Outperforming Sub-2-µm Totally Porous Particles using Fused-Core Technology

Robert E. Moran, Stephanie A. Schuster, Barry E. Boyes, William L. Miles

Advanced Materials Technology Inc., Wilmington, DE 19810

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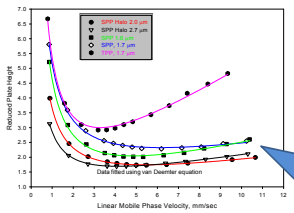
Abstract

Fused-core particles have shown distinct advantages over comparable totally porous particles (TPP) for separating molecules of all sizes and characteristics. The introduction of a new 2.0 µm particle size to the Fused-core product line continues to advance high speed, high efficiency separations into the future. These 2.0 µm Fused-core particles permit separation speeds and resolution improvements greater than sub-2-µm totally porous particles, but with about 20% lower column back pressure. Based on chromatographic theory, the trend of using smaller particle sizes has been accepted as a way to increase efficiency. This report describes the effect of these new particles on multiple factors of separation performance, including reduced plate height, column efficiency, back pressure, and resolution.

Comparing van Deemter Plots (h)

Reduced Plate Height vs. Mobile Phase Velocity Plots

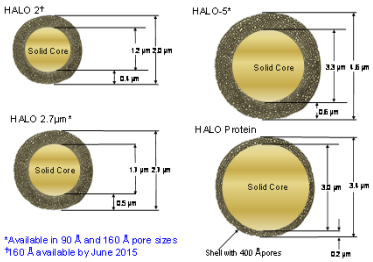
Columns: 50 x 2.1 mm; Instrument: Shimadzu Nexera; Solute: naphthalene
Mobile phase: Halo - 50/50 ACN/water, k = 6.3;
1.6 µm SPP - 48.5/51.5 ACN/water, k = 6.3; 1.7 µm SPP - 47/53 ACN/water, k = 6.2
1.7 µm TPP - 48.5/51.5 ACN/water, k=6.3; Temperature: 35 °C; Injection volume: 0.2 µL



Industry leading efficiency over broad range of flow rates

Engineered for high performance

HALO® Fused-Core Particle Designs

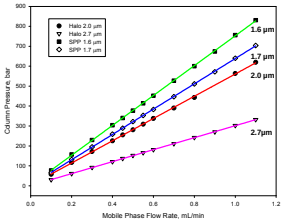


*Available in 90 Å and 160 Å pore sizes
†160 Å available by June 2015

Pressure vs. Flow Rate

Mobile Phase/Column Pressure Plots

Columns: 50 x 2.1 mm, C18; Instrument: Shimadzu Nexera; Solute: naphthalene
Mobile phase: Halo, 50/50 ACN/water, k=6.3; SPP 1.6 µm, 48.5/51.5 ACN/water, k=6.3
SPP 1.7 µm, 47/53 ACN/water, k=6.2; Temperature: 35 °C; Injection volume: 0.2 µL



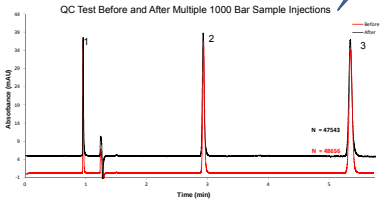
Incredible high pressure stability

Halo 2µm High Pressure Stability

Column : 3.0 x 150 mm Penta-HILIC
Instrument : Shimadzu Nexera
Injection Volume : 1.0 µL
Detection : 254 nm
Temperature : 25°C
Flow Rate : 0.6 mL/min

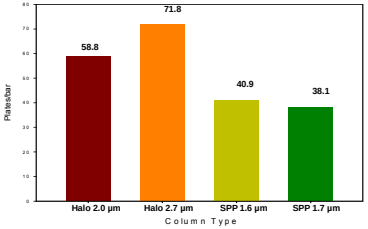
Mobile Phase :
88/12 ACN/ 0.1M Ammonium Formate pH:3
Pressure : 220 bar
Sample : QCH+
1. Acenaphthene
2. Adenosine
3. Cytidine

QC Test Before and After Multiple 1000 Bar Sample Injections



Plates per Bar

Columns: 2.1 x 50 mm C18; Instrument: Shimadzu Nexera; Solute: naphthalene
Mobile phase: 50/50 - 47/53 ACN/water; Flow rate: 0.5 mL/min; Temperature: 35 °C



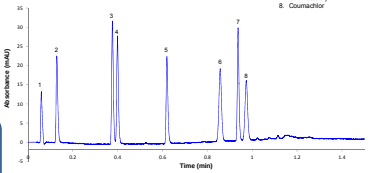
Ballistic Separation of Anticoagulants

Column : 2.1 x 30 mm HALO 2 C18, 2µm, 90 Å
Mobile phase A : 20 mM formic acid
Mobile phase B : 50/50 ACN/MeOH
Gradient :

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

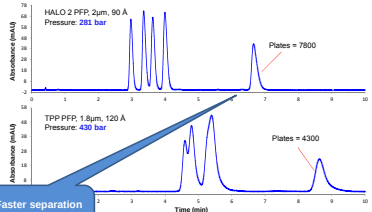
Flow rate : 1.1 mL/min
Temperature : 45 °C
Detection : 254 nm
Injection : 0.2 µL
Max Pressure : 430 bar

Peak Identities :
1. Uracil
2. 6,7-dihydroxycoumarin
3. 4-hydroxycoumarin
4. Coumarin
5. 6-chloro-4-hydroxycoumarin
6. Warfarin
7. Coumatetralyl
8. Coumachlor



Steroids Separation : HALO vs. TPP

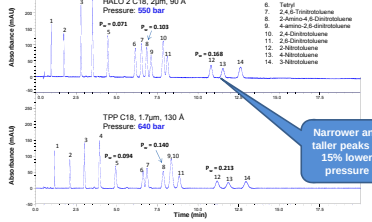
Column : 2.1 x 50 mm PFP
Mobile Phase : 57% H2O/ 43% MeOH
Flow rate : 0.3 mL/min
Temperature : 30 °C
Detection : 240 nm
Injection volume : 1.0 µL
Sample : Hydrocortisone, Prednisolone, Cortisone, Prednisone, Dexamethasone



Faster separation with high efficiency

Explosive Separation : HALO vs. TPP

Column : 2.1 x 100 mm
Mobile Phase : 72/28 H2O/MeOH
Instrument : Shimadzu Nexera
Flow rate : 0.4 mL/min
Temperature : 42 °C
Detection : 254 nm
Injection volume : 1.0 µL
Sample :
1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. Tetryl
7. 2,4,6-Trinitrotoluene
8. 2-Amino-4,6-Dinitrotoluene
9. 4-amino-2,6-dinitrotoluene
10. 2,4-Dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2-Nitrotoluene
13. 4-Nitrotoluene
14. 3-Nitrotoluene



Narrower and taller peaks at 15% lower pressure

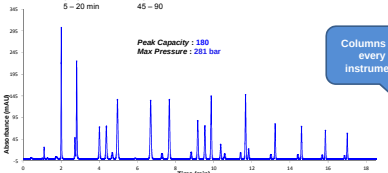
Carbonyl-DNPH High Resolution Separation

Column : 2.1 x 150 mm, HALO 2 C18, 2µm, 90 Å
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
Injection volume : 1.0 µL
Sample : DNPH Derivatized Carbonyl Compounds – Mix of Options #1 and #2 (19 out of 20 major components resolved)

Peak Capacity : 180
Max Pressure : 281 bar

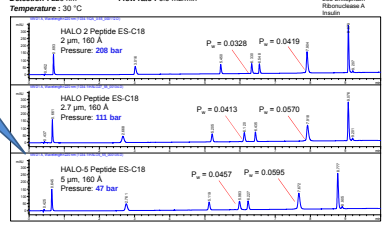


Peak Identities : Formaldehyde-2,4-DNPH, Acetaldehyde-2,4-DNPH, Acetone-2,4-DNPH, Acrolein-2,4-DNPH, Propionaldehyde-2,4-DNPH, Crotonaldehyde-2,4-DNPH, Butyraldehyde-2,4-DNPH, Benzaldehyde-2,4-DNPH, Cyclohexanone-2,4-DNPH, Isovaleraldehyde-2,4-DNPH, Valeraldehyde-2,4-DNPH, o-Tolualdehyde-2,4-DNPH, m-Tolualdehyde-2,4-DNPH, p-Tolualdehyde-2,4-DNPH, Hexaldehyde-2,4-DNPH, 2,5-Dimethylbenzaldehyde-2,4-DNPH, Heptaldehyde-2,4-DNPH, Octyl aldehyde-2,4-DNPH, Nonanal-2,4-DNPH, Decyl aldehyde-2,4-DNPH

Columns for every instrument

Peptide Separation : HALO comparison

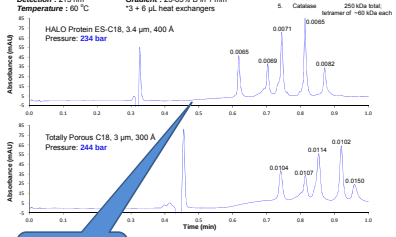
Columns : 3.0 x 50 mm
Instrument : Agilent 1100
Injection Volume : 2 µL
Detection : 220 nm
Temperature : 30 °C
Mobile Phase A : 90/10 H2O/ACN/0.1% TFA
Mobile Phase B : 30/70 H2O/ACN/0.1% TFA
Gradient : 0-55% B in 10 min.
Flow Rate : 0.6 mL/min
Sample: Gly-Tyr, Val-Tyr-Val, Met-Enkephalin, Angiotensin II, Leu-Enkephalin, Ribonuclease A, Insulin



Protein Separation : HALO vs. TPP

Columns : 4.6 x 100 mm
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
*3 + 6 µL heat exchangers

Peak Identities:
1. Ribonuclease A 13.7 kDa
2. Cytochrome c 12.4 kDa
3. Lysozyme 14.3 kDa
4. α-Lactalbumin 14.2 kDa
5. Catalase 250 kDa total; tetramer of ~60 kDa each



High resolution for protein separations

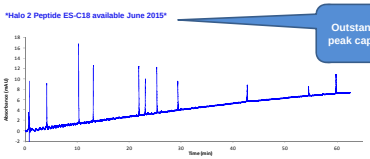
Peptide Separation : Peak Capacity

Columns : 2.1 x 150 mm
Instrument : Agilent 1200
Injection Volume : 2.5 µL
Detection : 215 nm
Temperature : 60 °C
Flow rate : 0.5 mL/min
Mobile Phase A : H2O + 0.1% TFA
Mobile Phase B : 80/20 ACN/H2O + 0.1% TFA
Gradient : 5 – 70% B in 90 min

Peak Identities (in order) :
1. Asp-Phe
2. Tyr-Tyr-Tyr
3. Angio 1-7 Amide
4. Bradykinin
5. Leu-Enk
6. Angiotensin II
7. Neurotensin
8. β-endorphin
9. Sauvagine
10. Melittin

HALO 2 Peptide ES-C18, 2µm, 160 Å
Peak Capacity : 453
Max Pressure : 501 bar

Halo 2 Peptide ES-C18 available June 2015

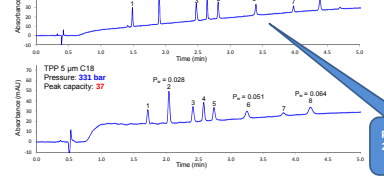


Outstanding peak capacity

Peptide Peak Capacity : HALO vs. TTP

Columns : 4.6 x 150 mm
Instrument : Agilent 1100
Injection Volume : 5 µL
Detection : 215 nm
Temperature : 40 °C
Flow rate : 4.0 mL/min
Mobile Phase A : H2O/0.1% TFA
Mobile Phase B : ACN/0.1% TFA
Gradient : 5 – 60% B in 5 min

Peak Identities (in order) :
1. Asp-Phe
2. Tyr-Tyr-Tyr
3. Bradykinin
4. Angiotensin II
5. Neurotensin
6. β-endorphin
7. Sauvagine
8. Melittin



Peak capacity 2.5 times TPP column

Conclusions

- Fused-Core columns consistently provide sharper peaks, lower reduced plate heights, and lower operating pressures when compared to totally porous particles of equivalent particle size
- Sharper peaks result in increased peak capacity and sensitivity for all separations
- 2µm Fused-Core columns deliver similar performance to sub-2µm columns at lower operating pressures
- Multiple particle sizes to fill the various objectives required in HPLC/UHPLC separations
  - Small particles for high speed and high resolution R&D separations
  - Larger particles for routine QA and QC separations
- Various pore sizes to accommodate the broad range of analyte molecular weights
  - 90Å – small molecules
  - 160Å – peptides
  - 400Å – proteins

Acknowledgments

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