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Are Sub-2 μm Particles Best for Separating Small Molecules? An Alternative

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Abstract

Superficially porous particles (SPP) in the 2.5 - 2.7 μm range provide almost the same efficiency and resolution of sub-2 μm totally porous particles (TPP), but at one-half to one-third of the operating pressure. The advantage of SPP has led to the introduction of sub-2 μm SPP as a natural extension of this technology. While short columns of both SPP and TPP sub-2 μm particles allow very fast separations, the efficiency advantages of these very small particles often are not realized nor sufficient to overcome some of the practical limitations and disadvantages of such small particles. Advantages and disadvantages of columns packed with sub-2 μm particles are described for comparison with the characteristics of larger particles. The authors conclude that while sub-2 μm particles have utility in research studies, columns of larger particles are often better suited for most applications. A suggested 2.0 μm superficially porous particle diameter retains many of the advantages of sub-2 μm particles, but minimizes some of the disadvantages. The characteristics of these new 2.0 μm SPP are described in studies comparing some present sub-2 μm SPP commercial columns for efficiency, column bed homogeneity and stability.

Keywords

HPLC columns; advantages and disadvantages of sub-2 μm particles; superficially porous particles; core shell particles; totally porous particles; column stability; 2.0 μm Fused-Core® particles

1. Introduction

The diameter of HPLC particles has been shrinking through the years so that sub-2 μm totally porous particles now are used widely for separating small molecules. Short columns of these particles permit very fast separations, and several manufacturers have made columns of these materials commercially available. Columns of superficially porous

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particles (SPP) have shown even further efficiency advantages, so that some users prefer these over totally porous particles (TPP) for the rapid separation of small molecules using reduced operating pressures [1-6]. Columns of sub-2 μm SPP (i.e., core-shell, Fused-Core®, porous shell, solid core etc.) have become available, and are especially used for research studies [7,8].

The question of whether columns of sub-2 μm SPP are really needed for separating small molecules has been discussed for some time. Theory certainly predicts efficiency advantages for such materials, based on smaller particle size [9]. Several years ago the authors synthesized sub-2 μm SPP and studied their properties and utility [10]. We found, as theory predicts, that these particles do allow for higher efficiency than larger particles, producing very fast separations with short columns. However, we also found some limitations that show some decided practical disadvantages of using columns of sub-2 μm SPP, not unlike those observed with totally porous particles of very small diameters. This presentation attempts to point out some advantages and disadvantages of sub-2 μm particles, and presents the authors' view on the use of these materials. We also offer a potential alternative that minimizes some of the disadvantages and retains some of the advantages of small particle technology.

Compared to columns of larger particles, columns of sub-2 μm particles, both totally porous and SPP, offer some distinct advantages which have attracted users:

- Efficient, fast separations permit more separations per unit time (improved productivity)
- Sharper peaks provide for higher detection sensitivity
- Faster separations use less mobile phase solvent per analysis
- Large plate numbers are obtained with longer columns for improved peak capacity (but requiring very high pressures)
- Provides the user with perceived state-of-the-art technology

On the other hand, literature reports and our own studies of columns with sub-2 μm Fused-Core particles have shown some of the limitations and practical disadvantages of this small particle technology:

- For optimum performance, columns of sub-2 μm particles need special, expensive high-pressure instruments capable of operation at 1000 bar or more (pressures of 400 - 600 bar often insufficient to obtain the mobile phase velocity at the plate height minimum)
- Instruments with minimum extra-column volumes are required for acceptable separation performance - modest band dispersion contributions will result in disappointing performance
- Small I.D. connecting tubing and a low-volume flow cell are required to reduce extra-column effects but add significantly to operational pressure
- Operation at high pressures can result in more frequent instrument repairs

- Instrument repairs and upkeep more costly and not as user-friendly as for instruments only capable of working at lower pressures
- Columns of sub-2 μm particles require very small-pore frits (0.2 - 0.5 μm) to retain particles. Compared to 2 μm pore size frits used for large particles, these narrow-pore frits are considered to be more easily fouled (plugged).
- 3 mm I.D. columns required to minimize frictional heating effects at high pressures that can cause loss of column efficiency [11-13]
- High pressures can cause changes in retention and separation selectivity [11] resulting in problems to convert separations made with small particles to columns of larger particles suited for routine analyses
- Columns may not exhibit the expected efficiency or stability because smaller particles are more difficult to pack into homogeneous beds [5,14]

In many practical situations, the disadvantages of using columns of sub-2 μm particles outweigh the advantages, particularly for routine analyses involving less technology-based personnel. In view of this we undertook a study of SPP that was designed to find a practical compromise: possess some of the advantages of smaller particles, but fewer of the disadvantages. We find that 2.0 μm (Fused-Core) SPP appear to be a practical compromise. This presentation points out some of the technical advantages of using 2.0 μm SPP as an effective approach for separating small molecules.

2. Experimental

2.1 Particles and packed beds

Figure 1 on the left shows a scanning electron micrograph (SEM) photograph of the 2.0 μm SPP synthesized in this laboratory. Also shown below the photograph for these new particles is a particle size analysis of these particles showing a mode of 2.006 μm , mean of 2.016 μm , median of 2.004 μm with a standard particle size deviation of 0.111 μm and coefficient of variation of 5.5 % measured using a Coulter Multisizer 3 instrument (Fullerton, CA). Manual size-measurements of 84 particles using the shown SEM photograph with the included scale produced a mean particle size of 2.01 μm with a standard deviation of 0.08 μm and a coefficient of variation of 3.8%. On the right of Figure 1 is a graphic of these SPP indicating that the particles have a 1.2 μm solid core and a 0.4 μm -thick porous outer shell with 90 Å pores. The surface area of these high-purity Type B silica particles is 120 m^2/g . Surface areas and related measurements were measured by nitrogen adsorption with a Micromeritics Tristar II instrument (Norcross, GA). Cross-section TEM images and pore size distribution plots were very much like the results shown for 2.7 μm fused-core particles in Reference 2 so are not included here.

Reproducibility for preparing packed beds of the 2.0 μm SPP is illustrated by the van Deemter plots in Figure 2. Data were obtained on two columns from one lot of particles and one column from another lot. The data for columns of the first lot is so close that the same van Deemter data fit was found. Very small differences are seen for the second lot, with aggregate data indicating good particle packed bed properties. As indicated later in this

presentation, a newly developed proprietary column filling method was employed for the 2.0 μm particle columns that ensures not only excellent column efficiency but good column bed stability when operated at the 1000 bar limit suggested for these columns.

The HPLC instrument used in this study was a low-dispersion Shimadzu Nexera, utilizing LC-30AD solvent pumps and the SPD-M30A photodiode array detector fitted with a 1 μL flow cell. Extra-column band broadening for this instrument was measured using the relationship [15]:

$$\mu'_{2,ex} = F_v^2 (W_{1/2})^2 / 5.545$$

where, $\mu'_{2,ex}$ = extra-column variance, μL^2 ; F_v = flow rate, mL/min and $W_{1/2}$ = peak width in minutes at half-height. The extra-column volume was calculated by:

$$E.C.V = F_v W_{4\sigma}$$

where $W_{4\sigma}$ is the four sigma peak width in minutes. These values were obtained by replacing the column with a zero dead volume connector using naphthalene as the test solute, with a mobile phase of 60/40 ACN/water at 35 $^{\circ}\text{C}$ and an injection volume of 0.20 μL . Table 1 compares the values found for the Nexera with the heat exchanger (H.E.) with data reported for other popular instruments [16]. The Nexera was measured to have a total extra-column instrument volume of about 6.7 μL with a $\mu'_{2,ex}$ range of 2.7 - 3.1 μL^2 from 0.2 - 2.00 mL/min. These results were used to correct for Nexera instrumental band dispersion for the non-corrected/corrected tests reported later in Figure 7. Corrections were made for each measurement in the data set.

2.2 Chemicals and other equipment

Silane for the C18 bonding reaction was obtained from Gelest, Inc. (Morrisville, PA). Acetonitrile (ACN) was obtained from Sigma-Aldrich (St. Louis MO), and trifluoroacetic acid (TFA) was from Pierce Chemicals (Rockford, IL). Solutes used for tests were from Sigma-Aldrich and used as received. Scanning electron micrographs were prepared by Micron, Inc. (Wilmington, DE).

Columns of superficially porous HALO 2.0 μm silica particles with C18 stationary phase were prepared at Advanced Materials Technology, Inc. (Wilmington, DE). Commercial columns of superficially porous particles and totally porous particles were obtained from Waters Corporation (Milford, MA) and Phenomenex (Torrance, CA). These columns were used as received (without any previous use) to obtain the data presented herein. Column dimensions for the various studies are given in figure captions. Corrections for instrumental extra-column band broadening were not applied to the data obtained in this study, except for results specifically designated. Peak widths (Full Width Half Max) were used for measuring plate numbers.

3. Results and Discussion

3.1 Effects of particles and particle size

It is well known and noted in the Introduction that as particle size is decreased, it becomes more difficult to pack and prepare the column particle bed to meet expected efficiency. This is illustrated by the data in Figure 3 showing the effect of particle size on the reduced plate heights of SPP (Fused-Core) in the 2.0 to 4.6 μm range. While more recent column filling procedures could produce slightly better results, the data clearly show that as the particle size is increased, reduced plate heights get smaller, indicating better homogeneity in packed beds for the larger particles. This effect has also been noted for totally porous particles (TPP), so the trend appears general [14]. The conclusion is that it is more difficult to pack columns of small particles with expected efficiency, based on results for larger particles. Future improvements in procedures for packing columns may eliminate this difficulty, but this is the current situation.

Plots showing plate heights versus mobile phase velocity (van Deemter plots) for columns of several SPP and a totally porous particle (TPP) are shown in Figure 4. As expected, the smaller SPP show the smallest value at the plate height minimum; larger particles show larger plate heights. The column of TPP does not provide the expected efficiency based on particle size for unknown reasons, but this observation is consistent with earlier reports [17]. Compared to results for the SPP, the steeper increase in plate height with mobile phase velocity increase for the conditions used suggests an additional mass transfer limitation for these totally porous hybrid particles. Alternatively, as previously suggested in several other studies [6,12], these totally porous particles have poorer heat transfer properties than SPP that have solid silica cores, so that larger deleterious effect can occur as a result of frictional forces at higher mobile phase velocities. For verification, the van Deemter plot for this TPP column was repeated one month later with the same results. It was not possible to calculate an accurate van Deemter A-term for this TPP column (A-term always approached a calculated zero value), so it appears that the results for this particle type do not fit the simple van Deemter relationship under the conditions tested. The same results were obtained with a fit of data to the Knox equation [9]. Other studies have indicated that the actual particle size of these 1.7 μm -designated particles is actually closer to 2.0 - 2.1 μm [18], which may partially account for the plate height results found.

Plots of reduced plate height versus mobile phase velocity (Figure 5) are more informative regarding the homogeneity of the column packed beds. In this case, the 2.0 and 2.7 μm SPP show almost the same and the smallest reduced plate height (about 1.75), suggesting closely similar and excellent packed bed homogeneity.

The A-, B-, and C-term coefficients of the van Deemter equation ($h = A + B/v + Cv$; where h = reduced plate height, v = mobile phase velocity) calculated for the plots in Figure 5 are given in Table 2. These coefficients more quantitatively represent the differences in the characteristics of the various columns studied. For example, the B-terms indicate that HALO SPP apparently have a smaller longitudinal diffusion contribution to the reduced plate height than the other particles studied, suggesting some favorable difference in diffusional properties within the porous structure.

Kinetic plots for some of the columns studied are shown in Figure 6 [19,20]. These plots of column dead time $\log t_0(\text{s})$ versus $\log$ plate number N were calculated using the maximum pressure specified by the manufacturer for each of the columns. As predicted by theory, columns with the smallest particles can produce the highest efficiency in the shortest time frame. However, because of lower pressures, longer columns with larger particles can be used to produce a larger plate number, but at a cost of increased separation time.

It might be argued that because of extra-column effects for the particular instrument used for these studies, one might expect columns of smaller particles to show better performance if an instrument with an even smaller extra-column volume was used. Only some specially-designed instruments show smaller extra-column volumes than the Nexera instrument used to gather these data. However, the Nexera actually has a smaller extra-column dead volume than many of the HPLC instruments in current use, as shown in Table 1. To test the effect of using the Nexera, the reduced plate heights of Figure 5 were corrected for band dispersion, and the results with corrected reduced plate heights for three of the study columns are shown in Figure 7. Data for the 1.6 and 2.0 μm SPP are shifted to slightly lower values, as expected with correction, but values for the 2.7 μm SPP are essentially unchanged. The same general results as in Figure 5 are obtained. Bed homogeneity for the 2.0 and 2.7 μm Fused-Core SPP columns appear superior to the 1.6 μm SPP, suggesting better packed beds than for smaller-diameter particle columns. The essentially equivalent minimum reduced plate heights for the 2.7 and 2.0 μm columns also suggests that the packed beds for these particles have strongly similar properties.

The effect of particle size for Fused-Core SPP on the reduced plate height is shown in Figure 8. As anticipated, the steepness in the C term dominated region of uncorrected data for 2.0 μm particles is lower with increased mobile phase velocity, compared to 2.7 and 4.6 μm particles, indicating a smaller C-term for the van Deemter relationship. Extra-column-volume corrected reduced plate height values produced plots (not shown) in which the reduced plate height minimum for all particle sizes was 1.54 ± 0.03 . This result confirms that the special column packing procedure utilized in this study is effective in producing a homogeneous packed bed for Fused-Core particles of all sizes, including 2.0 μm .

3.2 Pressure effects

Theory predicts that as the mobile phase flow rate is increased, column back pressure increases linearly [9]. This effect is confirmed for several of the particles investigated, as shown in Figure 9. As expected, lower pressures were found for larger particles and the highest pressure for the smallest particles studied.

Particle size and the homogeneity of the packed column bed can have a strong effect on the plate number that can be generated as a function of the pressure required.

Figure 10 shows the plates per bar pressure ratio at the plate height minimums for the SPP of this study. Because of lower pressures required and excellent packed bed homogeneity (see Figure 5), larger particles show the higher plates/bar, with the 2.7 μm SPP showing the maximum for the smaller particles studied. These results are influenced by the fact that while plate number increases linearly with decreasing particle size, the pressure increases

with the square of the decrease in particle size. Therefore, plates/pressure is favored for columns with larger particle sizes.

3.3 Frit effects

The porosity of the frits that are used to retain particles within a column often determines how long the column may be useful for separating “real” samples. Depending on the separation parameters and the type of samples to be separated, columns can last satisfactorily for a hundred to several thousand injections. Columns of larger particles (e.g., 2.7 and 5 μm) use inlet frits with 2 μm porosity which tend to resist fouling by pluggage with small solid particulates that often are present in samples and in mobile phases. This advantage for stable routine separations, coupled with the higher column efficiency compared to columns of totally porous particles, led to the development of 5 μm SPP [21]. On the other hand, columns of sub-2 μm particles use 0.5 - 0.2 μm porosity frits to retain these very small packing particles. These small-porosity frits can be more susceptible to fouling with particulates which can shorten the useful lifetime of such columns. Manufacturers of sub-2 μm columns often prescribe that the user should treat samples and mobile phases with 0.2 μm filters (or to centrifuge) before separations are attempted to minimize problems with column fouling [22]. Such actions are especially directed toward samples that originate from natural sources, such as plant and animal tissue, environment, etc. The 2.0 μm SPP columns described in this document use 1.0 μm porosity frits in the column inlet, which should reduce the plugging of frits by particulate fouling.

3.4 Column stability

Users are always concerned about the stability of columns, especially when high pressures are needed for the desired separation. To determine the stability of columns of the 2.0 μm SPP, a test was designed to measure the effect of sample injection at the maximum pressure recommended for these columns. For this study, the columns were first tested at about 200 bar with three replicate sample injections to obtain an original (BEFORE) plate number. The pressure was then increased to the maximum and the column again tested with three injections. A flow rate of 2.50 mL/min was required to reach the ~1000 bar level for these 50 $\times$ 2.1 mm I.D. columns under the test conditions used. Therefore, the test presents the challenge of injection-induced pressure pulses at both high mobile phase flow rate and high pressure. In addition, very high speed data collection procedures are mandated to accurately capture peak shape and width. To ensure temperature and bed equilibrium, the column was then allowed to stand overnight without any flow, then again tested with three replicate injections at about 200 bar to produce a final efficiency value (AFTER). Table 3 shows results for six columns prepared from a single lot of particles. After the high pressure injections, the pressure of these columns increased very slightly (actually within the precision of measurements), and the efficiency of the columns showed a very small loss (8% in plate number or a <4% loss in resolution). These results confirm packed bed stability for these 2.0 μm SPP under unusual flow rate and pressure conditions. The high pressure stability of these 2.0 μm SPP columns also is illustrated by the chromatograms in Figure 11. The bottom trace (grey) is the initial separation under the initial (BEFORE) conditions, and the upper trace (black) is after sample injections (AFTER) into the column at ~ 1000 bar as just described.

3.5 Peak Capacity

Compared to columns of sub-2 μm particles, the lower back pressure of 2 μm SPP allows the use of longer columns at lower pressures, which then translates into higher peak capacities for complex separations. As illustrated in kinetic plots of Figure 6, 2 μm SPP columns generate higher efficiency at longer times (higher t_0 values) which allows the use of longer columns. Figure 12 shows the separation of a tryptic digest of apomyoglobin using two connected columns with HALO 2 C18 of 100 mm length each. The peak capacity n_{pc} obtained for this separation was 459 with an initial back pressure of ~ 700 bar. The following equation was used to calculate gradient peak capacities in this work:

$$n_{pc} = (t_f - t_i) / W_{4\sigma}$$

where, t_i is the time for initial measurable peak in the gradient, t_f is the time for final peak and $W_{4\sigma}$ is the average four-sigma width in time for the peaks in the chromatogram. All major peaks are separated with a resolution of unity or better. An equivalent separation using a 1.6 μm SPP column would require an initial back pressure of ~ 1100 bar, which cannot be achieved on instruments limited to operation at 1000 bar.

4. Conclusions

We conclude from this study, our previous experiences, and from literature references that for the separation of small molecules, columns of sub-2 μm particles are useful for some applications, but often are not required. The advantages of these very small particles often are not sufficient to overcome the practical disadvantages and limitations for many small molecule applications. It is well known that larger SPP (e.g., 2.5 - 2.7 μm) provide almost the same separation efficiency and resolution as sub-2 μm totally porous particles, but at one-half to one-third the operating pressure [1,2,23]. Other studies have shown that small molecules do not require the shorter diffusion paths of small particle-size SPP for adequate mass transfer and good column efficiency [12,24], further suggesting that sub-2 μm SPP are not required for small molecule separations. The credibility of 2.7 μm SPP for separating small molecules has been further extended by the recent commercialization of 2.7 μm SPP by companies that have been prime promoters of ultra high-pressure liquid chromatography (UHPLC) separations using sub-2 μm totally porous particles.

In this study we suggest the utility of 2.0 μm SPP that retains many of the advantages of sub-2 μm particles and minimizes some of the disadvantages. Columns of these 2.0 μm SPP display unusual high efficiency, showing reduced plate heights equivalent to larger (2.7 μm SPP), indicating excellent and similar packed bed homogeneity as particle size was reduced. Columns of these particles are packed with special proprietary techniques that not only provide high bed homogeneity, but also maintain bed stability after sample injections at pressures up to ~ 1000 bar. These 2.0 μm SPP operate with significantly lower pressures than SPP or TPP sub-2 μm particles, allowing the use of longer columns with higher plate numbers for increased peak capacity separations.

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Highlights

- 2.0 μm superficially porous particles (SPP) show advantages of sub-2- μm particles with fewer disadvantages.
- Sub-2- μm column performance reduced by instrument and column packing limitations.
- 2.0 μm SPP columns exhibit reduced plate heights equivalent to larger particles.
- 2.0 μm SPP UHPLC columns are stable to 1000 bar.
- 2.0 μm SPP have a favorable efficiency to back pressure ratio compared to sub-2- μm particles.

SEM image of 2 μm HALO particles

Figure 1.

HALO 2.0 μm Particle Design. Left: Scanning electron micrograph of particles. Mode: 2.006 μm; Mean: 2.016 μm; Median: 2.004 μm; Standard Deviation: 0.111 μm; CV: 5.5 %; Surface area: 120 m²/g. Right: Graphic of particle with dimensions.

Figure 2.

Column Production Reproducibility. Columns: 50 mm $\times$ 2.1 mm HALO 2.0 μ m C18; Solute: naphthalene; Instrument: Shimadzu Nexera; Mobile phase: 50/50 ACN/water, $k = 6.3$; Temperature: 35 $^{\circ}$ C.

Figure 3.

Reduced Plate Height van Deemter Plots for Different Size Particles. Column: 50 mm $\times$ 3 mm; Mobile phase: 60/40 ACN/water; Instrument: modular Shimadzu pump and detector (Model SPD-10A VP), semi-micro flow cell (2.5 μL), low-volume Rheodyne 8125 manual injector; Solute: naphthalene, $k = 3.5$; Temperature: 22 $^{\circ}\text{C}$.

Figure 4.

Plate Height van Deemter Plots for Different Size Particles. Columns: 50 mm $\times$ 2.1 mm; Instrument: Shimadzu Nexera; Solute: naphthalene; Mobile phase: HALO: 50/50 ACN/water; $k = 6.3$; 1.6 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, $k = 6.3$. Injection volume: 0.2 μL ; Temperature: 35 $^{\circ}\text{C}$.

Figure 5. Reduced Plate Height van Deemter Plots for Different Size Particles. Conditions same as Figure 4.

Figure 6.
Kinetic Plots for Particles. Calculated from data in Figure 5.

Figure 7. Corrected Reduced Plate Height Plots for SPP Columns. Conditions same as for Figure 4, except plate heights corrected for instrument extra-column band broadening effects.

Figure 8. Effect of Particle Size for Fused-Core SPP on Reduced Plate Height. Conditions same as for Figure 4.

Figure 9.
Column Pressures as a Function of Mobile Phase Flow Rate. Conditions same as for Figure 4.

Figure 10.

Plate Numbers per Pressure for SPP Columns. Conditions same as for Figure 4.

Figure 11.

Column Stability at High Pressure. Column: 50 mm $\times$ 2.1 mm HALO 2.0 μ m C18; Instrument: Shimadzu Nexera; Mobile phase: 85/15 ACN/water; Flow rate: 0.5 mL/min; Temperature: 25 $^{\circ}$ C; Detection: 254 nm; Sample injections as described in text; Peak identities: 1 - uracil, 2 - pyrene, 3 - decanophenone; 4 - dodecanophenone; grey trace: before 950 bar; black trace: after 950 bar.

Figure 12.

High Peak Capacity on Coupled Columns. Columns: 2 coupled 100 mm $\times$ 2.1 mm HALO 2.0 μ m C18; Instrument: Shimadzu Nexera; Mobile phase A: water/0.1% TFA; Mobile Phase B: 80/20 ACN/water/0.1% TFA; Gradient: 5-45% B in 60 min; Flow rate: 0.5 mL/min; Temperature: 60 $^{\circ}$ C; Detection: 215 nm; Injection volume: 2 μ L of [2 μ g/ μ L] apomyoglobin tryptic digest. Pressure: $\sim$ 700 bar at the start of gradient; Peak capacity: 459. Insets show high resolution of segments from 0 – 4.5 min and 15 – 23 min.

Table 1

Comparison of Extra-column Band Broadening for Instruments

Instrument	Flow rate range, mL/min	μ' , ex Range, μL^2	E.C.V., μL
Nexera	0.20 - 2.00	2.7 - 3.1	6.7
Agilent 1290 Infinity ^a	0.05 - 0.80	2 - 9	10.2
Waters Classic Acquity ^a	0.05 - 0.80	1.5 - 4	7.4
Waters H-Class Acquity ^a	0.05 - 0.80	0.7 - 2.1	3.9
Waters I-Class Acquity ^a	0.05 - 0.80	0.2 - 0.6	2.1

^aData from Reference 16

Table 2

Van Deemter Coefficients for Figure 2

Particle	A-term ^a	B-term	C-term
2.0 µm HALO SPP	0.700	2.85	0.0956
2.7 µm HALO SPP	0.723	2.02	0.117
1.6 µm SPP	0.349	4.11	0.175
1.7 µm SPP	1.016	4.03	0.106
1.7 µm TPP	N/A	4.92	0.457

^a Coefficient data obtained by fitting the van Deemter equation using SigmaPlot v. 10.0 software N/A = Data fit for A-term for this column was not found

Table 3**Column Stability Test**

Columns	Ave. Injection Pressure, bar	Ave. Test Pressure, bar	Ave. Plate Number	Ave. Plate Number Loss
6 - Halo 2.0 μm	980 $\pm$ 22	BEFORE: 181 $\pm$ 4 AFTER: 186 $\pm$ 4	15570 $\pm$ 330 14320 $\pm$ 550	- 8%