC: 92812-705
HALO HILIC: 92812-701
Mobile Phase: A= 95/5 ACN/$H_2$0 with 5 mM NH_4formate, pH=3.0 (adj.)
Mobile Phase: B= 50/50 ACN/$H_2$0 with 5 mM NH_4formate, pH=3.0 (adj.)
Gradient: HALO Penta-HILIC: 85-65% B in 10 min.
HALO HILIC: 85-70% B in 10 min.
Flow Rate: 0.5 mL/min.
Pressure: 195 bar with HALO Penta-HILIC
163 bar with HALO 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. Cephalothin
2. Cefoxitin
3. Cefotaxime
4. Cefazolin
5. Cefaclor
6. Cephalexin
7. Cephradine
8. Cefadroxil
9. Ceftazidime
10. Cephalosporin C

REVERSED-PHASE SEPARATION OF CEPHALOSPORINS USING HALO ES-CN

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



TEST CONDITIONS:

Column: 4.6 x 100 mm 2.7 µm HALO ES-CN
Part Number: 92814-404
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
ECV: about 14 µL
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

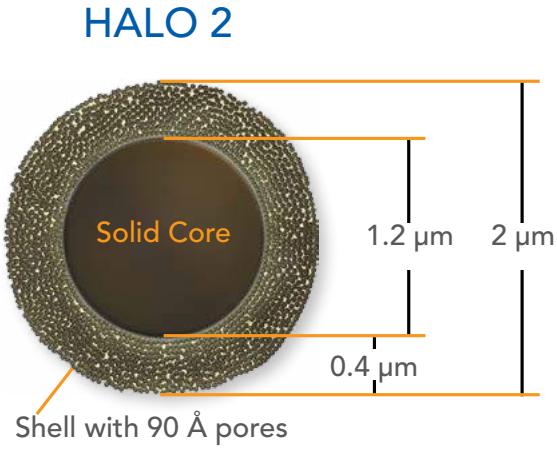
HALO® 2 COLUMNS

HALO 2 (UHPLC)

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

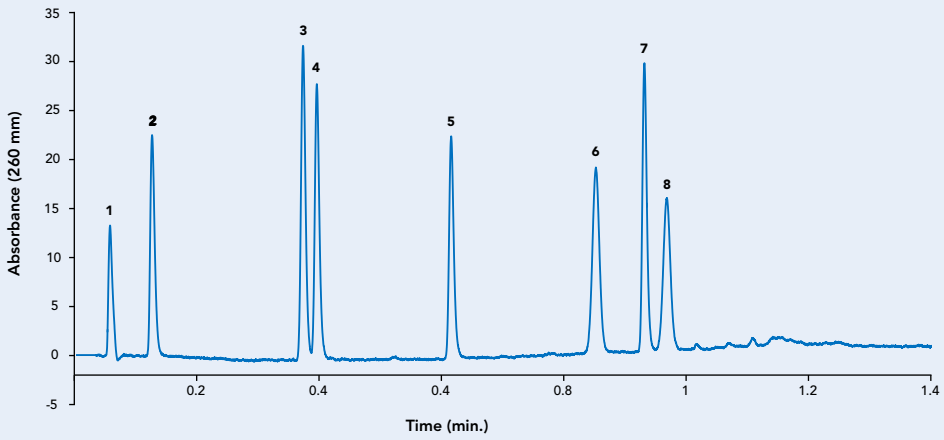
- Use when the highest efficiency is needed
- Excellent for fast method development and column/condition screening
- Best performance obtained with instrumentation having extracolumn volume (IBW< 10 µL)
- Ruggedness for R&D
- 1 µm inlet frit
- Pressure limit, 1000 bar/14,500 psi

Note: IBW is instrumental band width and is a measure of extracolumn dispersion.



ULTRA-FAST SEPARATION OF ANTICOAGULANTS USING HALO 2 C18

Figure S. This separation of anticoagulants is completed in one minute using a short 2.1 x 30 mm HALO 2 C18 column using a Shimadzu Nexera UHPLC system.



TEST CONDITIONS:

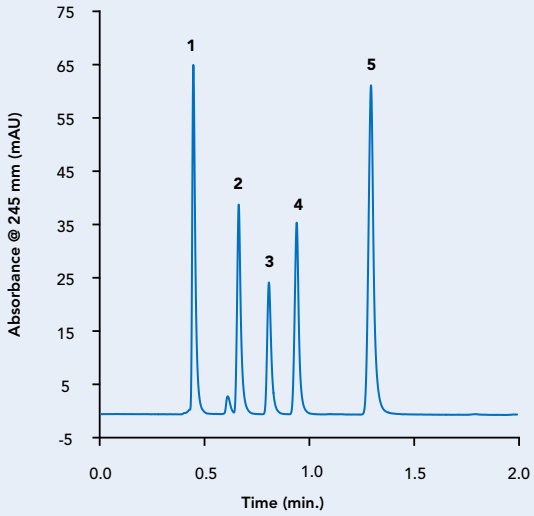
Column: 2.1 x 30 mm, HALO 2 C18
Part Number: 91812-302
Mobile Phase A: 20 mM Formic acid
Mobile Phase B: 50/50 Acetonitrile/Methanol
Gradient: Time %B
0-0.06 20
0.06-1.06 20-75
Flow Rate: 1.1 mL/min.
Temperature: 45 °C
Detection: 254 nm
Injection Volume: 0.2 µL
Maximum Pressure: 430 bar

PEAK IDENTITIES:

1. Uracil (t_o)
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 PENTA-HILIC

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



TEST CONDITIONS:

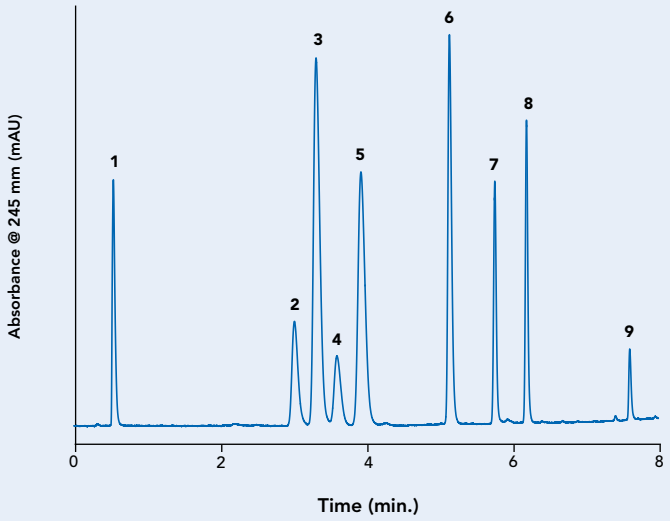
Column: 2.1 x 100 mm, HALO 2 Penta-HILIC
Part Number: 91812-605
Isocratic: 92/8: ACN/water with 5mM Ammonium Formate buffer, pH 3
Flow Rate: 0.5 mL/min.
Temperature: 30 °C
Detection: UV 245 nm, photodiode array detector
Injection Volume: 1.0 µL
Sample Solvent: 90/10 ACN/0.1 M ammonium formate buffer 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 PFP

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



TEST CONDITIONS:

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

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

Flow Rate: 0.4 mL/min.
Temperature: 35 °C
Pressure: 180 bar initial
Detection: UV 280 nm, VWD
Injection volume: 2 µL
Sample Solvent: methanol
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
ECV: about 14 µL
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 part numbers.

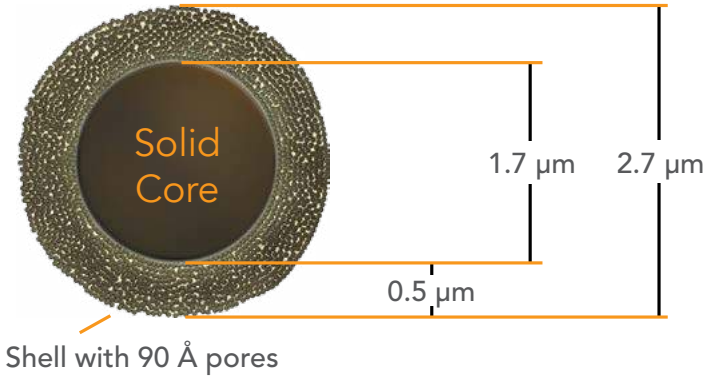
HALO® COLUMNS 2.7

HALO 2.7 (UHPLC AND HPLC)

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

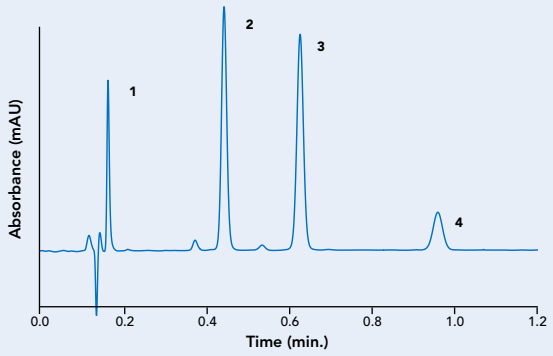
- Use for high speed or high resolution with UHPLC or HPLC applications
- Excellent for R&D and routine analyses
- 2 µm inlet frit
- Pressure limit, 600 bar/9000 psi

HALO 2.7



ULTRAFAST SEPARATION OF STATIN DRUGS

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

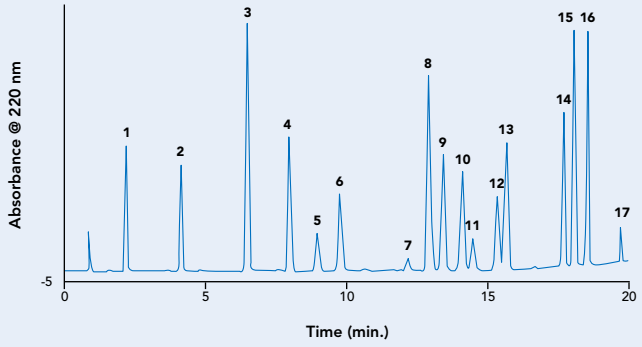


TEST CONDITIONS:
Column: 4.6 x 50 mm, HALO 2.7 Phenyl-Hexyl
Part Number: 92814-406
Mobile Phase: 43/57: A/B
A: 0.02 M formic acid in water
B: Acetonitrile
Flow Rate: 2.5 mL/min.
Pressure: 228 Bar
Temperature: 26 °C
Detection: UV 240 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 80/20 methanol/water (20 mM formic acid)
Response Time: 0.02 sec.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: ~14 µL

PEAK IDENTITIES:
1. Pravastatin
2. Atorvastatin
3. Mevastatin
4. Simvastatin

HIGH RESOLUTION SEPARATION OF EXPLOSIVES

Figure W. In this example, a 4.6 x 150 mm HALO 2.7 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 modelingand simulation using DryLab® software.



TEST CONDITIONS:
Column: 4.6 x 150 mm, HALO C18, 2.7 µm
Part Number: 92814-702
Mobile Phase A: Water
Mobile Phase B: Methanol
Gradient:

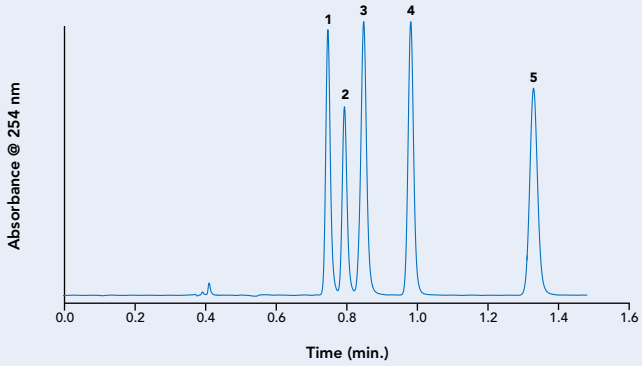
Time	%B
0.0	25
14.0	35
20.0	62

Flow Rate: 1.5 mL/min.
Temperature: 43 °C
Detection: UV 220 nm, 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
ECV: about 14 µL

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)

ULTRAFAST SEPARATION OF TRICYCLIC ANTIDEPRESSANTS

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

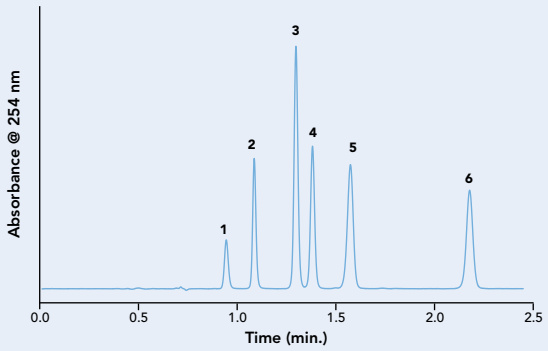


TEST CONDITIONS:
Column: 4.6 x 100 mm, HALO Penta HILIC
Part Number: 92814-605
Mobile Phase: 7/93: A/B
A: 0.1 M Ammonium formate, pH=3.5 (adj.)
B: Acetonitrile
Flow Rate: 2.5 mL/min.
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 0.5 µL
Sample Solvent: 10/90: Water/acetonitrile
Response Time: 0.02 sec.
Maximum Pressure: 165 Bar
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR
ECV: about 14 µL

PEAK IDENTITIES:
1. Trimipramine
2. Amitriptyline
3. Doxepin
4. Nortriptyline
5. Amoxapine

HIGH RESOLUTION OF NEONICOTINOIDS ON HALO 2.7 ES-CN

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



TEST CONDITIONS:
Column: 4.6 x 100 mm, HALO ES-CN
Part Number: 92814-604
Mobile Phase: 70/30: A/B
A: 0.1% Formic acid in water
B: Acetonitrile
Flow Rate: 1.5 mL/min.
Pressure: 205 Bar
Temperature: 35 °C
Detection: UV 254 nm, 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
ECV: ~14 µL

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

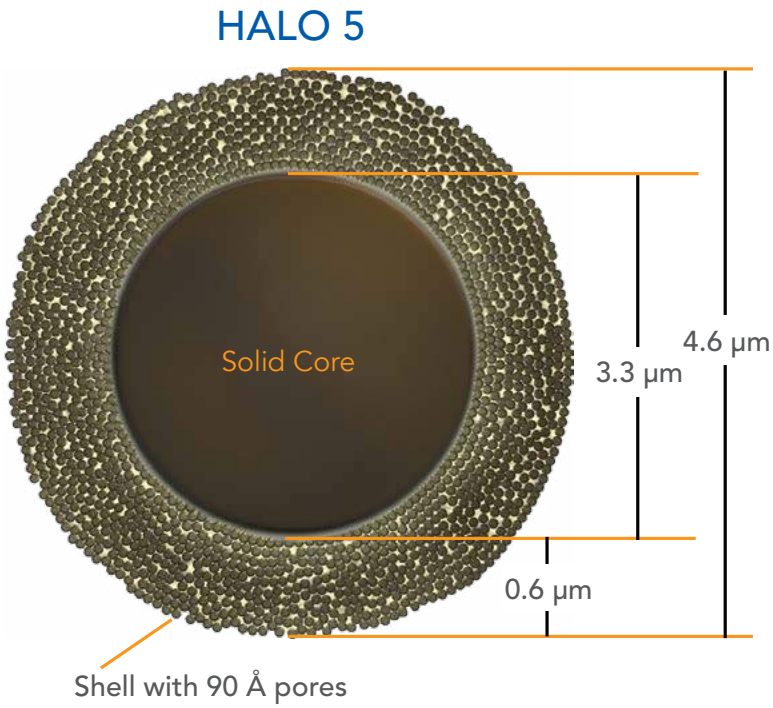
See page 33 for full list of HALO 2.7 part numbers.

HALO 5 (HPLC)

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

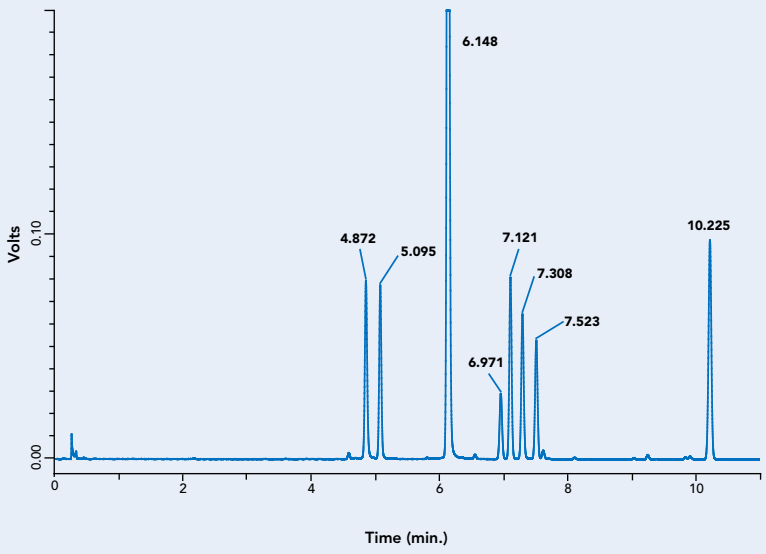
Ideal for:

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



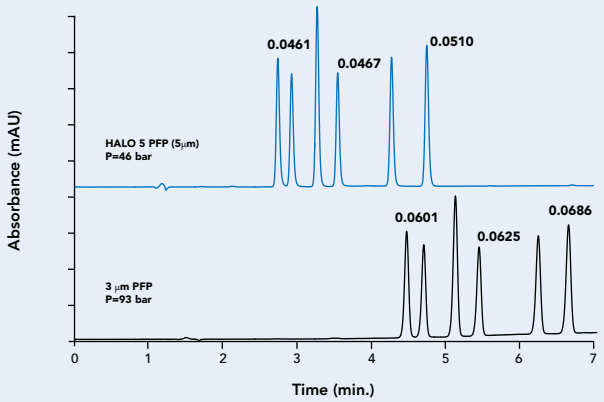
SAMPLE:
Mixture of 8 flavonoids, 1 µL in MeOH

TEST CONDITIONS:
Column: 2.1 x 150 mm, HALO 5 C18
Part Number: 95812-702
Flow Rate: 1.0 mL/min.
Temperature: 40 °C
Gradient: 5% CH_3CN for 0.5 min.
5-60% CH_3CN/10 mM NH_4COO (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 PFP

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

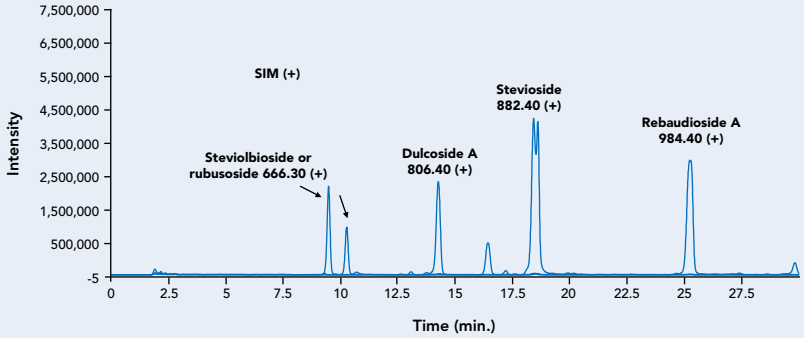
PEAK IDENTITIES:
1. Oxazepam
2. Lorazepam
3. Nitrazepam
4. Clonazeparm
5. Flunitrazepam
6. Diazepam

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

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

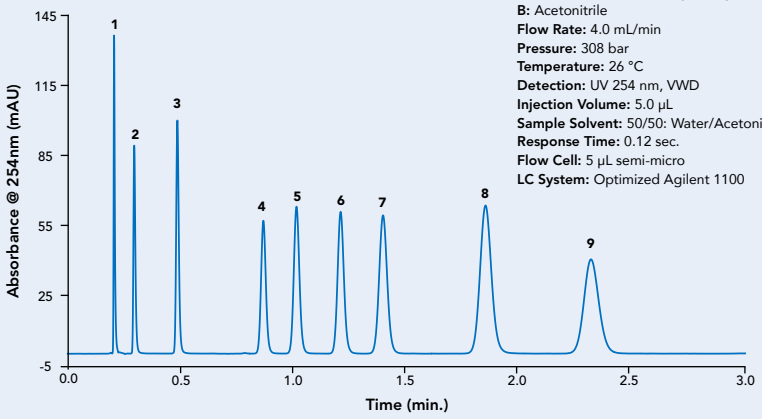
Figure BB.
LC-MS analysis of stevia glycosides from a Stevia natural sweetener extract is easily accomplished using the HALO 5 Penta-HILIC column due to its unique bonded phase containing five OH groups.

TEST CONDITIONS:
Column: 3.0 x 250 mm, HALO 5 Penta-HILIC
Part Number: 95813-905
Mobile Phase A: 50/50 Water/acetonitrile with 5 mM Ammonium formate, pH 3
Mobile Phase B: 5/95 Water/acetonitrile with 5 mM Ammonium formate, pH 3
Gradient: 90% B to 67% B over 30 min.
Flow Rate: 0.5 mL/min.
Pressure: 60 bar
Temperature: Ambient
Injection Volume: 5 µL
Sample Solvent: 80/20: Acetonitrile/water
LC System: Shimadzu Nexera
MS: Shimadzu LCMS 2020 (single quadrupole)
ESI: +4.5 kV
Scan Range: 200-1200 m/z
Scan Rate: 2 pps
Capillary: 250 °C
Heat Block: 350 °C
Nebulizing Gas Flow: 1.5 L/min.
Drying Gas Flow: 15 L/min.



POLAR AROMATIC COMPOUNDS ON HALO 5 RP-AMIDE

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



TEST CONDITIONS:
Column: 4.6 x 100 mm, HALO-5 RP-Amide
Part Number: 95814-607
Mobile Phase: 70/30: A/B
A: 20 mM Potassium Phosphate, pH 7
B: Acetonitrile
Flow Rate: 4.0 mL/min
Pressure: 308 bar
Temperature: 26 °C
Detection: UV 254 nm, VWD
Injection Volume: 5.0 µL
Sample Solvent: 50/50: Water/Acetonitrile
Response Time: 0.12 sec.
Flow Cell: 5 µL semi-micro
LC System: Optimized Agilent 1100

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

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

HALO PROTEIN, PEPTIDE AND GLYCAN

Today, researchers are keenly interested in both fast and high resolution separations of numerous biomolecules to support the development of novel therapeutic proteins and peptides in pharmaceutical drug development, to advance understanding in modern university laboratories, to characterize protein post-translation modifications, and to fully assess subtle differences in biosimilars and other products of bioengineering and manufacture. HALO BioClass columns have been developed to make such tasks easier and more effective, and are aimed at the following areas of bioseparations:

- 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

Currently, the HALO BioClass column family is comprised of the following products. Their features and benefits are described below, and their specifications are shown in Table F.

HALO Protein C4

- 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

HALO Protein ES-C18

- Extremely stable 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

HALO Peptide ES-C18

- Fast, high resolution separations
- Extremely stable up to 90˚C
- High peak capacity
- Rugged, reliable performance
- Use with either UHPLC or HPLC

HALO Peptide ES-CN

- Same benefits as Peptide ES-C18
- Alternative selectivity to Peptide ES-C18 for peptide mapping and proteomic applications

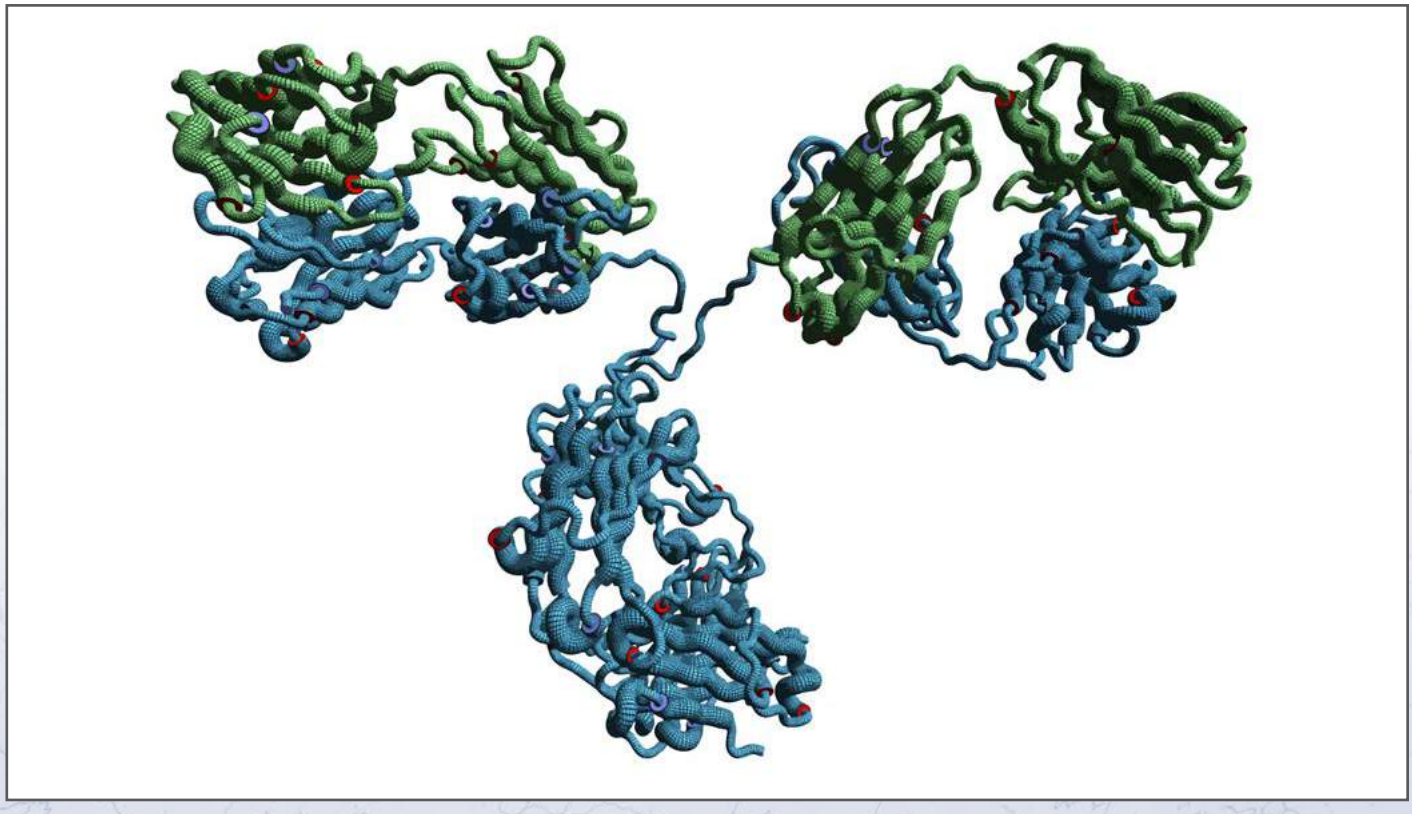
HALO Glycan

- Improved retention of acidic and zwitterionic analytes
- Very low sensitivity to buffer concentration
- Able to separate isobaric oligosaccharides with different linkages

Table F. HALO BioClass Column Specifications

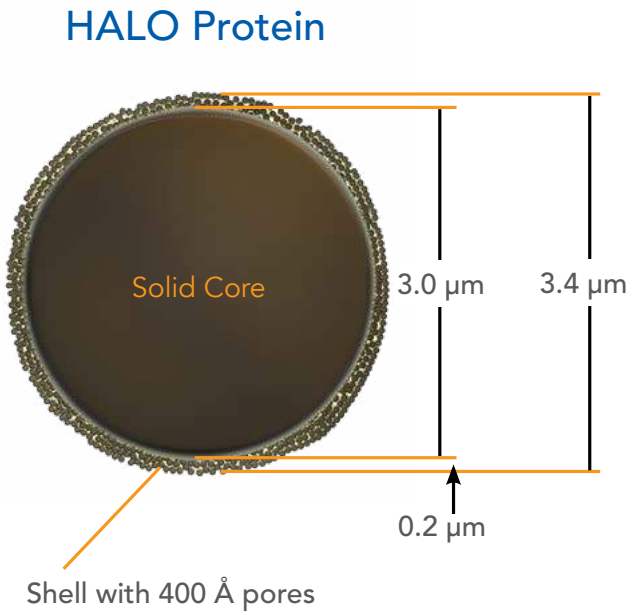
	Particle Size(s) (µm)	Pore Size (Å)	Carbon Load (%)	Surface Area (m^2/g)	Low pH Limit	High pH Limit	Max. Temp. Lower pH Limit	Max. Temp. Upper pH Limit	Endcapped
HALO Protein C4	3.4	400	0.4	15	2	9	90	40	Yes
HALO Protein ES-C18	3.4	400	1.0	15	1	8	90	40	Yes
HALO Peptide ES-C18	2.7 5	160	4.6 4.0	90 60	1	8	90	40	No
HALO Peptide ES-CN	2.7 5	160	2.2 1.5	90 60	1	8	90	40	Yes
HALO Glycan	2.7	90	3.2	135	2	9	65	40	No

Structure of an antibody showing its heavy chain in blue and its light chain in green.



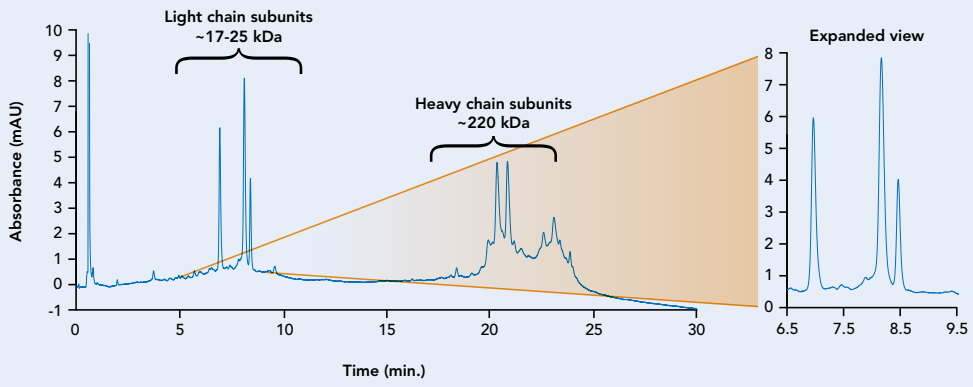
HALO PROTEIN

- 400 Ångstrom pores to provide unrestricted pore access for polypeptides and proteins as large as 500 kDa
- 3.4 µm Fused-Core particles with a very thin 0.2 µm outer porous shell
  - 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 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



LARGE PROTEIN SEPARATION USING HALO PROTEIN C4 FUSED-CORE COLUMN

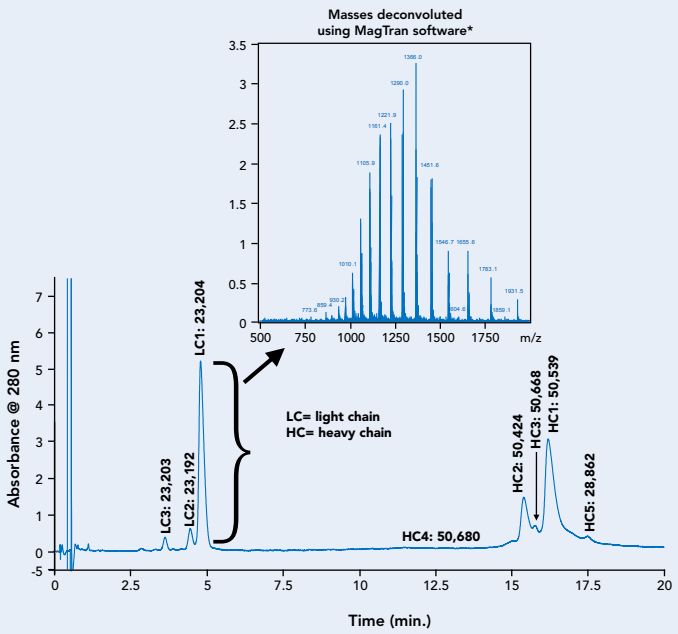
Figure DD. High resolution separation of light and heavy chains of a denatured contractile protein (whole myosin from purified rabbit skeletal muscle) using HALO Protein C4 at 80 ˚C.



TEST CONDITIONS:
Column: 2.1 x 100 mm, HALO 3.4 µm Protein C4
Part Number: 93412-614
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

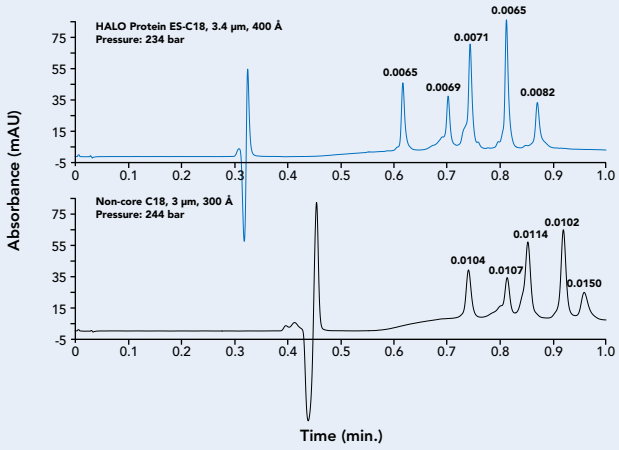
Figure EE. Very high resolution is obtained between variants of light and heavy chains of a reduced and alkylated monoclonal antibody (IgG1) sample using a HALO Protein C4 column.



TEST CONDITIONS:
Column: 2.1 x 100 mm, 3.4 µm HALO Protein C4
Part Number: 93412-614
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)

ULTRAFAST PROTEIN SEPARATION USING HALO PROTEIN ES-C18

Figure FF. An ultrafast protein separation is achieved on a HALO Protein ES-C18 400 Ångstrom column in less than 1 minute.



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

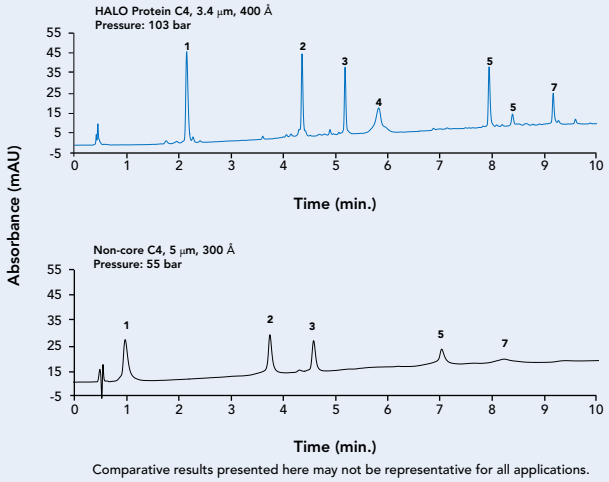
TEST CONDITIONS:
Column: 4.6 x 100 mm HALO Protein ES-C18
Part Number: 93414-602
Instrument: Agilent 1200 SL
Injection Volume: 5 µL
Detection: 215 nm
Temperature: 60 °C
Flow Rate: 3 mL/min.
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: 23–85% B in 1 min.
Using 3 and 6 µL heat exchangers in column compartment

PEAK IDENTITIES
1. Ribonuclease A
2. Cytochrome c
3. Lysozyme
4. α-Lactalbumin
5. Catalase

NOTE:
Peak widths at half height are shown above respective peaks.

HALO PROTEIN C4 PROVIDES NARROWER AND TALLER PEAKS THAN TOTALLY POROUS COLUMN

Figure GG. HALO Protein C4 dramatically outperforms a conventional non-core C4 column,delivering narrower and taller peaks with improved recovery of holotransferrin, apomyoglobin, catalase and enolase.



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

TEST CONDITIONS:
Column: 2.1 x 100 mm HALO Protein C4
Part Number: 93414-614
Instrument: Agilent 1200 SL
Injection Volume: 1 µL
Detection: 215 nm
Temperature: 60 °C
Flow Rate: 0.5 mL/min.
Mobile Phase A: water/0.1% TFA
Mobile Phase B: acetonitrile/0.1% TFA
Gradient: 25–52% B in 10 min.

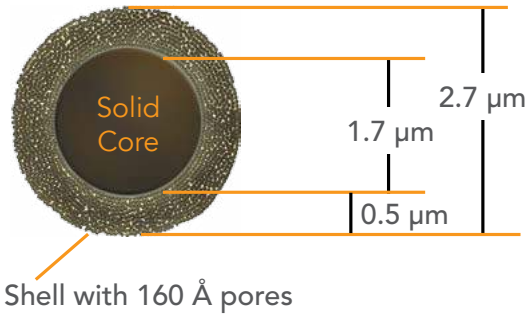
PEAK IDENTITIES
1. Ribonuclease A
2. Cytochrome c
3. Lysozyme
4. Holotransferrin
5. Apomyoglobin
6. Catalase
7. Enolase

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

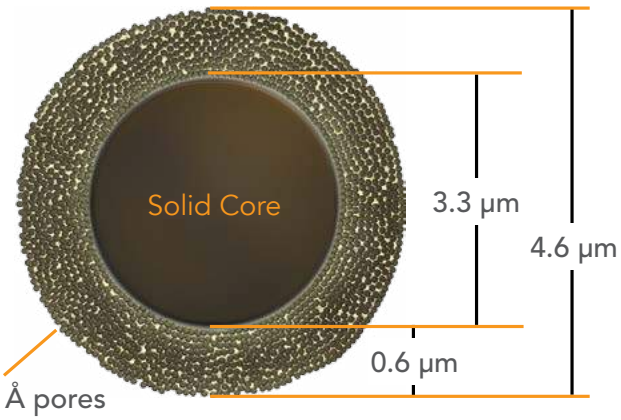
HALO® PEPTIDE

- HALO 160 Ångstrom columns currently available in two particle sizes (2.7 and 5 µm) and in two sterically-protected phases: ES-C18 and ES-CN (Table F)
- Ideal for both ultrafast and ultrahigh resolution separations of peptides and polypeptides up to 20 kDa
- Outperforms non-core 3 µm, 300 Ångstrom columns in terms of peak width, peak capacity and peak height (Figure HH)
- Offers comparable peak capacity to sub-2 µm non-core columns at 40–50% back pressure
- 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 II)
- HALO Peptide ES-CN offers different selectivity and improved retention for polar peptides (Figure JJ)
- 2 µm inlet frit
- Pressure limit, 600 bar/9000 psi

HALO 2.7 Peptide

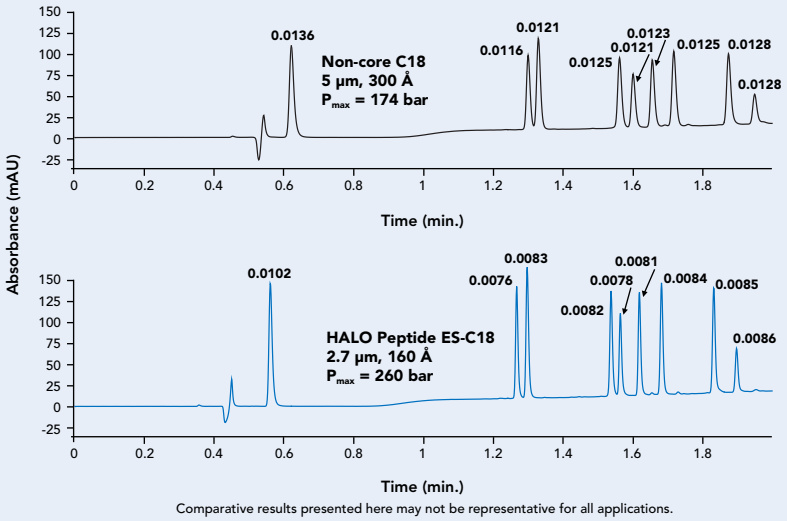


HALO 5 Peptide



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

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

PEAK IDENTITIES:

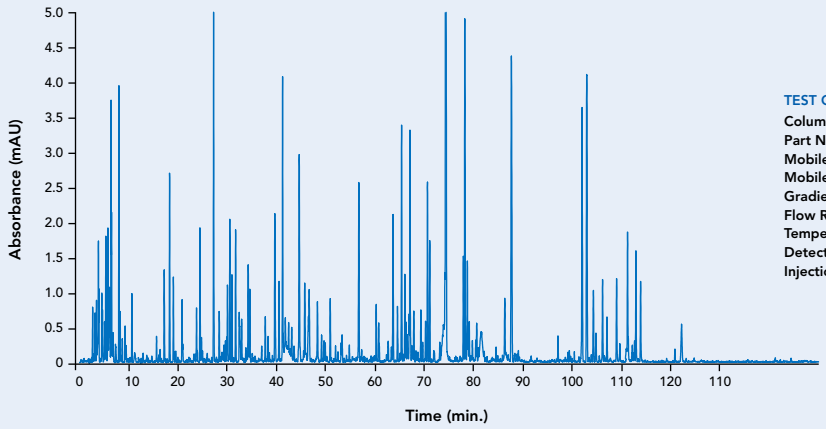
1. Gly-Tyr
2. Angiotensin 1/2 (1-7) amide
3. Val-Tyr-Val
4. Met-Enk
5. Angiotensin 1/2 (1-8) amide
6. Angiotensin II
7. Leu-Enk
8. Angiotensin (1-12) human
9. Angiotensin (1-12) mouse

NOTE:

Peak widths at half height are shown above respective peaks.

COUPLED HALO PEPTIDE COLUMNS FOR MAXIMUM PEAK CAPACITY

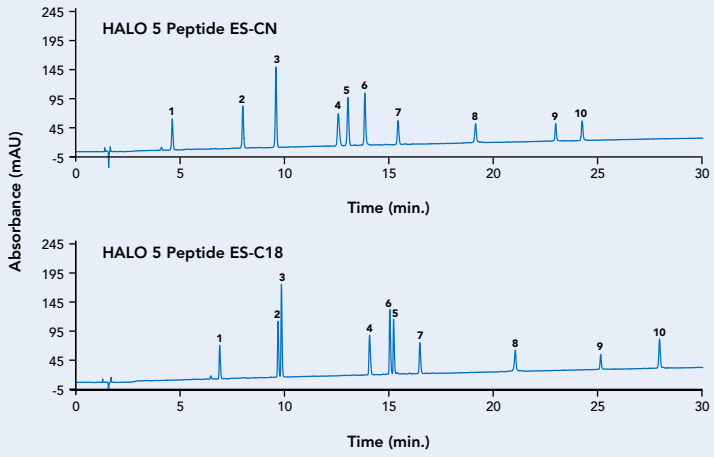
Figure II. Three HALO Peptide ES-C18 150-mm columns (450 mm total length) were connected in series to achieve a peak capacity of 560 for this mixture of tryptic digests of α-1-glycoprotein and apotransferrin.



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

ALTERNATE SELECTIVITY USING HALO 5 PEPTIDE ES-CN

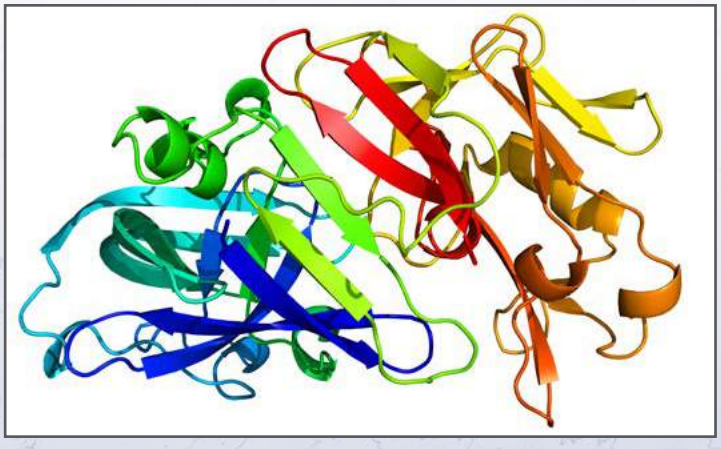
Figure JJ. HALO 5 Peptide ES-CN offers alternative selectivity to HALO 5 ES-C18 for this mixture of 10 peptides and polypeptides.



TEST CONDITIONS:
Column: 4.6 x 150 mm
Part Numbers: HALO 5 Peptide ES-CN: 95124-704
HALO 5 Peptide ES-C18: 95124-702
Instrument: Optimized Agilent 1100
Injection Volume: 10 µL
Detection: 215 nm
Temperature: 40 °C
Flow Rate: 1.0 mL/min
Mobile Phase A: water/0.1% TFA
Mobile Phase B: ACN/0.1% TFA
Gradient: 5–50% B in 30 min.

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

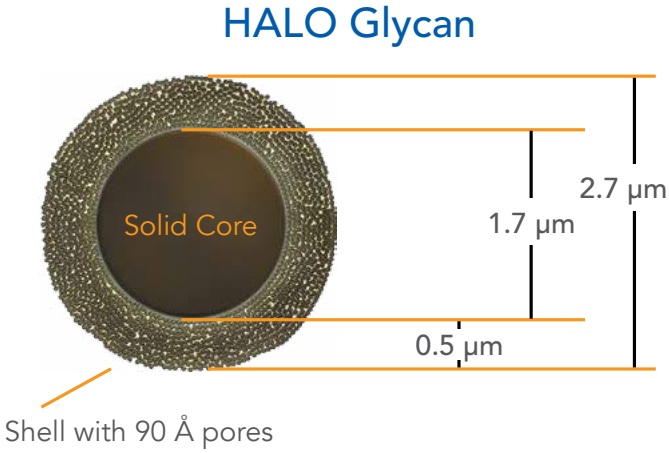
Graphical representation of a polypeptide structure having beta-pleated sheet and alpha-helical regions



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

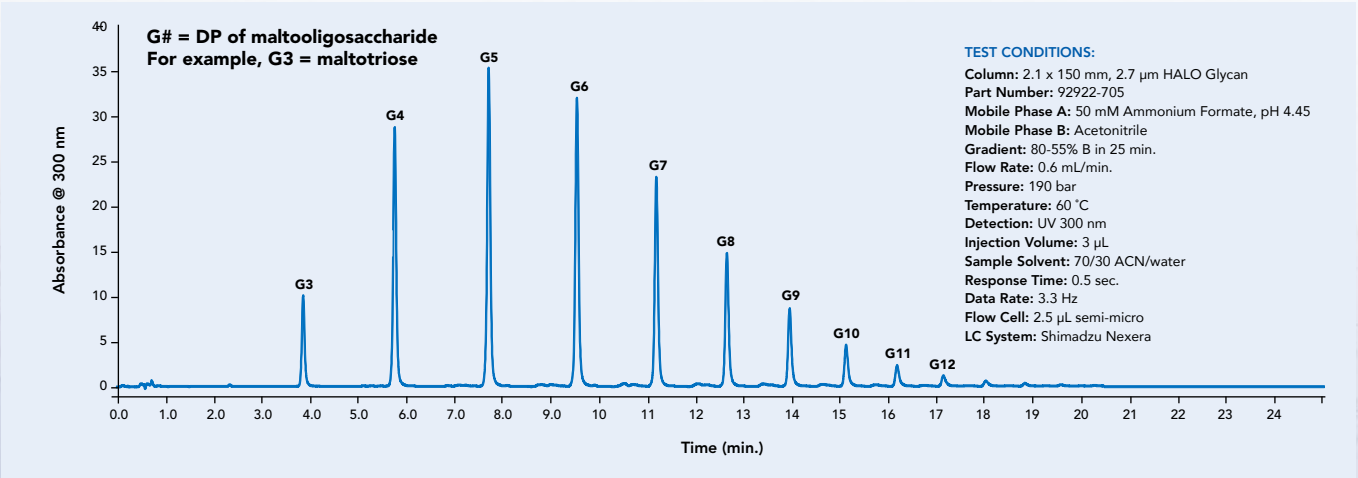
HALO® GLYCAN

- 90 Ångstrom pore size
- Incorporates a highly polar ligand that contains 5 hydroxyl groups tethered to 2.7 µm Fused-Core silica particles via novel, proprietary linkage chemistry (Table F)
- 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 KK) by separation of a procainamide-reducing-end-labeled glycan ladder of oligosaccharides having 2–25 glucose units (GU).
  - Peaks for oligosaccharides composed of 5 and 10 GU must meet tight specifications for retention and peak width before lot is approved for glycan analysis
- 2 µm inlet frit
- Pressure limit, 600 bar/9000 psi



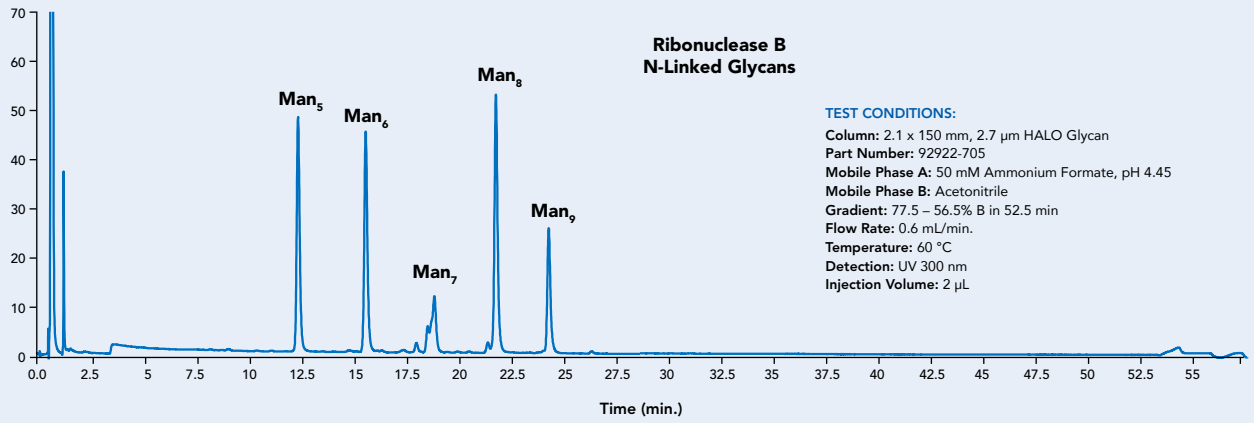
QA ANALYSIS OF HALO GLYCAN

Figure KK. 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

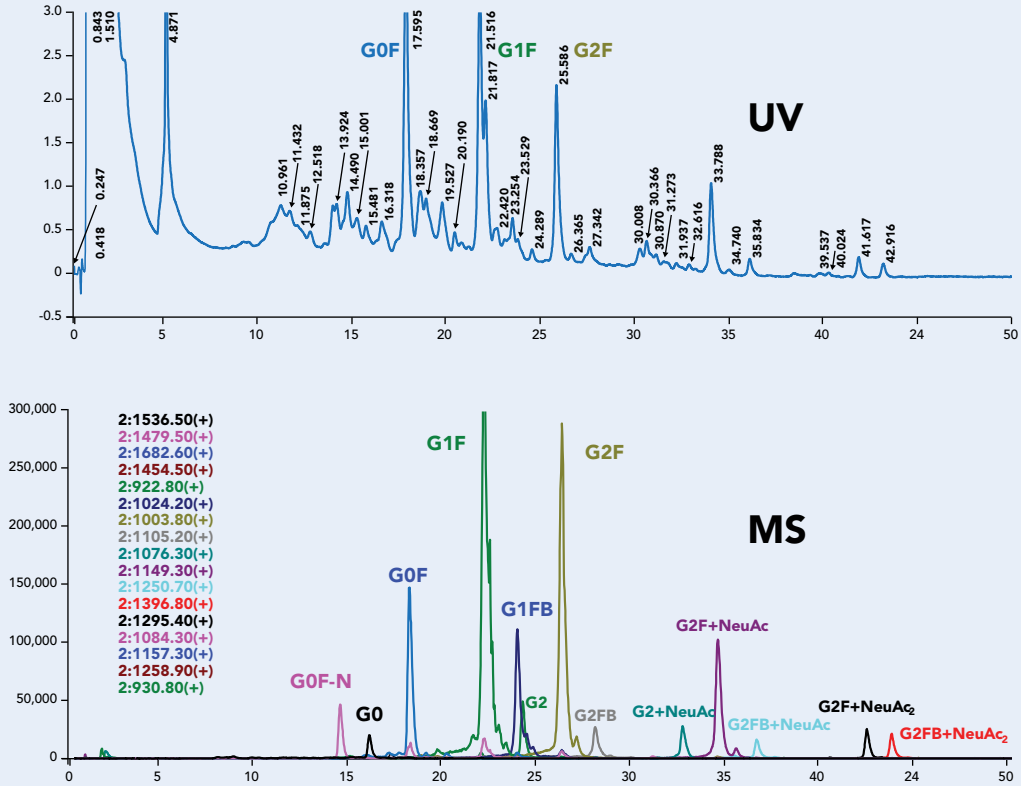
Figure LL. Gradient HILIC-MS separation of N-linked glycans, which had been released using PNGase from ribonuclease B, using the HALO Glycan column.



TEST CONDITIONS:
Column: 2.1 x 150 mm, 2.7 µm HALO Glycan
Part Number: 92922-705
Mobile Phase A: 50 mM Ammonium Formate, pH 4.45
Mobile Phase B: Acetonitrile
Gradient: 77.5 – 56.5% B in 52.5 min
Flow Rate: 0.6 mL/min.
Temperature: 60 °C
Detection: UV 300 nm
Injection Volume: 2 µL

SEPARATION OF N-LINKED GLYCANS FROM HUMAN IgG

Figure MM. 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.



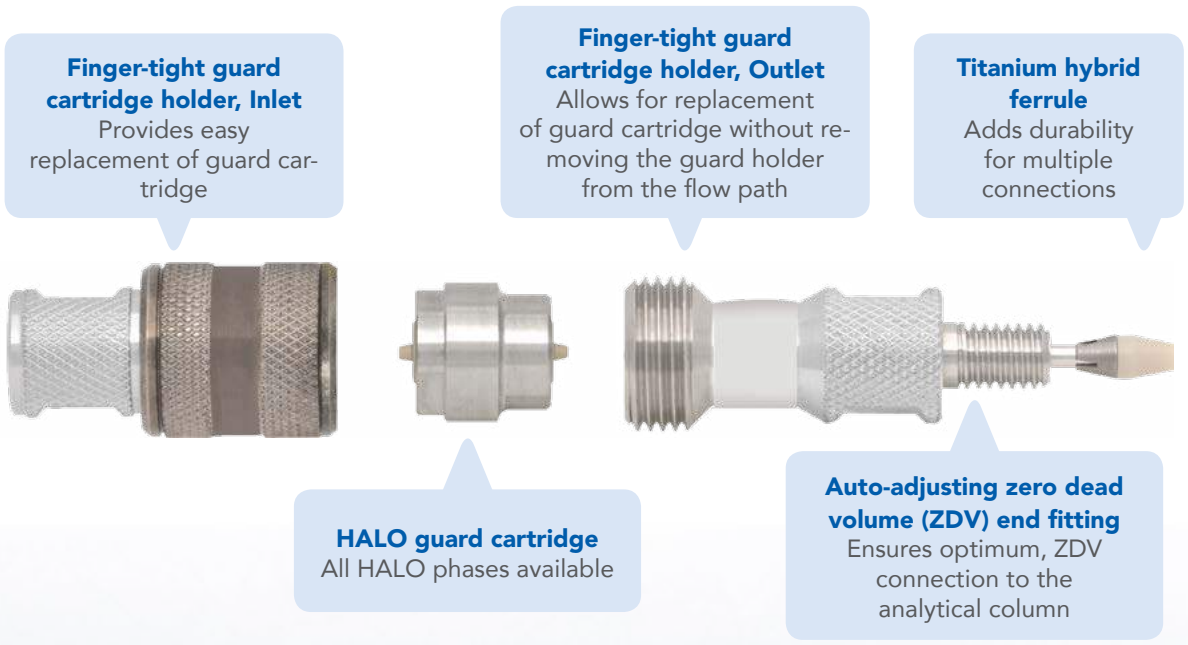
TEST CONDITIONS:
Column: 2.1 x 150 mm, HALO 2.7 µm Glycan
Part Number: 92922-705
Mobile Phase A: 50 mM Ammonium Formate, pH 4.4
Mobile Phase B: Acetonitrile
Gradient: 77.5 – 60% B in 60 min
Flow Rate: 0.5 mL/min.
Temperature: 60 °C
Detection: UV 300 nm
Injection Volume: 4 µL
LC System: Shimadzu Nexera
MS: Shimadzu LCMS 2020 (single quadrupole)
ESI: +4.7 kV
Scan range: 500-2000 m/z
Scan rate: 2 pps

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

HALO UHPLC AND HPLC GUARD COLUMNS

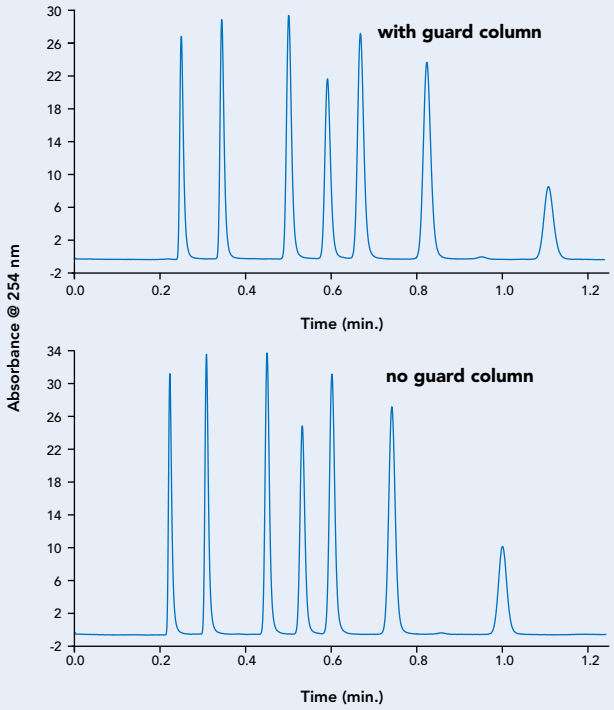
- Collect strongly retained compounds from the sample and minimizes column fouling
- Ultra-low dispersion, easy to use, operate at pressures up to 1000 bar
- Finger-tight, direct-connect units that auto-adjust to any column with a 10–32 inlet port
- Easily replace guard cartridge without removing guard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

See figure 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 NN.
HALO guard columns provide optimum protection for your HALO HPLC and UHPLCcolumn without sacrificing column efficiency.



TEST CONDITIONS:

Column: 4.6 x 50 mm 2.7 µm HALO C18
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

HALO 2 COLUMNS

The part numbers for HALO 2 columns are presented below. HALO 2 columns are available in the following analytical internal diameters (2.1 and 3.0 mm). Guard columns are also available for HALO 2 columns for these IDs for UHPLC to provide additional protection when necessary.

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

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HALO 2.7 COLUMNS

HALO 2.7 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.

2.7 µm, 90 Å								
Dimensions (in mm)	C18	C8	Phenyl Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
0.075 x 50	98219-402	98219-408	98219-406	98219-407	98219-409	98219-404	98219-405	98219-401
0.075 x 100	98219-602	98219-608	98219-606	98219-607	98219-609	98219-604	98219-605	98219-601
0.075 x 150	98219-702	98219-708	98219-706	98219-707	98219-709	98219-704	98219-705	98219-701
0.1 x 50	98218-402	98218-408	98218-406	98218-407	98218-409	98218-404	98218-405	98218-401
0.1 x 100	98218-602	98218-608	98218-606	98218-607	98218-609	98218-604	98218-605	98218-601
0.1 x 150	98218-702	98218-708	98218-706	98218-707	98218-709	98218-704	98218-705	98218-701
0.2 x 50	98217-402	98217-408	98217-406	98217-407	98217-409	98217-404	98217-405	98217-401
0.2 x 100	98217-602	98217-608	98217-606	98217-607	98217-609	98217-604	98217-605	98217-601
0.2 x 150	98217-702	98217-708	98217-706	98217-707	98217-709	98217-704	98217-705	98217-701
0.3 x 50	98216-402	98216-408	98216-406	98216-407	98216-409	98216-404	98216-405	98216-401
0.3 x 100	98216-602	98216-608	98216-606	98216-607	98216-609	98216-604	98216-605	98216-601
0.3 x 150	98216-702	98216-708	98216-706	98216-707	98216-709	98216-704	98216-705	98216-701
0.5 x 50	98215-402	98215-408	98215-406	98215-407	98215-409	98215-404	98215-405	98215-401
0.5 x 100	98215-602	98215-608	98215-606	98215-607	98215-609	98215-604	98215-605	98215-601
0.5 x 150	98215-702	98215-708	98215-706	98215-707	98215-709	98215-704	98215-705	98215-701
1.0 x 30	92811-302	92811-308	92811-306	92811-307	92811-309	92811-304	92811-305	92811-301
1.0 x 50	92811-402	92811-408	92811-406	92811-407	92811-409	92811-404	92811-405	92811-401
1.0 x 75	92811-502	92811-508	92811-506	92811-507	92811-509	92811-504	92811-505	92811-501
1.0 x 100	92811-602	92811-608	92811-606	92811-607	92811-609	92811-604	92811-605	92811-601
1.0 x 150	92811-702	92811-708	92811-706	92811-707	92811-709	92811-704	92811-705	92811-701
2.1 x 20	92812-202	92812-208	92812-206	92812-207	92812-209	92812-204	92812-205	92812-201
2.1 x 30	92812-302	92812-308	92812-306	92812-307	92812-309	92812-304	92812-305	92812-301
2.1 x 50	92812-402	92812-408	92812-406	92812-407	92812-409	92812-404	92812-405	92812-401
2.1 x 75	92812-502	92812-508	92812-506	92812-507	92812-509	92812-504	92812-505	92812-501
2.1 x 100	92812-602	92812-608	92812-606	92812-607	92812-609	92812-604	92812-605	92812-601
2.1 x 150	92812-702	92812-708	92812-706	92812-707	92812-709	92812-704	92812-705	92812-701
2.1 x 250	92812-902	92812-908	92812-906	92812-907	92812-909	92812-904	92812-905	92812-901
3.0 x 20	92813-202	92813-208	92813-206	92813-207	92813-209	92813-204	92813-205	92813-201
3.0 x 30	92813-302	92813-308	92813-306	92813-307	92813-309	92813-304	92813-305	92813-301
3.0 x 50	92813-402	92813-408	92813-406	92813-407	92813-409	92813-404	92813-405	92813-401
3.0 x 75	92813-502	92813-508	92813-506	92813-507	92813-509	92813-504	92813-505	92813-501
3.0 x 100	92813-602	92813-608	92813-606	92813-607	92813-609	92813-604	92813-605	92813-601
3.0 x 150	92813-702	92813-708	92813-706	92813-707	92813-709	92813-704	92813-705	92813-701
3.0 x 250	92813-902	92813-908	92813-906	92813-907	92813-909	92813-904	92813-905	92813-901
4.6 x 20	92814-202	92814-208	92814-206	92814-207	92814-209	92814-204	92814-205	92814-201
4.6 x 30	92814-302	92814-308	92814-306	92814-307	92814-309	92814-304	92814-305	92814-301
4.6 x 50	92814-402	92814-408	92814-406	92814-407	92814-409	92814-404	92814-405	92814-401
4.6 x 75	92814-502	92814-508	92814-506	92814-507	92814-509	92814-504	92814-505	92814-501
4.6 x 100	92814-602	92814-608	92814-606	92814-607	92814-609	92814-604	92814-605	92814-601
4.6 x 150	92814-702	92814-708	92814-706	92814-707	92814-709	92814-704	92814-705	92814-701
4.6 x 250	92814-902	92814-908	92814-906	92814-907	92814-909	92814-904	92814-905	92814-901
10.0 x 50	92810-402	92810-408	92810-406	92810-407	92810-409	92810-404	92810-405	92810-401
10.0 x 75	92810-502	92810-508	92810-506	92810-507	92810-509	92810-504	92810-505	92810-501
10.0 x 100	92810-602	92810-608	92810-606	92810-607	92810-609	92810-604	92810-605	92810-601
10.0 x 150	92810-702	92810-708	92810-706	92810-707	92810-709	92810-704	92810-705	92810-701
2.7 µm, 90 Å Guard columns, 3-pack								
Dimensions (in mm)	C18	C8	Phenyl Hexyl	RP-Amide	PFP	ES-CN	Penta-HILIC	HILIC
2.1 x 5	92812-102	92812-108	92812-106	92812-107	92812-109	92812-104	92812-105	92812-101
3.0 x 5	92813-102	92813-108	92813-106	92813-107	92813-109	92813-104	92813-105	92813-101
4.6 x 5	92814-102	92814-108	92814-106	92814-107	92814-109	92814-104	92814-105	92814-101
Guard Column Holder		94900-001						

HALO 5 COLUMNS

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

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

HALO PROTEIN COLUMNS

Part numbers for nano, capillary, analytical and semi-preparative HALO Protein columns in both C4 and ES-C18 phases are provided below. Guard columns are available for HALO Protein columns in 2.1, 3.0 and 4.6 mm IDs for UHPLC and HPLC applications to provide additional column protection when desired.

Dimensions (in mm)	HALO Protein C4	HALO Protein ES-C18
0.075 x 50	94319-414	94319-402
0.075 x 100	94319-614	94319-602
0.075 x 150	94319-714	94319-702
0.1 x 50	94318-414	94318-402
0.1 x 100	94318-614	94318-602
0.1 x 150	94318-714	94318-702
0.2 x 50	94317-414	94317-402
0.2 x 100	94317-614	94317-602
0.2 x 150	94317-714	94317-702
0.3 x 50	94316-414	94316-402
0.3 x 100	94316-614	94316-602
0.3 x 150	94316-714	94316-702
0.5 x 50	94315-414	94315-402
0.5 x 100	94315-614	94315-602
0.5 x 150	94315-714	94315-702
1.0 x 30	93411-314	93411-302
1.0 x 50	93411-414	93411-402
1.0 x 75	93411-514	93411-502
1.0 x 100	93411-614	93411-602
1.0 x 150	93411-714	93411-702
2.1 x 20	93412-214	93412-202
2.1 x 30	93412-314	93412-302
2.1 x 50	93412-414	93412-402
2.1 x 75	93412-514	93412-502
2.1 x 100	93412-614	93412-602
2.1 x 150	93412-714	93412-702
3.0 x 20	93413-214	93413-202
3.0 x 30	93413-314	93413-302
3.0 x 50	93413-414	93413-402
3.0 x 75	93413-514	93413-502
3.0 x 100	93413-614	93413-602
3.0 x 150	93413-714	93413-702
4.6 x 20	93414-214	93414-202
4.6 x 30	93414-314	93414-302
4.6 x 50	93414-414	93414-402
4.6 x 75	93414-514	93414-502
4.6 x 100	93414-614	93414-602
4.6 x 150	93414-714	93414-702
10.0 x 50	93410-414	93410-402
10.0 x 75	93410-514	93410-502
10.0 x 100	93410-614	93410-602
10.0 x 150	93410-714	93410-702
Guard Columns, 3-pack		
Dimensions (in mm)	HALO Protein C4	HALO Protein ES-C18
2.1 x 5	93412-114	93412-102
3.0 x 5	93413-114	93413-102
4.6 x 5	93414-114	93414-102
Guard Column Holder	94900-001	

HALO GLYCAN COLUMNS

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

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

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HALO PEPTIDE COLUMNS

The part numbers are provided below for the nano, capillary, analytical and semi-preparative HALO 2.7 and HALO 5 Peptide columns in both ES-C18 and ES-CN phases. Guard columns are available for 2.1, 3.0 and 4.6 mm internal diameters for UHPLC and HPLC applications, if additional protection is desired.

Dimensions ID x Length (in mm)	HALO 2.7 Peptide ES-C18	HALO 2.7 Peptide ES-CN	HALO-5 Peptide ES-C18	HALO-5 Peptide ES-CN
0.075 x 50	91229-402	91229-404	91529-402	91529-404
0.075 x 100	91229-602	91229-604	91529-602	91529-604
0.075 x 150	91229-702	91229-704	91529-702	91529-704
0.1 x 50	91228-402	91228-404	91528-402	91528-404
0.1 x 100	91228-602	91228-604	91528-602	91528-604
0.1 x 150	91228-702	91228-704	91528-702	91528-704
0.2 x 50	91227-402	91227-404	91527-402	91527-404
0.2 x 100	91227-602	91227-604	91527-602	91527-604
0.2 x 150	91227-702	91227-704	91527-702	91527-704
0.3 x 50	91226-402	91226-404	91526-402	91526-404
0.3 x 100	91226-602	91226-604	91526-602	91526-604
0.3 x 150	91226-702	91226-704	91526-702	91526-704
0.5 x 50	91225-402	91225-404	91525-402	91525-404
0.5 x 100	91225-602	91225-604	91525-602	91525-604
0.5 x 150	91225-702	91225-704	91525-702	91525-704
1.0 x 30	92121-302	92121-304	95121-302	95121-304
1.0 x 50	92121-402	92121-404	95121-402	95121-404
1.0 x 75	92121-502	92121-504	95121-502	95121-504
1.0 x 100	92121-602	92121-604	95121-602	95121-604
1.0 x 150	92121-702	92121-704	95121-702	95121-704
2.1 x 20	92122-202	92122-204	95122-202	95122-204
2.1 x 30	92122-302	92122-304	95122-302	95122-304
2.1 x 50	92122-402	92122-404	95122-402	95122-404
2.1 x 75	92122-502	92122-504	95122-502	95122-504
2.1 x 100	92122-602	92122-604	95122-602	95122-604
2.1 x 150	92122-702	92122-704	95122-702	95122-704
2.1 x 250	92122-902	92122-904	95122-902	95122-904
3.0 x 20	92123-202	92123-204	95123-202	95123-204
3.0 x 30	92123-302	92123-304	95123-302	95123-304
3.0 x 50	92123-402	92123-404	95123-402	95123-404
3.0 x 75	92123-502	92123-504	95123-502	95123-504
3.0 x 100	92123-602	92123-604	95123-602	95123-604
3.0 x 150	92123-702	92123-704	95123-702	95123-704
3.0 x 250	92123-902	92123-904	95123-902	95123-904
4.6 x 20	92124-202	92124-204	95124-202	95124-204
4.6 x 30	92124-302	92124-304	95124-302	95124-304
4.6 x 50	92124-402	92124-404	95124-402	95124-404
4.6 x 75	92124-502	92124-504	95124-502	95124-504
4.6 x 100	92124-602	92124-604	95124-602	95124-604
4.6 x 150	92124-702	92124-704	95124-702	95124-704
4.6 x 250	92124-902	92124-904	95124-902	95124-904
10.0 x 50	92120-402	92120-404	95120-402	95120-404
10.0 x 75	92120-502	92120-504	95120-502	95120-504
10.0 x 100	92120-602	92120-604	95120-602	95120-604
10.0 x 150	92120-702	92120-704	95120-702	95120-704
10.0 x 250			95120-902	95120-904
Guard Columns, 3-pack				
Dimensions ID x Length (in mm)	HALO 2.7 Peptide ES-C18	HALO 2.7 Peptide ES-CN	HALO-5 Peptide ES-C18	HALO-5 Peptide ES-CN
2.1 x 5	92122-102	92122-104	95122-102	95122-104
3.0 x 5	92123-102	92123-104	95123-102	95123-104
4.6 x 5	92124-102	92124-104	95124-102	95124-104
Guard Columns	94900-001			

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REFERENCES

1. Adapted from book, "Introduction to Modern Liquid Chromatography", 3rd Edition, L. R. Snyder, J. J. Kirkland and J. W. Dolan, 2010, p.29, Wiley & Sons.
2. "Orthogonal" separations for reversed-phase liquid chromatography; J. Pellett, P. Lukulay, Y. Mao, W. Bowen, R. Reed, M. Ma, R.C. Munger, J.W. Dolan, L. Wrisley, K. Medwid, N.P. Toltl, C.C. Chan, M. Skibic, K. Biswas, K. A. Wells, and L.R. Snyder; Journal of Chromatography A, 1101 (2006) 122–135.
3. Column selectivity in reversed-phase liquid chromatography I. A general quantitative relationship; N.S. Wilson, M.D. Nelson, J.W. Dolan, L.R. Snyder, R.G. Wolcott and P.W. Carr; Journal of Chromatography A, 961 (2002) 171–193.
4. Column selectivity in reversed-phase liquid chromatography IV. Type-B alkyl-silica columns; J. J. Gilroy, J. W. Dolan and L. R. Snyder; Journal of Chromatography A, 1000 (2003) 757–778.
5. http://www.hplccolumns.org/
6. http://molnar-institute.com/drylab/ ("ColumnMatch").
7. D.V. McCalley and U.D. Neue, J. Chromatogr. A 1192, 225–229 (2008).
8. A.J. Alpert. Anal. Chem. 80, 62–76 (2008).
9. D.V. McCalley, J. Chromatogr. A 1171, 46–56 (2007).
10. A.J. Alpert et al., Anal. Chem. 82, 5253–5259 (2010).

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