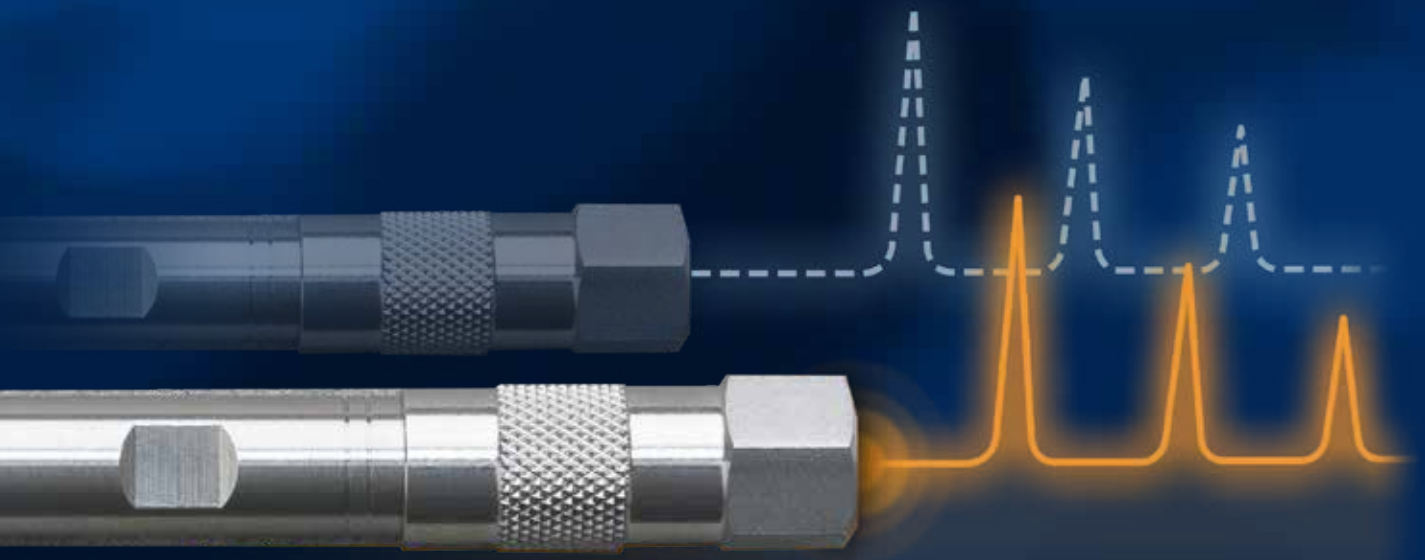
HALO®

METHOD CONVERSION GUIDEBOOK



HALO®

METHOD CONVERSION GUIDEBOOK

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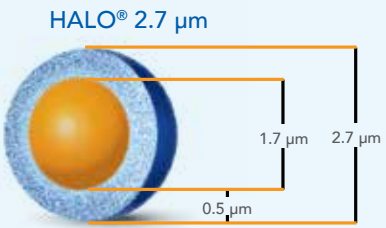
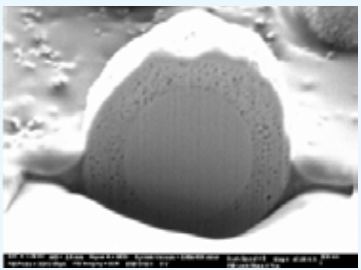
HALO®

Introduction to HALO® Fused-Core® Technology, Its Benefits, and How to Take Advantage of Superficially Porous Particles for Method Improvements

Chromatographers continue to push separation limits due to increasing productivity demands. How can I achieve an improved separation in a faster time, or with less solvent consumption, increased sensitivity, or all of the above? The specific drivers may be different, but the overall goal is the same – '*I need more, but without sacrificing anything*'. This dilemma is not new and it gained prime attention back in the early 2000's when UHPLC instrumentation first arrived on the scene. Separation scientists were presented with a possibility to reduce run times by up to 70% and still maintain high resolution with the adoption of smaller particle size columns and new hardware that could accommodate the generated increase in back pressure required by these smaller particles.

In 2006, Advanced Materials Technology took a different path towards this goal and introduced a novel technology where particles had an overall diameter of 2.7 µm with a solid silica core surrounded by a porous layer that maintained 75% of the total particle volume. This little HALO® Fused-Core® particle started one large revolution and technology has been accepted as the new standard in high performance LC and LCMS separations!





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.

How HALO® Delivers Higher Efficiency

Theoretical plates (N) are a measure of the column efficiency. By normalizing plates to the column length (L), the height equivalent theoretical plate, called HETP or H (L/N) is obtained. The van Deemter equation describes three factors which impact H: eddy diffusion (A term), longitudinal diffusion (B term), and mass transfer (C term). The amount to which these factors contribute to H is dependent on mobile phase velocity. Other factors influence H, particularly particle size and particle morphology [fully porous particles (FPP) versus superficially porous particles (SPP)]. Effective use of the van Deemter equation enables operation at the optimum mobile phase velocity usually with the lowest H and highest N.

The simplest van Deemter Equation Relating Theoretical Plate Height to Linear Velocity (µ), Eddy Dispersion (A), Longitudinal Dispersion (B), and Resistance to Mass Transfer (C) Terms.

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

Higher efficiencies (H) for SPP columns are due to a combination of all three van Deemter A, B and C terms being smaller for SPP particles. *See van Deemter curves in Figure A.*

- 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 (25–30% smaller van Deemter "B term")
  - due to the presence of the solid core inside the particles
- Flatter van Deemter plot and higher optimum linear velocity (μ_{opt}, $\propto$ flow rate, "C term")
  - due to the shorter diffusion distances into the particles

van Deemter curves for 2.7 µm HALO® and various FPP columns

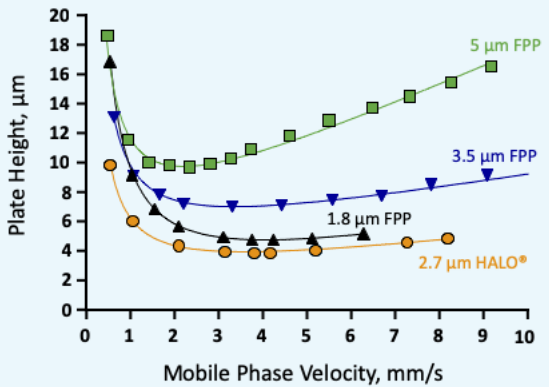


Figure A.

The original HALO® Fused-Core® columns are able to perform competitively versus sub-2 µm fully porous columns and substantially outperform 3 and 5 µm fully porous columns delivering the goal of increased performance without the consequence of high back pressures!

The 2.7 µm HALO® column has *less than half the back pressure* with competitive performance, to the 1.7 µm FPP column and *far superior performance* to the 5 µm FPP column with only moderate increase in back pressure.

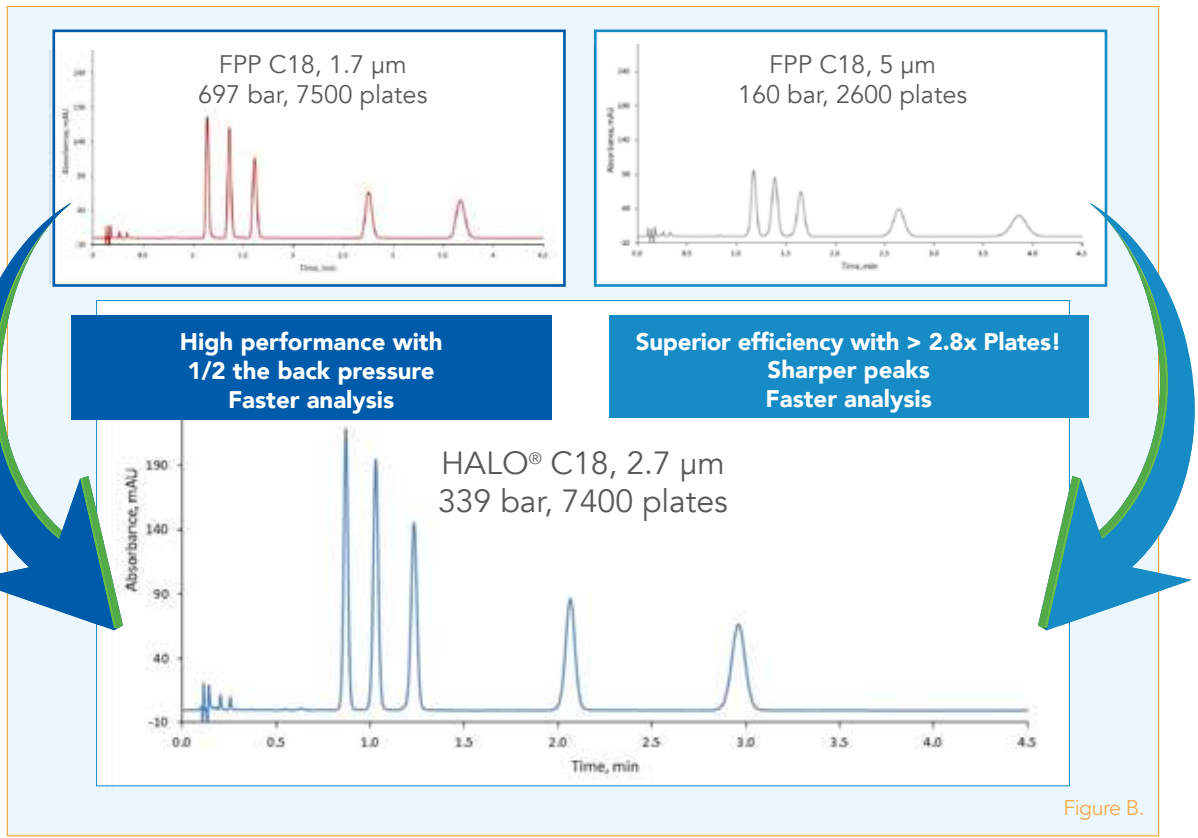


Figure B.

Acceptance, Advancement and Adoption of Fused-Core® Technology

As the realization of SPP benefits became more mainstream, recognized at first by thought leaders and then more universally adopted, ongoing development of the particle morphology has been underway.

A 5 µm particle was designed to offer performance improvements as direct replacements in older, FPP methods. The 5 µm HALO® columns deliver high efficiency of a 3 µm FPP but at lower back pressure of a 5 µm particle. In practicality that means the 5 µm HALO® provides more robust assays. A 2 µm HALO® option was also introduced to allow users who had adopted UHPLC technology to gain additional resolving power as these 2 µm SPP columns significantly outperform sub-2 µm FPP columns. The ultra high efficiency 2 µm is ideal for complex separations and those with ultra HPLC instruments who seek highest efficiencies and robust performance at lower back pressure.

As separation demands evolve so does Advanced Materials Technology's industry leading innovation.

2006 **Original HALO® 2.7 µm SPP**
changed the perception of what is required for high efficiency separations

2012 **HALO® 5 µm SPP**
robust replacement to conventional 5 µm particle columns with SPP benefits

2013 **HALO® BioClass Line Introduced**
Protein, Peptide and Glycan solutions to meet the challenges of biomolecule separations

2014 **HALO® 2 µm SPP**
the go-to SPP for highest efficiency separations with UHPLC technology

2017 **HALO® 1000 Å Protein**
First 1000 Å pore size providing the widest pore available in an SPP that delivered significant gains in resolution of large protein complexes.

SPP column technology has already been widely adopted by new method developers and recognized regulatory bodies in method modernization efforts to replace less efficient FPP methods. Modernization includes the purview of USP, EP and JP. While the latest regulations must be followed for method change acceptance criteria, Fused-Core® technology is here to stay!

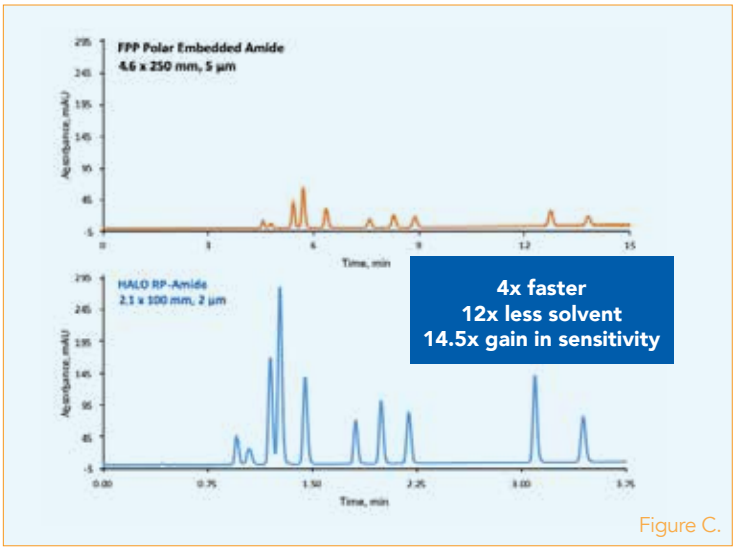


Figure C.

So what are your drivers for method improvement? Faster run times, reduced solvent consumption, increased sensitivity? You can achieve all of these with HALO® Fused-Core® technology! In Figure C., the separation of 10 components is transferred from a 5 µm FPP column to a 2 µm HALO® column demonstrates these improvements. As in many cases, nearly identical selectivity was observed for the same stationary phase.

Advanced Materials Technology remains intently focused on Fused-Core® technology development and the separation potentials still being discovered.

Taking Advantage of HALO® Fused-Core® Technology

SMALL MOLECULE

2 micron particle | 2.7 micron particle | 5 micron particle

HALO® particle sizes and properties

	Particle Size		
	2 µm	2.7 µm	5 µm
Best Uses	complex separations when ultimate resolution is required with a low system volume UHPLC	best all-purpose rugged particle providing high efficiencies with minimal back pressure	when more performance or lower back pressures from 3 µm or 5 µm FPP methods are desired; samples with dirty matrices
Performance Potential (plates/meter)	300,000+	230,000+	160,000+
Efficiency Comparison to FPP	outperforms <2 µm FPP	performance of <2 µm FPP	performance of 3 µm FPP
Instrumentation	UHPLC	UHPLC/HPLC	HPLC

Implementing Fused-Core® Technology

To facilitate converting conventional reversed-phase FPP separations to ultra-fast separations, we have created this method conversion guide. It is intended to assist you in selecting a HALO® column and modifying conditions for a faster run time. This guide will help you estimate how the new HALO® conditions will affect run time, resolution, and back pressure. Because peaks elute from HALO® columns faster and with much smaller volumes than conventional FPP columns, modifications may also have to be made to conventional HPLC equipment to obtain the full benefits that these columns offer.

An important chromatographic parameter that needs to be considered is selectivity. While selectivity is beyond the scope of this guide, you should be aware that converting between various columns will sometimes be accompanied by a change in selectivity. Small changes to mobile phase compositions may be necessary to obtain the desired resolution.

Typical efficiencies expected from each HALO® particle size.

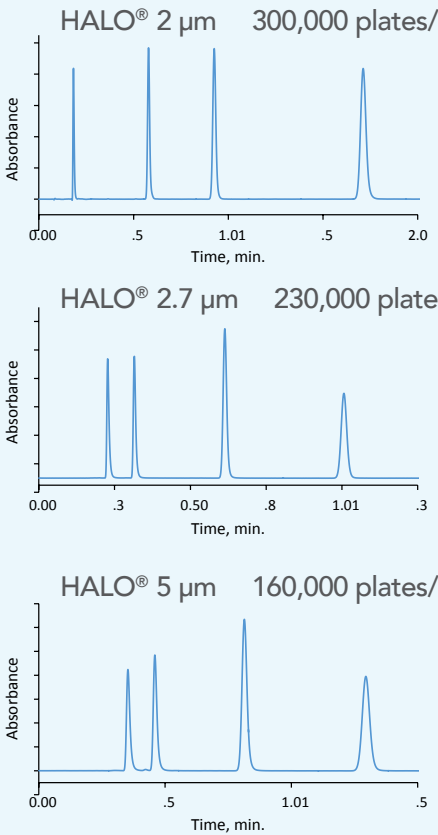
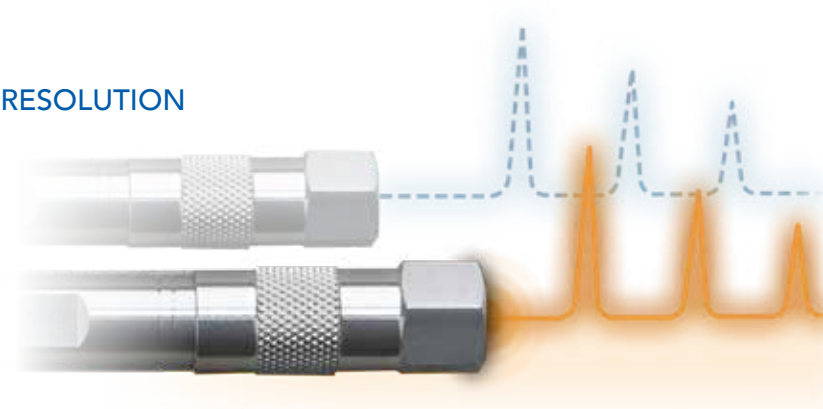


Figure D.

Suggested Steps for Converting a Conventional Reversed-Phase FPP Separation to an Ultra-Fast HALO® Separation

1. SELECT AN ULTRA-FAST HALO® COLUMN LENGTH
2. ESTIMATE BACK PRESSURE
3. CONFIRM SELECTIVITY AND RESOLUTION
4. OPTIMIZE FLOW RATE
5. ADJUST GRADIENT TIME
6. ADJUST THE SAMPLE INJECTION VOLUME



STEP 1. SELECT AN ULTRA-FAST HALO® COLUMN LENGTH

Select the shortest HALO® column that can provide resolution equivalent to or better than the conventional FPP column. *See Figure E.*

INSTRUCTIONS: This chart plots resolving power (the ability of a column to separate components in a mixture) versus column length for 6 different columns. Resolving power or resolution is proportional to the square root of N, assuming that selectivity and retention are constant across different columns. Three of the columns are packed with fully porous particles (5 µm, 3 µm and 1.8 µm) and three are packed with superficially porous HALO® particles (5 µm, 2.7 µm, and 2 µm). As column length increases, so does resolving power, but run time also increases. Notice that the **5 µm HALO® columns** provide more resolving power in much shorter column lengths compared to the 5 µm fully porous particle columns and slightly more resolving power compared to the 3 µm fully porous particle columns. **2.7 µm HALO® columns** offer increased resolving power in shorter column lengths over both 3 and 5 µm fully porous particle columns. **2 µm HALO® columns** offer the highest resolving power in the shortest column lengths similar to 1.8 µm FPP columns, but with lower relative back pressure.

Select the HALO® column in a particle size that provides resolving power equal to or better than the FPP column it is replacing, provided that your system is capable of the pressure that will be generated by the column *(See step 2.)*. This will allow you to minimize run time and maintain acceptable resolution.

Resolving Power as a Function of Particle Size and Column Length

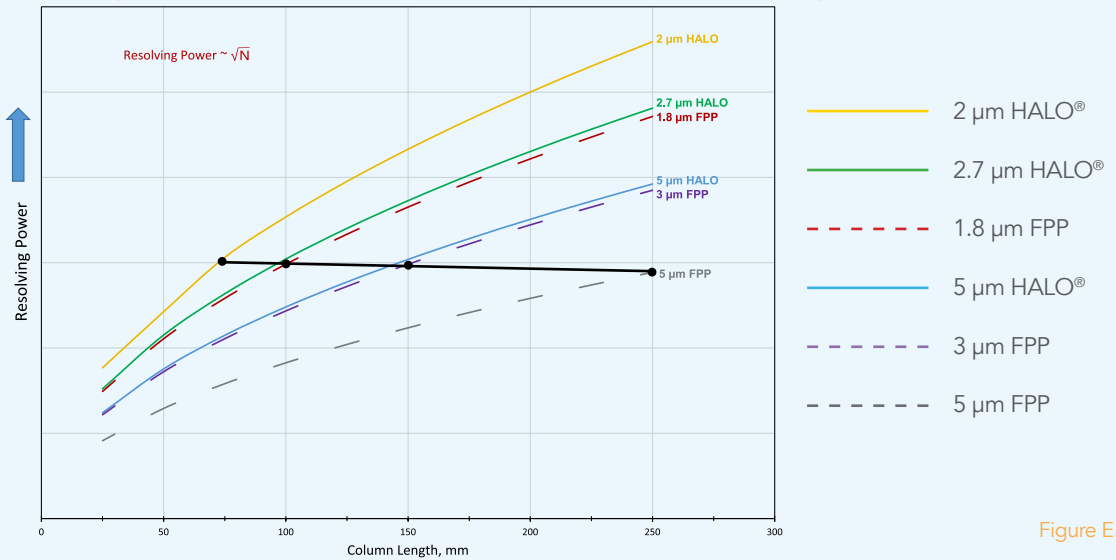


Figure E.

Example 1: A 100 mm column packed with 2.7 µm HALO® particles meets the criteria of providing equal or better resolving power compared to a 250 mm column packed with 5 µm FPP particles. The HALO® column is an appropriate choice for replacing the 250 mm length FPP column in an ultra-fast method.

Example 2: Similar to Example 1, a 75 mm column packed with 2 µm HALO® particles could be used to replace a 150 mm length column packed with 3 µm FPP particles since both of these columns have similar resolving power.

STEP 2. ESTIMATE BACK PRESSURE

Estimate the back pressure for the selected HALO® column. See Figure F. If the pressure approaches or exceeds the maximum acceptable pressure for your system, select an alternate column with lower back pressure, most likely one packed with larger particles. You could elect to operate at a lower flow rate to keep the pressure acceptable, but this would also increase the run time, negating the purpose of converting to an ultra-fast HALO® column.

INSTRUCTIONS: For the HALO® column configuration selected in Step 1 (length, particle size), estimate the expected back pressure on this column by multiplying the pressure observed on the FPP column by the ratio of the "Relative Pressure" of the HALO® column to the FPP column and then by the ratio of the column lengths.

Note: This calculation assumes that the mobile phase velocity is the same for both the FPP column and the HALO® column.

$$P_2 = P_1 \times \frac{RP_2}{RP_1} \times \frac{L_2}{L_1}$$

P_2: Estimated back pressure of the HALO® column
RP_2: Relative back pressure of the HALO® column
L_2: Length of the HALO® column
P_1: Measured back pressure of FPP column
RP_1: Relative back pressure of the FPP column
L_1: Length of the FPP column

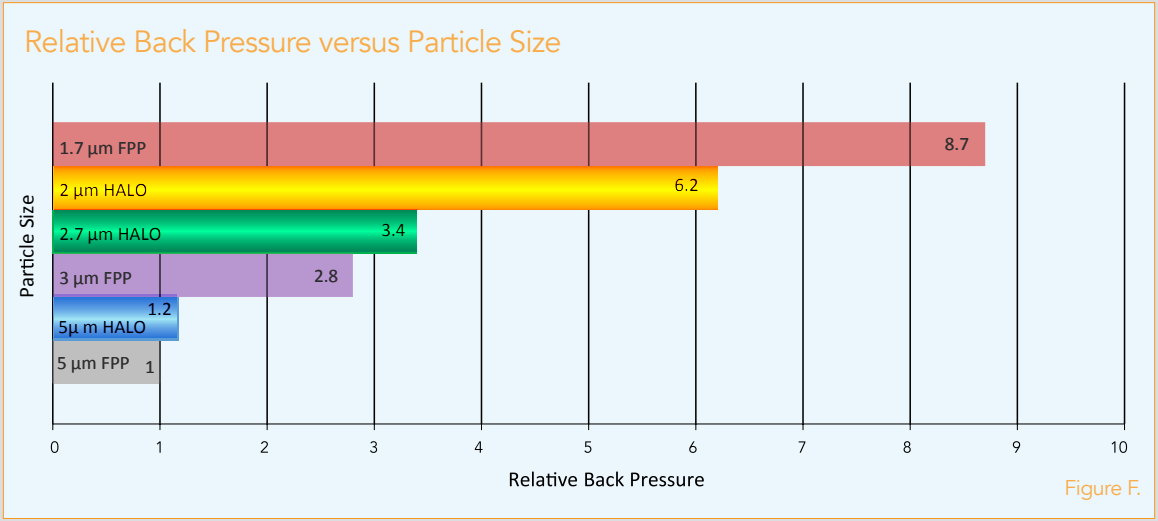


Figure F.

Example 1: A 100 mm HALO® column packed with 2.7 µm particles will generate approximately 1.36 times the back pressure of a 250 mm conventional column packed with 5 µm particles.

$$P_{HALO\ Column} = P_1 \times \frac{3.4}{1} \times \frac{100\ mm}{250\ mm} = 1.36 \times P_{FPP\ Column}$$

Example 2: A 75 mm HALO® column packed with 2 µm particles will generate approximately 1.11 times the back pressure of a 150 mm conventional column packed with 3 µm particles.

$$P_{HALO\ Column} = P_1 \times \frac{6.2}{2.8} \times \frac{75\ mm}{150\ mm} = 1.11 \times P_{FPP\ Column}$$

STEP 3. CONFIRM SELECTIVITY AND RESOLUTION

Confirm that the selectivity and resolution of the HALO® column is adequate. Since the selectivity of the HALO® column may differ slightly from the selectivity of the conventional column, run your separation with the HALO® column and calculate resolution. If the resolution does not meet the minimum required resolution, you may have to choose a longer column, use a smaller particle, or slightly modify your mobile phase conditions if your method allows, in order to achieve acceptable resolution. If the resolution exceeds requirements, you may be able to use an even shorter HALO® column, or at least operate at a higher mobile phase flow rate to reduce the run time even further.

INSTRUCTIONS: Calculate resolution, use the following equation:

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

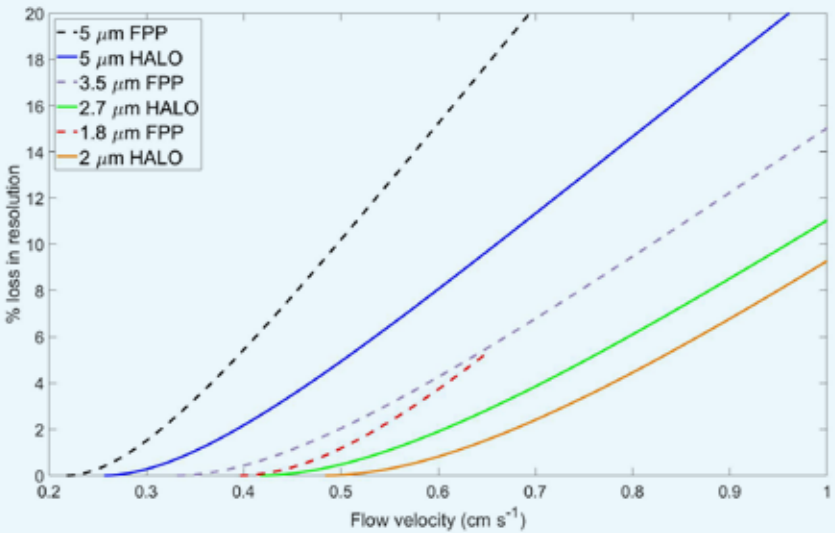
N = plates
α = selectivity
k = retention factor

STEP 4. OPTIMIZE FLOW RATE

Once a column provides acceptable resolution and pressure, increase flow rate to minimize run time while maintaining acceptable resolution and pressure. Considerable time savings and greater sample throughput can be achieved with HALO® columns as they are amenable to operating at higher flow rates with minimal efficiency losses compared to FPP.
See Figure G.

INSTRUCTIONS: If the resolution on the selected HALO® column exceeds the minimum required resolution for the separation and does not exceed the pressure limit, you will be able to reduce analysis time further by increasing the flow rate. Since the optimum flow velocity (for maximum resolution) of a 2.7 µm HALO® column is 3 to 4 times faster than for a 5 µm FPP column, you may be able to both reduce run time and increase resolution by operating at a higher flow rate. This chart estimates change in resolution with changes in mobile phase velocity. Not only do ultra-fast HALO® columns have their optimum efficiency at higher mobile phase velocities, they also sacrifice less of their efficiency as mobile phase velocity is increased beyond their optimum.

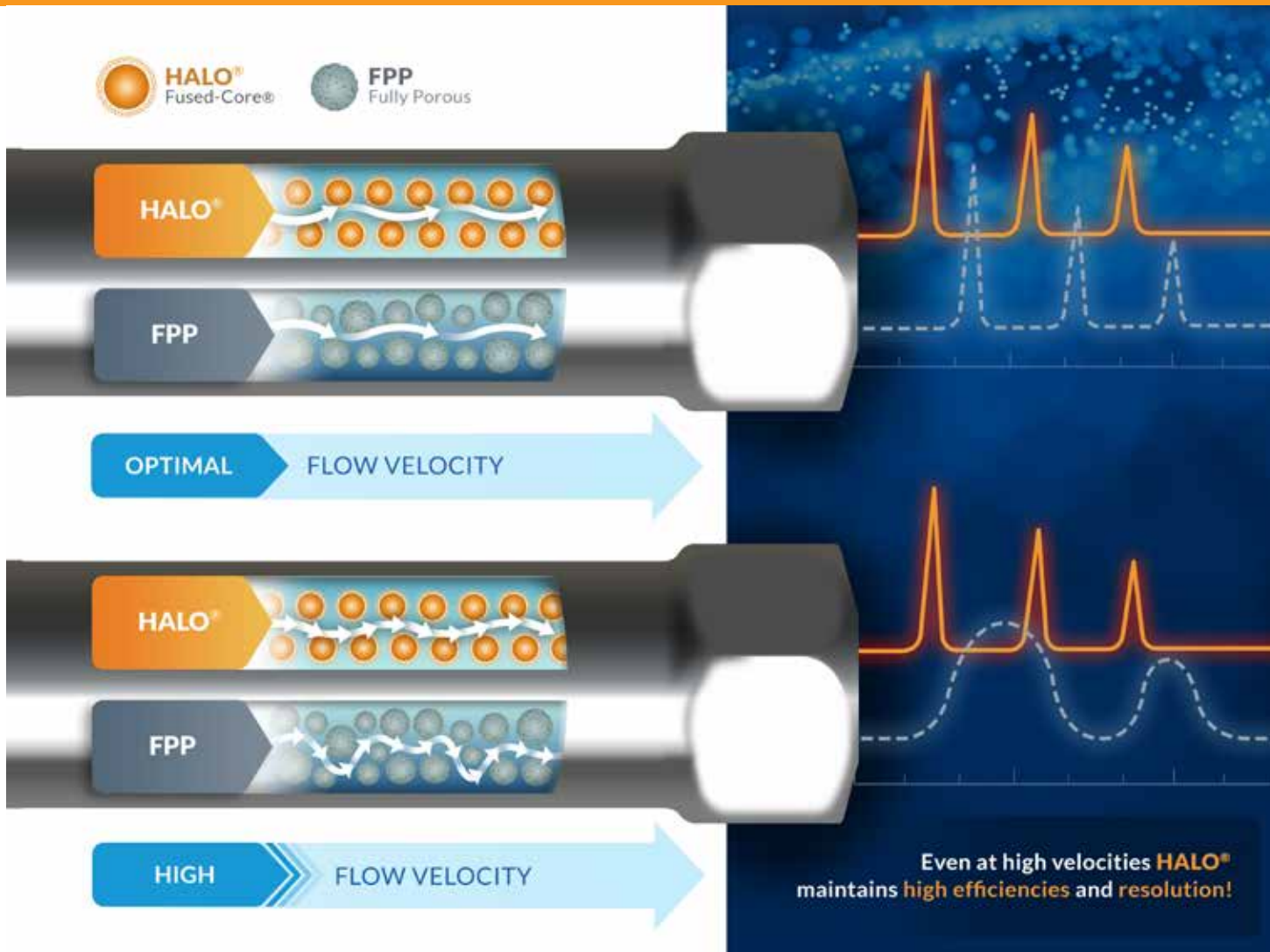
Resolution versus Mobile Phase Flow Rate



	Flow Velocity (cm s^{-1})		
	0.2	0.7	1
Column ID (mm)	**Equivalent Flow Rate (mL / min)**		
2.1	0.26	0.92	1.3
3.0	0.93	1.9	2.7
4.6	1.3	4.4	6.3

Figure G.

Example: A traditional FPP column packed with 5 µm fully porous particles run at 0.6 cm/s would retain only about 84% of its resolving power however, a 5 µm HALO® column run at the same flow rate would maintain 92% of its resolving power. A 2.7 µm HALO® column can be operated at a relatively fast mobile phase velocity of 0.6 cm/s and still retain over 98% of its resolving power. With a 2 µm HALO® column, the optimal velocity is higher, which means that it can be run at 0.7 cm/s and still retain over 98% of its resolving power!


HALO®
Fused-Core®
FPP
Fully Porous
HALO®
FPP
OPTIMAL
FLOW VELOCITY
HALO®
FPP
HIGH
FLOW VELOCITY
Even at high velocities HALO®
maintains high efficiencies and resolution!

References for Converting Mobile Phase Velocity (cm/s) to Column Flow Rate (mL/min)

Semi-micro, Analytical and Semi-prep Column IDs

	Column ID (mm)						Column ID (mm)				
	1	2.1	3	4.6	10		1	2.1	3	4.6	10
Linear Velocity (cm/s)	Flow rate (mL/min)					Linear Velocity (cm/s)	Flow rate (mL/min)				
0.1	0.03	0.13	0.27	0.63	3.0	0.6	0.18	0.79	1.6	3.8	18
0.2	0.059	0.26	0.53	1.3	6.1	0.7	0.21	0.92	1.9	4.4	21
0.3	0.089	0.39	0.8	1.9	9.0	0.8	0.24	1	2.1	5	24
0.4	0.12	0.52	1.1	2.5	11.8	0.9	0.27	1.2	2.4	5.7	27
0.5	0.15	0.65	1.3	3.1	14.7	1	0.3	1.3	2.7	6.3	30

table is used for semi-micro, analytical and semi-prep column IDs

Capillary Column IDs

	Column ID (µm)						Column ID (µm)				
	75	100	200	300	500		75	100	200	300	500
Linear Velocity (cm/s)	Flow rate (µL/min)					Linear Velocity (cm/s)	Flow rate (µL/min)				
0.1	0.17	0.3	1.2	2.7	7.5	0.6	1.0	1.8	7.2	16	45
0.2	0.33	0.59	2.4	5.3	14.8	0.7	1.2	2.1	8.4	19	53
0.3	0.50	0.89	3.6	8.0	22.3	0.8	1.4	2.4	9.6	22	60
0.4	0.68	1.2	4.8	10.8	30.0	0.9	1.5	2.7	11	24	68
0.5	0.84	1.5	6.0	13.5	37.5	1.0	1.7	3.0	12	27	75

STEP 5. ADJUST GRADIENT TIME

If the separation uses gradient elution, you will need to adjust the gradient time (t_G) to the volume of the HALO® column and for any changes in flow rate.

INSTRUCTIONS: Calculate the adjusted gradient time using the equation below:

Important Note: The system dwell volume (gradient mixing volume) can also have a significant effect on the chromatography when using gradients because it adds an isocratic hold to the beginning of the gradient. The time of this "hold" is equal to the dwell volume divided by the flow rate. When the flow rate is changed, this isocratic hold will also change. This change in gradient hold will generally have more of an effect on early eluting peaks, but it will also affect all peaks in the chromatogram to some extent. To minimize the effect on your separation, keep the dwell volume as small as possible by using micro gradient mixers and keeping the tubing volume in the system to a minimum.

$$t_{G2} = t_{G1} \times \frac{V_{m2}}{V_{m1}} \times \frac{F_1}{F_2}$$

t_{G2}: Gradient time for the HALO® separation
V_{m2}: Column volume of the HALO® column (see Table 1)
F_2: Flow rate for the HALO® separation
t_{G1}: Gradient time for the FPP separation
V_{m1}: Column volume of the FPP column (see Table 1)
F_1: Flow rate for the FPP separation

Example 1: A FPP method uses a 4.6 x 150 mm 5 µm column (1.57 mL column volume), a flow rate of 1.0 mL/min, and a gradient of 15% B to 35% B in 20.0 minutes. The gradient time for a HALO® method that uses a 4.6 x 50 mm (2.7 µm column, 0.42 mL column volume) column and a flow rate of 2.0 mL/min is:

$$t_{G\,HALO} = 20\ minutes \times \frac{0.42\ mL}{1.57\ mL} \times \frac{1.0\ mL/min}{2.0\ mL/min} = 2.7\ min$$

Example 2: A FPP method uses a 2.1 x 100 mm 5 µm column (0.218 mL), a flow rate of 0.25 mL/min, and a gradient of 20% B to 65% B in 15.0 minutes. The gradient time for a HALO® method that uses a 2.1 x 50 mm (2 µm column, 0.087 mL column volume) column and a flow rate of 0.5 mL/min is:

$$t_{G\,HALO} = 15\ minutes \times \frac{0.087mL}{0.218\ mL} \times \frac{0.25\ mL/min}{0.5\ mL/min} = 3.0\ min$$

Reference Table 1, page 29 for column volume (V_m) estimates.

STEP 6. ADJUST SAMPLE INJECTION VOLUME

Adjust the sample injection volume to the HALO® column's volume. SPP columns have less surface area compared to FPP columns of the same particle size so the injection volume must be reduced to avoid overloading the column.

Note: A simple accepted practice is to reduce the injection volume by about 30% which adjusts for the slightly lower sample loadability of SPP columns. This is generally more than what is needed. If more sensitivity is needed, the injection volume may be empirically increased.

INSTRUCTIONS: Adjust the Sample Injection Volume for Changes in Column Dimension. See equation below.

$$S_{V2} = S_{V1} \times \frac{V_{m2}}{V_{m1}}$$

S_{V2}: Injected sample volume for the HALO® column
S_{V1}: Injected sample volume for the FPP column
V_{m2}: Volume of the HALO® column (see Table 1)
V_{m1}: Volume of the FPP column (see Table 1)

Example: A conventional method uses a sample injection volume of 20 µL on a column 4.6 x 150 mm.

The sample volume that should be injected on a 4.6 x 50 mm 2.7 µm HALO® column is:

$$S_{V\,HALO} = 20\ \mu L \times \frac{0.42\ mL}{1.57\ mL} = 5\ \mu L$$

See Table 1, page 29 for column volume (V_m) estimates.

If the column volumes are not known, the following simplified equation may be used:

$$V_{i1} = V_{i2} \times \left(\frac{d_2}{d_1}\right)^2 \times \left(\frac{L_2}{L_1}\right) \times 0.7$$

V_{i2}: Injected sample volume for the HALO® column
V_{i2}: Injected sample volume for the FPP column
d_2: Diameter of the HALO® column
d_1: Diameter of the FPP column
L_2: Length of the HALO® column
L_1: Length of the FPP column

$$V_{i1} = 20\ \mu L \times \left(\frac{4.6\ mm}{4.6\,mm}\right)^2 \times \left(\frac{50\ mm}{150\ mm}\right) \times 0.7 = 5\ \ \mu L$$

Implementation Example: Converting a Conventional FPP Column Separation to an Ultra-Fast HALO® Separation

Original HPLC Separation Conditions

COLUMN: 3.0 x 250 mm, 5 µm FPP
FLOW RATE: 0.65 mL/min
MOBILE PHASE: Isocratic
RUN TIME: 10 minutes
PRESSURE: 1,624 psi, 112 bar
Maximum acceptable pressure = 4,000 psi, 275 bar
RESOLUTION: 2.3
SAMPLE INJECTION VOLUME: 10 µL

Converting to HALO® Separation Conditions

1. Select an ultra-fast HALO® column length

Select the shortest HALO® column that provides resolution equivalent to or better than the FPP column. (See Relative Resolution chart in Figure E.) A HALO® column 3.0 x 100 mm packed with 2.0 µm particles is selected for further investigation.

2. Estimate back pressure.

(See relative pressure table in Figure F.)

$$P_{HALO\ Column} = P_1 \times \frac{6.2}{1} \times \frac{100\ mm}{250\ mm} = 2.48 \times P_{FPP\ Column} = 4028\ psi,\ 278\ bar$$

Since this column exceeds our maximum acceptable back pressure (4,000 psi), a different ultra-fast column is selected for investigation. The alternative HALO® column selected is 3.0 x 100 mm packed with 2.7 µm Fused-Core® particles. The back pressure on this column is:

$$P_{HALO\ Column} = P_1 \times \frac{3.4}{1} \times \frac{100\ mm}{250\ mm} = 1.36 \times P_{FPP\ Column} = 2209\ psi,\ 152\ bar$$

This size HALO® column provides both acceptable resolution and acceptable back pressure for our method.

3. Confirm selectivity and resolution

For simplicity, we will assume that the selectivity of this HALO® column is almost identical to the selectivity of the FPP column and, therefore, the resolution is adequate. If not, modify the mobile phase conditions slightly if your protocols allow this.

4. Optimize flow rate

(See Figure G. to estimate changes in resolution with changes in flow rate.)

We can further reduce run time by operating the HALO® column at a higher flow rate. We just have to make sure we stay within the requirements of minimum resolution and maximum pressure. The HALO® column we selected has low enough back pressure that we can operate at a faster flow rate of 1.1 mL/min and still stay within our defined limits of pressure and resolution.

$$P_{HALO\ Column} = 2209\ psi \times \frac{1.1\ mL/min}{0.65\ mL/min} = 3738\ psi,\ 258\ bar$$

5. Adjust gradient time

This is an isocratic separation, so no adjustment to gradient time is required.

6. Adjust the sample injection volume

(See Table 1 with estimated column volumes.)

$$S_{V\ HALO} = 10\ \mu L \times \frac{0.356\ mL}{1.11\ mL} = 3.0 - 4.0\ \mu L$$

Time Savings with HALO®

COLUMN: 3.0 x 100 mm, 2.7 µm HALO®
FLOW RATE: 1.1 mL/min
RUN TIME:

$$10\ min \times \frac{0.356\ mL}{1.11\ mL} \times \frac{0.65\ mL/min}{1.1\ mL/min} = 1.9\ min^{*}$$

RESOLUTION: 2.8
PRESSURE: 3,738 psi
SAMPLE INJECTION VOLUME: 3-4 µL

*Run time for the ultra-fast separation can be estimated by multiplying the run time on the conventional column by the ratio of the volumes of ultra-fast column to the conventional column and then by the inverse ratio of the flow rates on the two columns. *(See Table 1, page 29)*

Figure H. shows simulated chromatograms to illustrate the example conversion to a HALO® column.

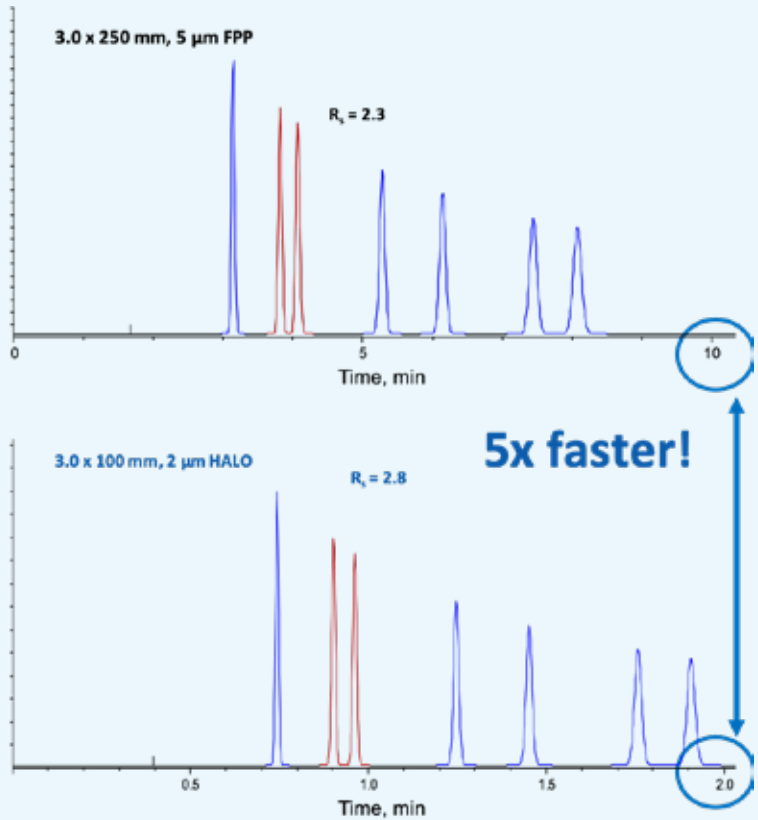

3.0 x 250 mm, 5 µm FPP
R_s = 2.3
0
5
10
Time, min
3.0 x 100 mm, 2 µm HALO
R_s = 2.8
5x faster!
0.5
1.0
1.5
2.0
Time, min

Figure H.

The following example shows a separation on a 5 µm FPP column that was transferred to a 5 µm HALO® column, a 2.7 µm HALO® column, and a 2 µm HALO® column. For low back pressure, increased resolution, and higher plates, select a 5 µm HALO® column. For additional resolution and efficiency, select the 2.7 µm HALO® column. For ultimate speed, select the 2 µm column in a shorter dimension.

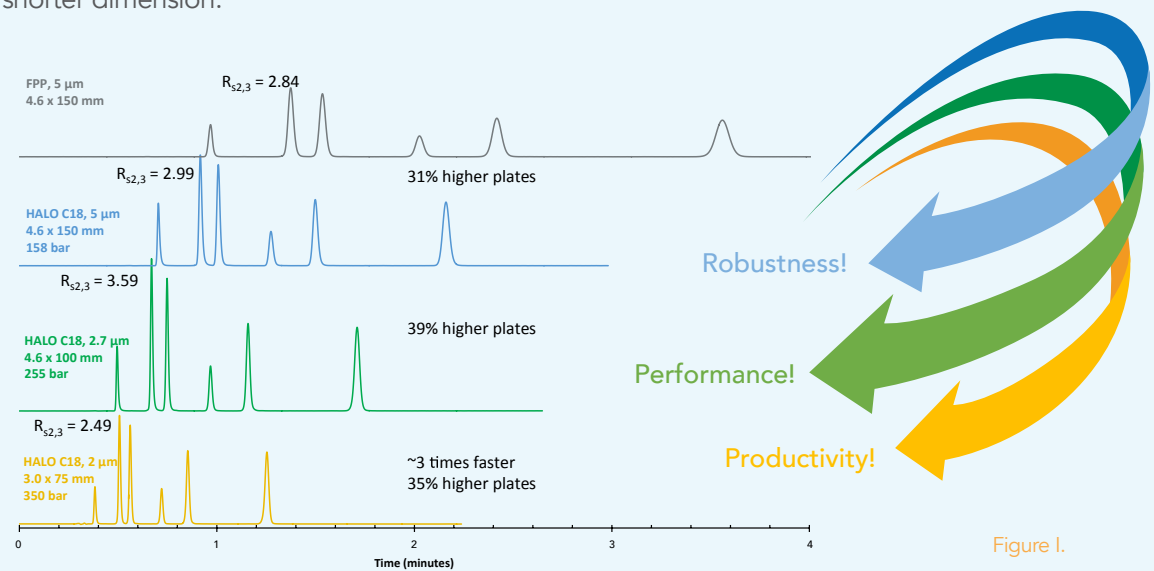


Figure I.

Choose HALO® columns when converting legacy LC methods to improve Robustness, Performance, and Productivity!

Reference Tables and Equations for Quick Estimates

TABLE 1. Estimated volume, V_m, for a variety of available column dimensions

ID (mm)	Length (mm)	V_m (mL)	V_m HALO (mL)	ID (mm)	Length (mm)	V_m (mL)	V_m HALO (mL)
1.0	20	0.010	0.008	3.0	20	0.089	0.071
1.0	30	0.015	0.012	3.0	30	0.134	0.107
1.0	50	0.025	0.020	3.0	50	0.223	0.178
1.0	75	0.037	0.030	3.0	75	0.334	0.267
1.0	100	0.050	0.040	3.0	100	0.445	0.356
1.0	150	0.074	0.059	3.0	150	0.668	0.534
1.0	250	0.124	0.099	3.0	250	1.11	0.89
2.1	20	0.044	0.035	4.6	20	0.209	0.168
2.1	30	0.066	0.052	4.6	30	0.314	0.251
2.1	50	0.109	0.087	4.6	50	0.524	0.419
2.1	75	0.164	0.131	4.6	75	0.785	0.628
2.1	100	0.218	0.175	4.6	100	1.05	0.84
2.1	150	0.327	0.262	4.6	150	1.57	1.26
2.1	250	0.546	0.436	4.6	250	2.62	2.09

Column volumes listed here are estimates only.
However, most commercial columns can be expected to have volumes within about 5% of what is reported here.

TABLE 2. Column plate number, N, for columns packed with different size/type particles

Particle	Plates/meter of Column Length	Particle	Plates/meter of Column Length
5 µm FPP	80,000	5 µm Fused-Core	160,000
3 µm FPP	133,000	2.7 µm Fused-Core	230,000
1.8 µm FPP	240,000	2 µm Fused-Core	300,000

TABLE 3. Reference Equations

$$Rs_{HALO} = Rs_{FPP} \times \sqrt{\frac{N_{HALO}}{N_{FPP}}}$$

$$RT_{HALO} = RT_{FPP} \times \frac{V_{HALO}}{V_{FPP}} \times \frac{F_{FPP}}{F_{HALO}}$$

$$t_{g\ HALO} = t_{g\ FPP} \times \frac{V_{HALO}}{V_{FPP}} \times \frac{F_{FPP}}{F_{HALO}}$$

$$Sv_{HALO} = Sv_{FPP} \times \frac{V_{HALO}}{V_{FPP}}$$

R_S = Resolution
N = Column Plate Number
RT = Run Time
V = Column Volume
F = Flow Rate
S_V = Injected Sample Volume
t_g = Gradient Time

Experimental Conditions

Figure A. 4.6 x 50 mm, 24 °C ; 40/60 water/ACN; Solute: naphthalene

Figure B. 2.1 x 50 mm, 1 mL/min; 40 °C; 70/30 Water/ACN; 230 nm; analytes in elution order: nandrolone, methandienone, testosterone, epitestosterone, norethandrolone

Figure C. 5 µm Polar Embedded Amide, 4.6 x 250 mm, 1.5 mL/min; 35 °C; Mobile Phase A: 20mM Phosphoric Acid; Mobile Phase B: Methanol; 30-60% B in 15 min; HALO 90 Å RP-Amide, 2 µm, 2.1 x 100 mm, 0.5 mL/min; 35 °C; 30-60% B in 3.75 min; analytes in elution order: homovanillic acid, caffeic acid, syringic acid, vanillic acid, chlorogenic acid, sinapic acid, ferulic acid, p-coumaric acid, trans-cinnamic acid, resveratrol

Figure D. HALO 90 Å C18, 2 µm, 3.0 x 50 mm, 1.0 mL/min; 30 °C; 15/85 water/ACN; 254 nm; analytes in elution order: uracil, pyrene, decanophenone, dodecanophenone; HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm, 1.8 mL/min; 30 °C; 40/60 water/ACN; 254 nm; analytes in elution order: uracil, phenol, 1-chloro-4-nitrobenzene, naphthalene; HALO 90 Å C18, 5 µm, 3.0 x 50 mm; 0.5 mL/min; 30 °C; 40/60 water/ACN; 254 nm; analytes in elution order: uracil, phenol, 1-chloro-4-nitrobenzene, naphthalene

Figure E.

Particle	Column Length (mm)	Flow Rate (mL/min)	Sample inj (ul)
5 µm FPP, C18	4.6 x 150	2.00	2
HALO 90 Å C18, 5 µm	4.6 x 150	2.00	2
HALO 90 Å C18, 2.7 µm	4.6 x 100	2.00	2
HALO 90 Å C18, 2 µm	3.0 x 75	0.85	0.5

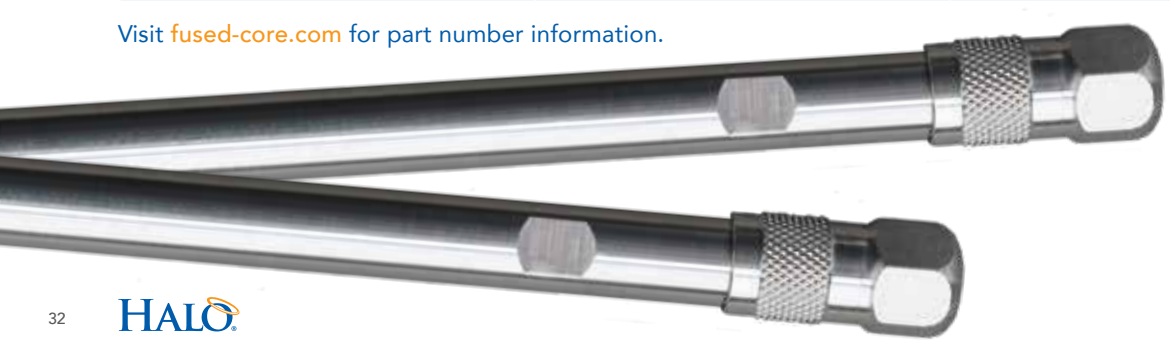
Analytes in elution order: aspirin, tolmetin, naproxen, fenoprofen, diclofenac, mefenamic acid, 35 °C; Mobile Phase A: 20 mM Phosphate buffer, pH 2.8; Mobile Phase B: ACN; 40/60 A/B

HALO® Columns

HALO 90 Å, 2 µm Columns

Available Bonded Phases	Available Column ID (mm)	Available Column Lengths (mm)
C18	2.1	5
AQ-C18	3.0	20
C8		30
Biphenyl		50
Phenyl-Hexyl		75
RP-Amide		100
PFP		150
ES-CN		250
Penta-HILIC		
HILIC		

Visit fused-core.com for part number information.



HALO 90 Å, 2.7 µm Columns

Available Bonded Phases	Available Column ID (mm)	Available Column Lengths (mm)
C18	0.075	5
AQ-C18	0.1	20
C8	0.2	30
C30	0.3	50
Biphenyl	0.5	75
Phenyl-Hexyl	1.0	100
RP-Amide	2.1	150
PFP	3.0	250
ES-CN	4.6	
Penta-HILIC	10	
HILIC		

Visit fused-core.com for part number information.

HALO 90 Å, 5 µm Columns

Available Bonded Phases	Available Column ID (mm)	Available Column Lengths (mm)
C18	0.075	5
AQ-C18	0.1	20
C8	0.2	30
C30	0.3	50
Biphenyl	0.5	75
Phenyl-Hexyl	1.0	100
RP-Amide	2.1	150
PFP	3.0	250
ES-CN	4.6	
Penta-HILIC	10	
HILIC		

Visit fused-core.com for part number information.

HALO®

HALO® Global Distribution Network

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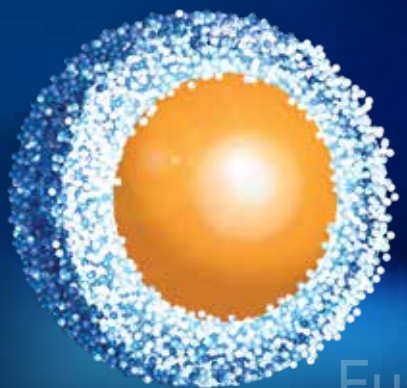

Highest efficiency RP-Amide ES-CN
C8 C18 Biphenyl Penta-HILIC PFP
2 µm Phenyl-Hexyl HILIC 5 µm AQ-C18
Fused-Core® 2.7 µm Small molecule

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