

Evaluation of Six Core Shell Columns Based on Separation Behaviour and Physical Properties

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In this study, the separation behaviour and physical properties of Kinetex C18, Accucore C18, Cortecs C18, PoroShell EC-C18, Ascentis Express C18 and SunShell C18 core shell particle columns were evaluated. The evaluation and performance measurements were done using the Tanaka Method [1] with reference to retention factor, hydrogen bonding capacity, hydrophobicity and steric selectivity. In addition to peak shape profiles, the core shell columns were evaluated for loading capacity of amitriptyline under neutral pH condition. The stability of the core shell columns at acidic pH 1 and basic pH 10 conditions, at elevated temperatures was evaluated. In addition to determination of particle physical properties such as specific surface area, pore volume, pore diameter and carbon loading of each C18 packing material, we studied the forced degradation of alkyl chains by sintering at 600 degree Celsius for 8 hours. This study revealed significant differences for the six core shell C18 particles with regard to separation behaviour, stability and physical properties. When compared to fully porous silica C18 sorbents, this difference may be attributed to the diversity of each manufacturing method and bonding technique. Of the six columns tested, SunShell C18 showed the largest retention factor and the highest physical stability despite moderate carbon loading (7%), while the lowest retention factor and the lowest stability in regards to chromatographic performance and endurance at elevated temperature and pH could be correlated with the core shell column which had the lowest carbon loading and the lowest specific surface area.

Introduction

Columns packed with 2.6 μm and 2.7 μm core shell particles (also alternatively termed solid core, porous shell or superficially porous particles) have been widely documented for HPLC and UHPLC. These core shell columns show comparable column efficiency to 1.8 μm fully porous particles along with an approximate 50% reduction in backpressure in comparison to sub-2 μm fully porous particle (2). Core shell particles consist of a solid core of 1.6 μm to 1.7 μm diameter and an outer porous silica layer of 0.5 μm thickness giving a larger particle measuring 2.6 μm to 2.7 μm and lower operating backpressure in comparison with sub-2 μm fully porous particle (back pressure largely being governed by particle size). The volume ratio between the porous silica layer of the core shell particle and the

fully porous particle of the same particle size is 75% and does not have a significant impact on the separation performance [2].

The advantage of the core shell particles is thought to arise from the structure of the core shell particle and narrow particle size distribution [3] as the packing material leads to lower values of the A term (Eddy Diffusion), B term (Longitudinal Diffusion) and C term (Mass Transfer) in the van Deemter equation. The lower values of the A term is due to a narrow particle size distribution and dense packing methodologies leading to a minimisation of the space among particles in the column. A higher value of the A term leads to increased dispersion of the analyte due to increased differences in the varying pathways around the particles [4]. The lower value of the B term is due to the limiting of the diffusion of the

solute within the space between core shell particles. The inner non porous core blocks diffusion of the solute, leading to reduction in longitudinal diffusion. The reduced diffusion path length on the thin porous silica layer lowers the C term due to more rapid analyte concentration equilibration due to lower mass transfer effects.

At present more than 15 types of core shell column are available on the market. Two types of processes are used to manufacture core shell silica particles. One is based on a mono-layer structure of porous silica like that manufactured by Advanced Materials Technology [5, 6] and the other is a multi-layer structure like that manufactured by Phenomenex [7]. The bonding chemistry or bonding technique is different among column manufacturers leading to differences

Table1: Separation parameter, back pressure and theoretical plate count

	Back pressure (MPa)	Theoretical- plates of pentylbenzene	Retention factor for pentylbenzene	Hydrogen bonding capacity α (Caffeine/Phenol)	Hydrophobicity α (Pentylbenzene/Butylbenzene)	Steric selectivity α (Triphenylene/o-Terphenyl)
Company A C18	26.1	30,800	5.4	0.48	1.54	1.20
Company B C18	22.7	31,600	7.4	0.35	1.56	1.50
Company C C18	18.5	23,300	7.7	0.38	1.59	1.32
Company D C18	30.6	30,200	9.0	0.42	1.57	1.25
Company E C18	22.2	31,800	9.7	0.44	1.60	1.31
SunShell C18	21.8	31,900	10.4	0.39	1.60	1.46

Table 2: Physical properties.

	Carbon loading (%)	Specific surface area ^a (m ² /g)	Pore volume ^a (mL)	Pore diameter ^a (nm)
Company A C18	4.9 (12 effective carbon loading) ^b	102 (200 effective surface area) ^b	0.237	9.25 (10) ^b
Company B C18	8.8 (9) ^b	130 (130) ^b	0.273	8.39 (8) ^b
Company C C18	7.3 (6.6) ^b	113	0.264	9.32
Company D C18	8.5 (8)b	135 (130) ^b	0.414	12.3 (12) ^b
Company E C18	8.0	133 (150) ^b	0.278	8.20 (9) ^b
SunShell C18	7.3 (7) ^b	125 (150) ^b	0.261	8.34 (9) ^b

a. Measured after C18 materials were sintered at 600 degree Celsius for 8 hours.

b. Value cited in company brochure or literature

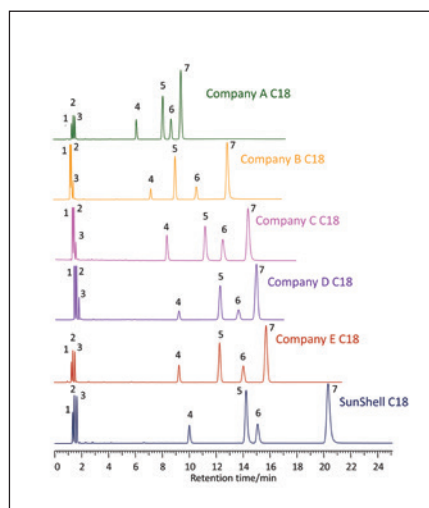


Figure 1: Comparison of standard test probes. Column as in figure, column dimensions: 150 x 4.6 mm i.d. Mobile phase: Methanol/water = 75/25. Flow rate: 1.0mL/min. Column temperature: 40°C. Sample: (1) uracil, (2) caffeine, (3) phenol, (4) butylbenzene, (5) o-terphenyl, (6) pentylbenzene, (7) triphenylene.

in separation selectivity. In this study, we investigate separation behaviour regarding retention factor, hydrogen bonding capacity, hydrophobicity, steric selectivity, peak shape and sample loadability of amitriptyline, as well as an evaluation of physical stability under acidic and basic pH conditions at elevated temperatures and pH for six different core shell C18 columns. Furthermore we also discuss measured physical properties such as carbon loading, specific surface area, pore volume, pore diameter and particle size distribution using the packing material taken from the different columns.

Materials and Methods

Chemicals and reagents

HPLC grade methanol, acetonitrile and water, analytical grade sodium phosphate, sodium borate, sodium hydroxide and trifluoroacetic acid were obtained from Wako Pure Chemical Industries (Osaka, Japan). Samples, of uracil,

caffeine, phenol, butylbenzene, o-terphenyl, pentylbenzene, triphenylene, 8-quinolinol, propranolol, nortriptyline and amitriptyline were also procured from Wako Pure Chemical Industries.

Instrument

Chromatographic experiments were carried out on a LaChrom Elite HPLC system (Hitachi High-Technologies, Tokyo, Japan). Particle size distribution was measured using a Beckman Coulter Multisizer 3 (Beckman Coulter, Brea, CA, USA). Carbon loading was measured using an EA 2400 SERIES II elemental analysis (PerkinElmer, Waltham, MA USA). Specific surface area, pore volume and pore diameter were measured using an Autosorb surface area and pore size analysis instrument (Quantachrome, Boynton Beach, FL USA).

Columns

The following core shell columns were investigated in the study:

- 1) Phenomenex "Kinetex™" C18 2.6 μm 150 x 4.6 mm i.d. and 50 x 2.1 mm i.d.
- 2) Thermo Fisher Scientific "Accucore™" C18 2.6 μm 150 x 4.6 mm i.d. and 50 x 2.1 mm i.d.
- 3) Waters "Cortecs™" C18 2.7 μm 150 x 4.6 mm i.d. and 50 x 2.1 mm i.d.
- 4) Agilent "Poroshell™" EC-C18 2.7 μm 150 x 4.6 mm i.d. and 50 x 2.1 mm i.d.
- 5) Sigma Aldrich "Ascentis Express™" C18 2.7 μm 150 x 4.6 mm i.d. and 50 x 2.1 mm i.d.
- 6) ChromaNik "SunShell™" C18 2.6 μm 150 x 4.6 mm i.d. and 50 x 2.1 mm i.d.

Additionally, a fully porous column was used for some of the column comparison experiments. This was a ChromaNik "Sunniest™" C18 3 μm 150 x 4.6 mm i.d.

Determination of separation parameters and physical properties

To determine separation parameters, a mixture

containing uracil (t0 marker), caffeine, phenol, butylbenzene, o-terphenyl, pentylbenzene and triphenylene, a mixture of 8-quinolinol and caffeine, and a mixture of uracil, propranolol, nortriptyline and amitriptyline were used to characterise the columns. The concentration of samples except for butylbenzene and pentylbenzene ranged from 15 μg/mL to 100 μg/mL. The concentration of butylbenzene and pentylbenzene was around 5 μL/mL. When measuring sample loadability of amitriptyline, the sample was diluted from 10 times to 100 times. C18 packing material from the core shell column was unpacked from the column following conclusion of the initial characterisation studies and carbon loading measurements. In regards to measurement of specific surface area, pore volume and pore diameter of each packing material, the forced degradation of alkyl chains was accomplished by sintering the particles at 600 degree Celsius for 8 hours and respective measurements undertaken.

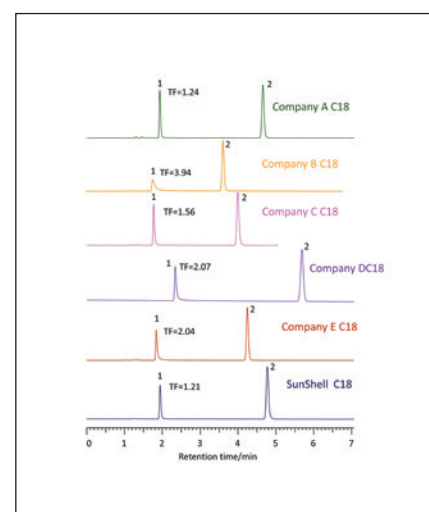


Figure 2: Comparison of metal chelating test probe. Column as in figure, column dimensions: 150 x 4.6 mm i.d. Mobile phase: Acetonitrile/20 mM phosphoric acid = 10/90. Flow rate: 1.0 mL/min. Column temperature: 40°C. Sample: (1) 8-quinolinol, (2) caffeine. TF on a chromatogram is USP tailing factor of 8-quinolinol.

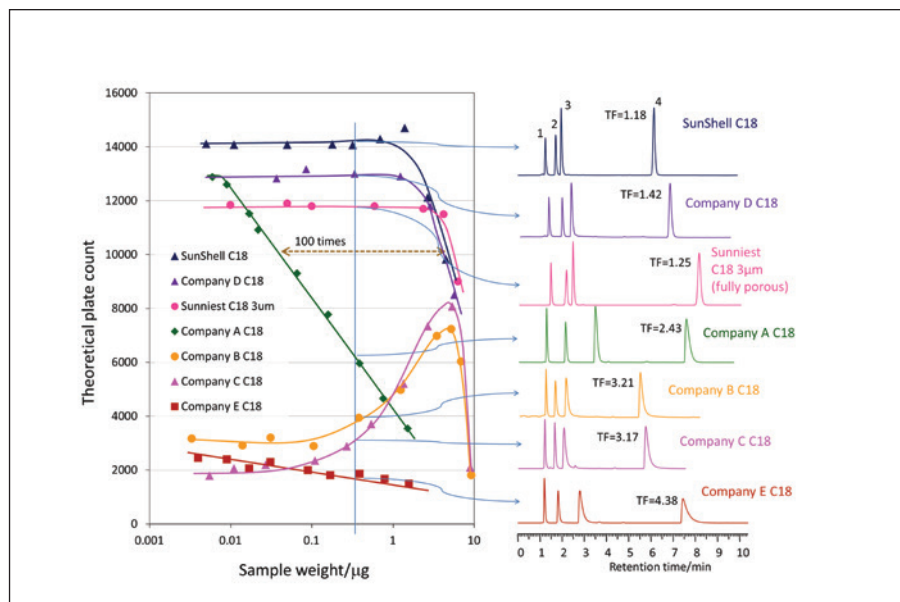


Figure 3: Loading capacity and peak shape for amitriptyline. Column as in figure, Sunniest C18 is a fully porous material, column dimensions: 150 x 4.6 mm i.d. Mobile phase: Acetonitrile/20mM phosphate buffer pH 7.0 = 60/40. Flow rate: 1.0mL/min. Column temperature: 40°C. Sample: (1) uracil, (2) propranolol, (3) nortriptyline, (4) amitriptyline. Theoretical plate value was calculated 5 method using peak width at 4.4% of peak height. Chromatograms shown were a 0.3 μg injection of amitriptyline. TF on a chromatogram is USP tailing factor of amitriptyline.

Results and Discussion

Chromatograms used for the comparison of separation parameters are shown in Figure 1. Theoretical plate count and retention factor of pentylbenzene, hydrogen bonding capacity measured using caffeine and phenol, hydrophobicity measurement using butylbenzene and pentylbenzene and steric selectivity using *o*-terphenyl and triphenylene were calculated from these chromatograms and data tabulated in Table 1 along with the recorded back pressure. The parameters of hydrogen bonding capacity, hydrophobicity and steric selectivity are known as the Tanaka Index [1]. Although the average particle size of each column was listed in the literature to be 2.6 or 2.7 μm, there were larger differences in back pressure of the six columns than expected. Company B and E's C18s and SunShell C18 showed comparable back pressure. Company A's C18 showed 20% higher back pressure and D's C18 showed 40% higher back pressure than SunShell C18, while company C's C18 showed 15% lower back pressure. With regard to theoretical plate count, all columns indicated similar values except for company C's C18 column. Company A's C18 showed much lower retention than the other five columns. Hydrogen bonding capacity, hydrophobicity and steric selectivity are illustrative of column separation characteristics. Although the Tanaka Index includes electrostatic interactions, the C18 columns show little difference in the electrostatic interactions measured by this

method. The peak shape and tailing of amitriptyline under neutral pH condition shown in Figure 3 are considered to be the electrostatic interactions. High hydrogen bonding capacity, low hydrophobicity and low steric selectivity are indicative of lower C18 ligand density. Company A's C18 showed the highest hydrogen bonding capacity, the lowest hydrophobicity and the lowest steric selectivity, so it may be rationally inferred that low C18 ligand density lead to the lowest retention factor.

The peak shape of 8-quinololol as a metal chelating compound is shown in Figure 2. Company A's C18 and the SunShell C18 showed excellent peak shape without peak tailing and indicates these to be inert for this metal chelating compound. The other core shell C18s showed significant tailing. The USP tailing factor (TF) of 8-quinololol on company B's C18 was 3.94. The packing material of company B's C18 was therefore assumed to include higher metal impurities than the other C18s.

Column loading capacity and peak shape for amitriptyline on the six columns is illustrated in Figure 3. A Sunniest C18 3 μm (ChromaNik) fully porous column was added as a reference for this work. For the amitriptyline peak, SunShell C18, company D's C18 and Sunniest C18 showed good peak shape. The other C18s showed a high USP tailing factor for amitriptyline. The relationship between sample weight and theoretical plate is indicative of column loading capacity. In the case of the

SunShell C18 2.6 μm, sample loadings greater than 1 μg led to a significant decrease in theoretical plate counts. Comparison of the fully porous C18 and the SunShell C18 2.6 μm showed only 20% lower loading capacity than the Sunniest C18 3 μm column. In the case of company A's C18, sample loadings of 0.01 μg or greater were found to overload the column. The difference between the loading capacities of the SunShell C18 and company A's C18 was 100 times. The reason for this may be attributed to the multi-layer structure of porous silica. The company A C18 has a multi-layer porous structure whereas the SunShell is a single silica monolayer. The company D C18 is considered to have a monolayer porous structure by observation of the micro-electroscope photo in the literature. Company D's C18 showed the same loading capacity as the SunShell C18. Company B and C's C18s showed unexpected behaviour. The higher the sample loading, the higher the observed theoretical plate count. It is postulated that both particles have some strongly active residual silanol groups that amitriptyline can adsorb to. Thus the influence of the silanol group reduces as these active sites become saturated as amitriptyline sample loading increases leading to improved peak shape. Company E's C18 showed low theoretical plate counts from 0.01 μg to 1 μg. It is considered that a basic compound showed a different behaviour on account of residual silanol groups in comparison to a neutral compound.

Stability under acidic and basic pH conditions are shown in Figure 4 and 5 respectively. The acidic conditions shown in Figure 4 were accelerated at pH 1 at 80 degree Celsius. Under acidic conditions, an alkyl chain

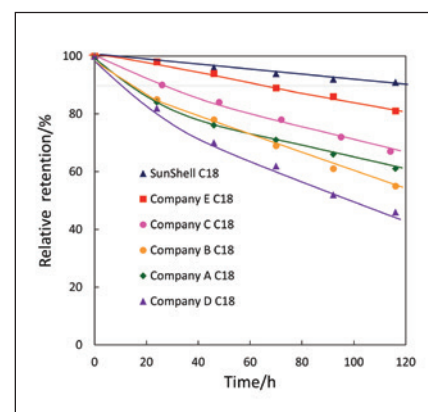


Figure 4: Stability under pH 1 at 80°C. Column as in figure, column dimensions: 50 x 2.1 mm i.d. Durable test condition: mobile phase: Acetonitrile/1.0% trifluoroacetic acid pH 1 = 10/90. Flow rate: 0.4mL/min. Column temperature: 80°C. Measurement condition: Mobile phase: Acetonitrile/water = 60/40. Flow rate: 0.4mL/min. Column temperature: 40°C. Sample: (1) uracil (t0 marker), (2) butylbenzene.

is cleaved from the silica surface, so that analyte retention time reduces. The vertical y-axis was assigned as relative retention of uracil and butylbenzene. At 90% relative retention, SunShell C18 was stable up to 120 hours, company E's C18 60 hours, company C's C18 25 hours and the other C18s only approximately 10 hours. Under basic pH conditions, the particle is deteriorated by dissolution of the silica gel. This leads to void formation along with a reduction of theoretical plate count. The y-axis in Figure 5 is listed as relative theoretical plate count. Company C's C18 literature states that the operable pH range for this column is 2 to 8, so this column was not tested at pH 10. The SunShell C18 was found to be stable for 5000 mL of elution volume. The SunShell C18 was found to be ten times more stable than company A, B and D's C18s under the basic pH condition (as well as acidic pH conditions). Company A and B's columns sales literature indicates they may be used from pH 1.5 to pH 10 and pH 1 to pH 11 respectively, while ChromaNik states the SunShell column is operable in the range pH 1.5 to pH 10 and also at elevated temperatures. Biba et al. [8] also reported the stability of SunShell C18 and Kinetex C18 for ion-pair reversed-phase liquid chromatography analysis of oligonucleotides at pH 7.0 and temperature of 65 degree Celsius. Their conclusion was in agreement with the above results.

After finishing the chromatographic evaluation of core shell columns, the packing materials were removed from each column and carbon loading of each packing material was measured. Portions of these packing materials were then sintered at 600 degree Celsius for 8 hours. The particle size distribution was measured using a Beckman Coulter

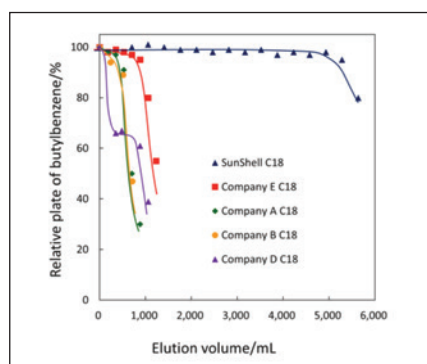


Figure 5: Column stability at pH 10 at 50 °C. Column as in figure, column dimensions: 50 x 2.1 mm i.d. Durable test condition: mobile phase: Methanol/20mM sodium borate/10mM sodium hydroxide pH 10 = 30/21/49. Flow rate: 0.4 mL/min. Column temperature: 50°C. Measurement condition: Mobile phase: Methanol/water = 70/30. Flow rate: 0.4mL/min. Column temperature: 40°C. Sample: Butylbenzene.

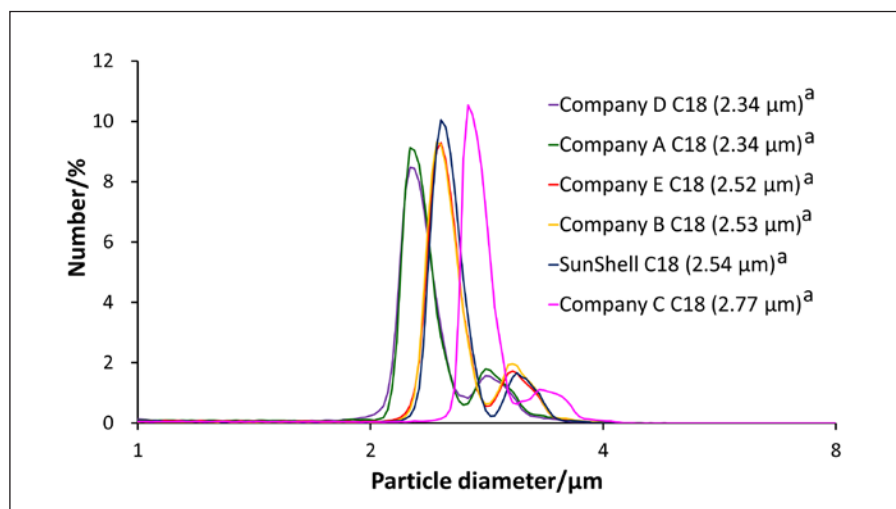


Figure 6: Comparison of particle size distribution. a: Median particle size

Multisizer 3 and the results shown in Figure 6. Specific surface area, pore volume and pore diameter were also measured and results listed in Table 2. The measured particle size diameters were all smaller than the values listed in the company literature. Different measurement techniques can give rise to different values for the same particles, and this may account for the differences in the measured values. However all core shell silica particles showed a similar narrow particle size distribution although the median particle size was different. Median particle size and back pressure in Table 1 showed a high correlation. Company C's C18 showed the lowest back pressure and the lowest theoretical plate count attributable to it having the largest particle size.

The values listed in Table 2 in parentheses are quoted values from each brochure or literature. Except for Company A's C18, the measured value was almost as same as the literature value. For comparison of the standard samples analysed in Figure 1, Company A's C18 showed approximately half the retention times in comparison with SunShell C18. Measured values for carbon loading and specific surface area of Company A's C18 were measured as 4.9% and 102 m²/g respectively. So retention time values of 50% for Company A's C18 in Figure 1 was acceptable in consideration of their measured values not the value written in their brochure. Company A lists effective carbon loading and effective surface area, not specific surface area in their brochure. It is not clear what 'effective' means or how this was measured.

Conclusion

Differences in retention, inertness to a metal chelating compound, residual silanol groups,

loading capacity, stability and particle size of six core shell C18s and a fully porous C18 was measured. SunShell C18 showed the longest retention, the best peak symmetry and the highest pH stability. The recommended pH values in the respective brochures are restricted to ambient temperature for all core shell columns with the exception of the SunShell column. In most cases the measured values of physical properties and the usable pH range of a mobile phase in some cases were not found to be the same as the values listed in the associated brochures and literature.

Reference

1. K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki and N. Tanaka, J. Chromatogr. Sci., 27 (1989) 721-728.
2. J. J. Kirkland, T. J. Langlois, and J.J. DeStefano, Am. Lab., 39 (2007) 18-21.
3. R. A. Henry, LCGC North Am., 32(4) (2014) 12-19.
4. F. Gritti, D. S. Bell and G. Guiochon, J. Chromatogr. A, 1355 (2014) 179-192.
5. J. J. DeStefano, S. A. Schuster, J. M. Lawhorn and J. J. Kirkland, J. Chromatogr. A, 1258 (2012) 76-83.
6. J. J. DeStefano, T. J. Langlois and J. J. Kirkland, J. Chromatogr. Sci. 46 (2008) 254-260.
7. F. Gritti, I. Leonardis, D. Shock, P. Stevenson, A. Shalliker and G. Guiochon, J. Chromatogr. A, 1217 (2010) 1589-1603.
8. M. Biba, C. J. Welch and J. P. Foley, J. Pharm. Biomed. Anal., 96 (2014) 54-57.