High Quality Reproducibility in SPP HPLC Product Manufacturing

Stephanie Schuster, Harry Ritchie, Stephanie Rosenberg, Timothy Langlois, Joseph DeStefano
Advanced Materials Technology Inc., Wilmington, DE

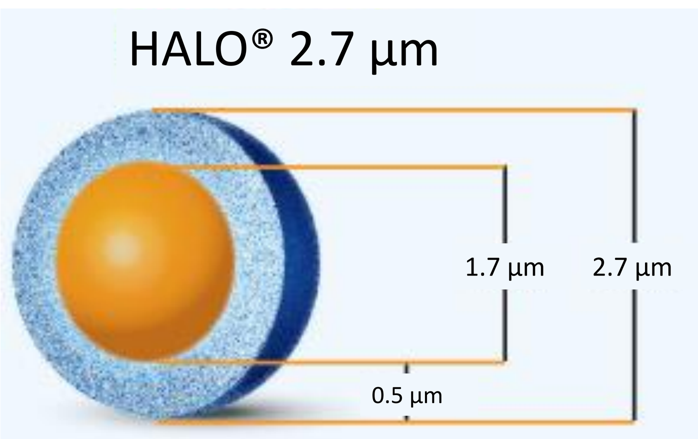
HPLC 2025 Bruges, Belgium
COL-07

Introduction

- Chromatographic column performance is impacted by particle size, particle type (fully porous/superficially porous), and particle size distribution
- Superficially porous particle (SPP) design enables equivalent separation efficiency in a larger particle size because the thickness of the porous shell is reduced relative to a fully porous particle, thus reducing the distance that analytes need to travel to fully access the stationary phase
- SPPs enables faster, more efficient mass transfer, which then translates to the ability to use faster flow rates
- Since the particle size is larger for equivalent efficiency compared to smaller diameter fully porous particles, the back pressure is lower and there is more opportunity to increase the flow rate to achieve rapid analyses
- Consistent, narrow particle size distribution that results from the manufacturing process of superficially porous particles leads to improved column bed stability and high efficiencies. The combination of these 3 properties (sub-3-µm size, superficially porous particle design, and narrow size distribution) give excellent column-to-column and lot-to-lot reproducibility as well as long column lifetimes for modern UHPLC methods.
- For Advanced Materials Technology (AMT), all of the manufacturing takes place in Wilmington, Delaware. High-quality raw materials are used throughout the particles' production as each step of the process is documented for traceability.

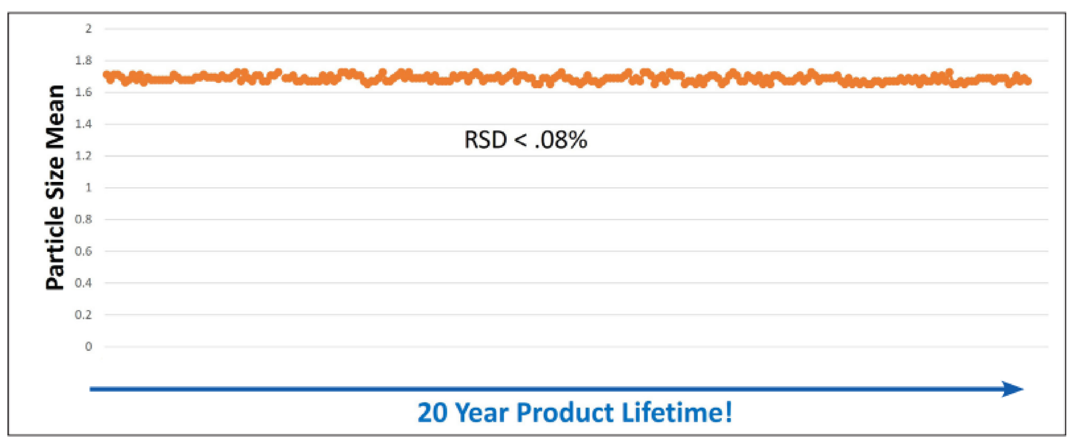
HALO® Particle Design

- HALO® particles are characterized by their solid silica core surrounded by a thin porous silica shell



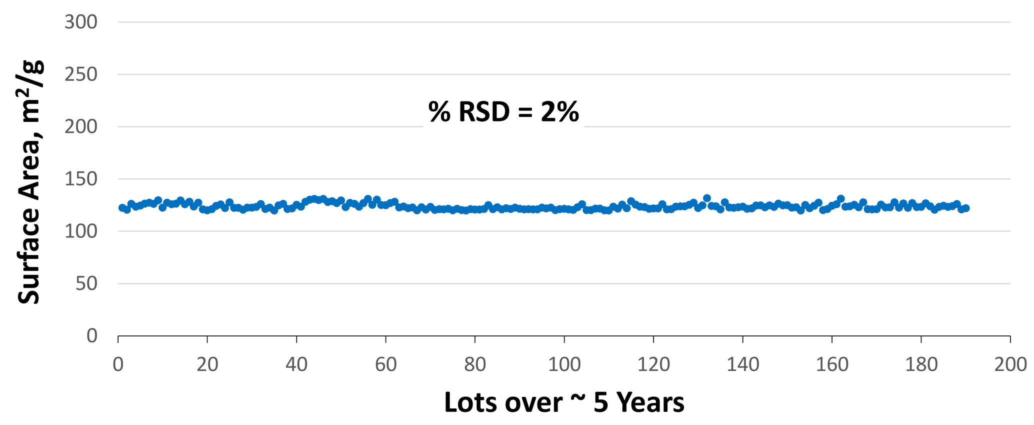
- AMT tightly controls the shell to particle size ratio
- Different combinations of shell and particle size are available with the porosity of the shell designed to accommodate various sizes of molecules from small drug pharmaceuticals to monoclonal antibodies and oligonucleotides
- 2, 2.7, 3.4, and 5 µm particle sizes are available
- Pore sizes of 90, 120, 160, 400, and 1000 Å are available

Creating the Core



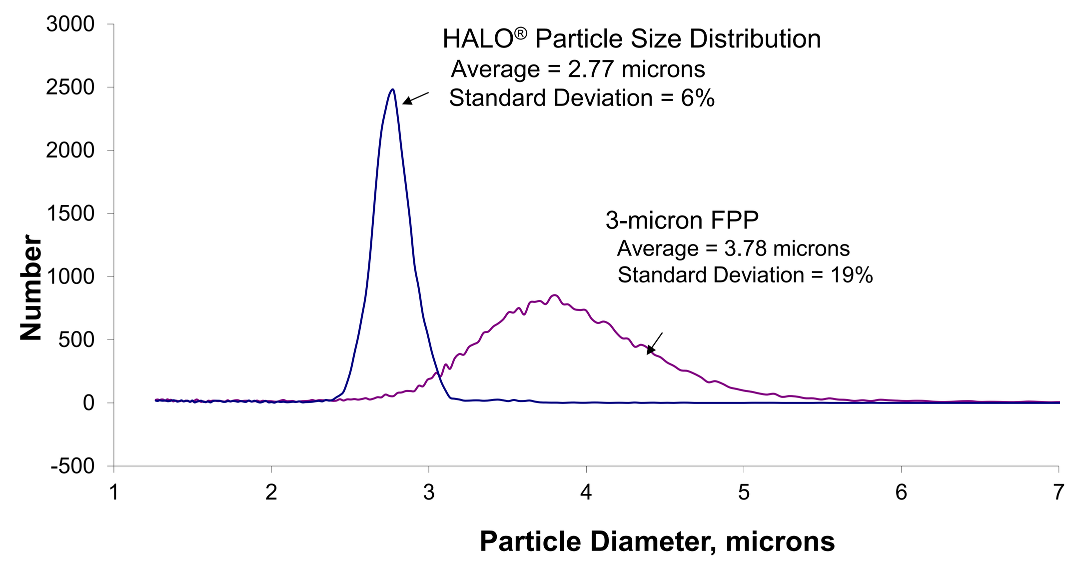
- Figure showing particle size mean vs. 20 years of time demonstrates how little variation there has been in the 1.7 µm core for the 2.7 µm HALO® particles over the span of the product lifetime
- Creating the solid silica core is characterized by more than 45 different in-process controls and 4 separate QC/QA tests
- Tight tolerances are in place to guarantee that the core is made reproducibly and consistently every time

Building the Shell



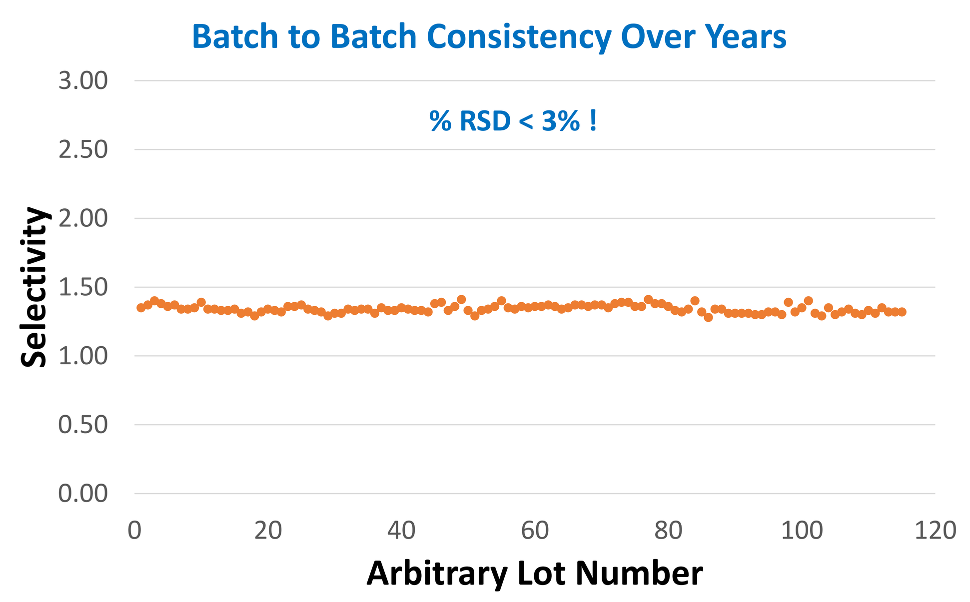
- Porous silica shell is added to the core by coating the core with several layers of highly pure, monosized silica nanoparticles
- AMT has complete control over the production and quality of the nanoparticles to produce a uniform pore size and shell thickness thus leading to a consistent and reliable surface area
- This consistency in surface area from lot to lot and year after year yields excellent chromatographic reproducibility
- Addition of the shell to the core consists of > 100 in-process controls and 7 rigorous QC/QA tests proving how diligently the thickness of the porous shell is controlled and monitored

Particle Size Distribution



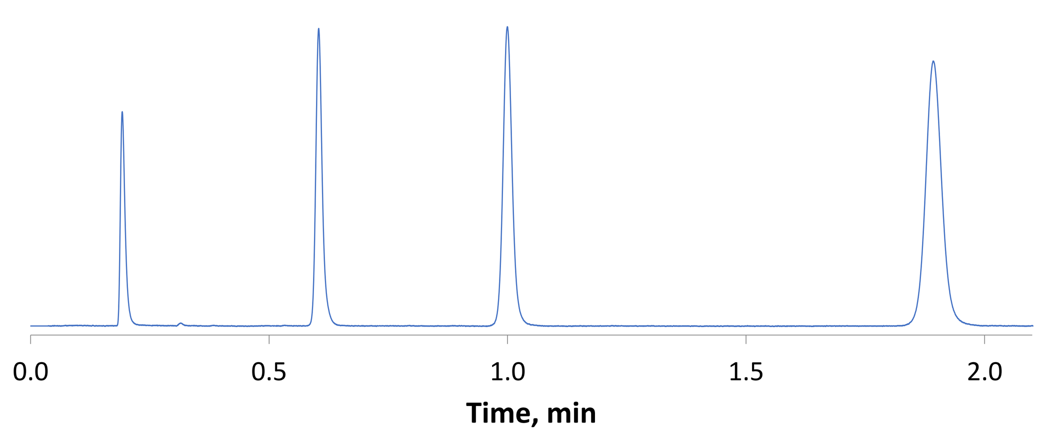
- Particle size distribution for 2.7 µm HALO® particles compared to typical 3 µm fully porous particles
- The AMT SPP process has a standard deviation more than 3 times smaller than the fully porous particle (FPP) manufacturing process
- The narrow particle size distribution contributes to high efficiency columns with stable chromatographic beds

Particle Surface Optimization



- AMT uses a proprietary method to create uniform and consistent surface silanols on the HALO® particles
- Once this step is complete the particles are ready for bonding or can be used as non-derivatized or bare silica
- AMT uses a challenging chromatographic test at pH 7 with a basic analyte to confirm the uniformity of the silanols on the particle surface

Bonding

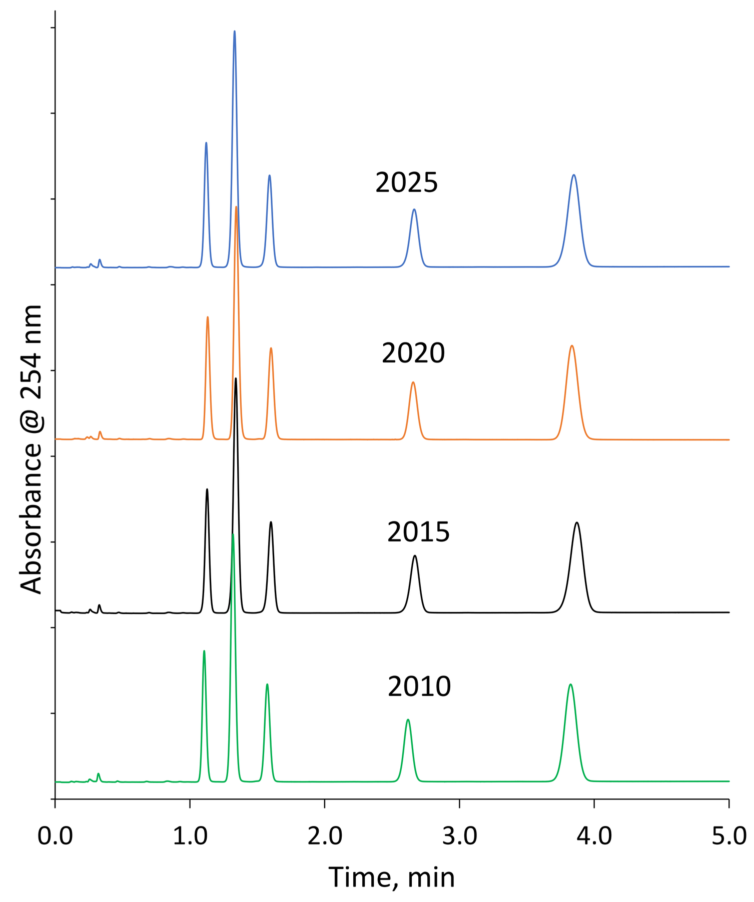


- Example QA test that is used for evaluating HALO® C18
- Test is run to qualify that the reaction to derivatize the surface with the stationary phase has been successfully completed as demonstrated by tight specifications for retention and selectivity
- Bonding process consists of 25 in-process controls and 20 QC/QA tests
- Various stationary phases are available for small molecule, biomolecule and application-specific columns, such as PFAS and PAH applications

2010 to 2025 C18 Lot-to-Lot Reproducibility

HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm, Isocratic 70/30 water/acetonitrile; Flow Rate: 0.8 mL/min; Temperature: 40 °C; Injection: 1.0 µL; Detection: 254 nm

Peak Identities:
1. Nandrolone
2. Methandienone
3. Testosterone
4. Epitestosterone
5. Norethandrolone

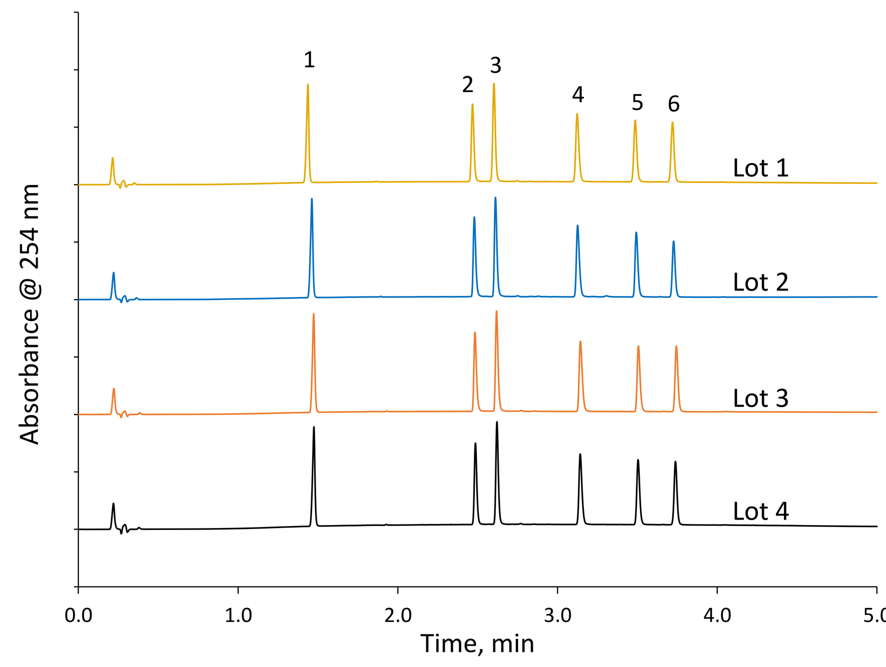


- To illustrate the lot-to-lot consistency of HALO® columns over a broad time span, columns loaded using archive lots of 2.7 µm HALO® C18 from 2010, 2015, and 2020 were tested and compared to a column loaded with a current lot of 2.7 µm HALO® C18 manufactured in 2025
- The figure shows the highly reproducible results for the HALO® C18 lots spanning 15 years
- Average %RSD over all peaks for retention time is 0.8%.

HALO® Elevate C18 Lot-to-Lot Reproducibility

2.1 x 50 mm, A: 0.1% NH_4OH, pH 10.7; B: Acetonitrile; Gradient: 5-95% B in 4 min;
Flow Rate: 0.4 mL/min; Back Pressure: 134 bar; Temperature: 40 °C; Injection: 0.5 µL
Sample Solvent: 65/35 0.1% NH_4OH/ACN; Wavelength: PDA, 254 nm, LC System: Shimadzu Nexera X2

Peak Identities:
1. Butyl Paraben (neutral)
2. Doxylamine (base)
3. Chlorpheniramine (base)
4. Doxepin (base)
5. Amitriptyline (base)
6. Trimipramine (base)



- Four different lots of HALO® Elevate C18 were tested using a mix containing a neutral compound and 5 basic compounds
- Average %RSD across all of the compounds for retention time was 0.4%

Loading and QC Testing

- More than 5 years' worth of efficiency data for 90 Å HALO® C18, 2.7 µm, 4.6 x 150 mm columns
- AMT uses a proprietary automated process to pack HALO® columns to eliminate any variation in packing quality
- 20 in-process controls and 6 QC/QA tests are used
- Every column is tested and must pass tight criteria for theoretical plates, tailing factor, and selectivity before it is qualified as acceptable

Summary

- Production of HALO® columns is tightly controlled throughout the entire manufacturing process: from core to final QC testing
- AMT column users should have confidence that the product received today will be of the highest quality and consistent throughout the lifetime of the methods developed with it
- Highly reproducible method results are attributed to the reliable quality that is built into SPP manufacturing by AMT