Comparison of the Reversed-Phase Selectivity of Solid Core HPLC Columns

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Key Words

Accucore, solid core, column characterization, selectivity, primary interactions, secondary interactions, Core Enhanced Technology, radar plots

Abstract

The selectivities of Thermo Scientific™ Accucore™ C18 and RP-MS phases are compared to six other solid core reversed-phase stationary phases. The retention properties of the stationary phases were categorized by analyzing primary modes of interaction (hydrophobicity, steric selectivity, and hydrogen bonding) and secondary modes of interaction (ion exchange and chelation).

Introduction

Accucore HPLC columns are based on Core Enhanced Technology™, which features solid core materials with a very tight particle size distribution and advanced bonding technology to functionalize the surface. The particles in the Accucore stationary phases can be described as a solid silica core surrounded by a porous outer layer. The very tight particle size distribution of these materials results in columns with high permeability. Therefore, for the same nominal pressure, Accucore particles provide better separations than fully porous materials.

In this technical note the retention properties of Accucore C18 and Accucore RP-MS (an optimized alkyl chain length bonded phase) materials are compared to other solid core reversed-phase C18 materials. To fully characterize the surface chemistry of the reversed-phase materials, a series of diagnostic chromatographic tests were used (based on those developed by Tanaka [1]). These tests characterize analyte/stationary phase interactions and combine probes to measure hydrophobicity, shape selectivity, hydrogen bonding, and secondary interactions with bases, acids, and chelators. The results from this characterization study will help users to select the best phase for their separations. These tests are described in Tables 1 to 3.

The phase characterization data obtained were summarized in radar plots, which allow visual comparison of the overall selectivity of the different stationary phase chemistries. Radar plots, also known as spider or star charts (because of their appearance), plot the values of each category along a separate axis that starts in the center of the chart and ends on the outer ring.

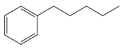
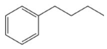
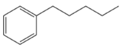
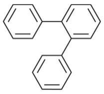
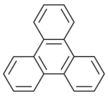
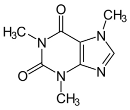
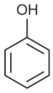
Parameter	Interaction Investigated	Test Molecules
HR	**Hydrophobic retention** is the retention factor of a hydrophobic hydrocarbon, pentylbenzene, which gives a broad measure of retention for a particular molecule. This is based on the available surface area, the ligand, and its density.	Pentylbenzene 
HS	**Hydrophobic selectivity** is the selectivity factor between pentylbenzene and butylbenzene and provides a measure for the selectivity of two molecules based on their hydrophobicity. It is affected by the surface coverage of the phase and the ligand density.	Butylbenzene  Pentylbenzene 
SS	**Steric selectivity** is the ability of the stationary phase to distinguish between molecules with similar structures and hydrophobicity but different shapes. The selectivity factor between o-terphenyl and triphenylene is indicative of steric selectivity, as the former has the ability to twist and bend, while the latter has a fairly rigid structure and will be retained quite differently. More rigid ligands will in general give greater selectivity.	o-Terphenyl  Triphenylene 
HBC	**Hydrogen bonding capacity** is the selectivity factor between caffeine and phenol, which provides a measure of the degree of hydrogen bonding a molecule will experience. Caffeine is a good hydrogen bonder and increasing the number of available silanol groups will increase the retention of the caffeine molecule but not the phenol molecule. This term is affected by the total number of silanols, which in turn is affected by the total surface area and also the degree of endcapping.	Caffeine  Phenol 

Table 1: Hydrophobic tests

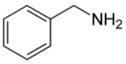
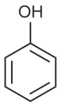
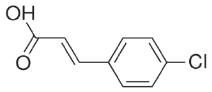
Parameter	Interaction Investigated	Test Molecules
IEX2.7	**Ion-exchange capacity at pH 2.7** is estimated by the selectivity factor between benzylamine and phenol, at pH 2.7. Tanaka [1] showed that the retention of protonated amines at pH < 3 could be used to get a measure of the ion exchange sites on the silica surface. Silanol groups (Si-OH) are undissociated at pH < 3 and therefore cannot contribute to the retention of protonated amines, but the acidic silanols in the dissociated form (SiO^-) can. Acidic silanols can be formed with the addition of impurities and also the different forms (in order of acidity: geminyl, bridged, vicinyl, metallic forms) of the silanols moiety. The more acidic silanols contribute to the retention of the protonated amines.	Benzylamine  Phenol 
AI	The capacity factor and **tailing factor of chlorocinnamic acid** are also measured to test the applicability of the stationary phase towards acidic interactions. These interactions are due to impurities in the substrate material and also to certain ligands that are used.	4-Chlorocinnamic acid 

Table 2: Secondary interactions and ion exchange tests at low pH

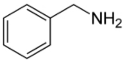
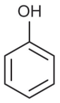
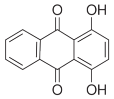
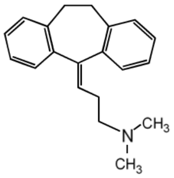
Parameter	Interaction Investigated	Test Molecules
IEX7.6	**Ion-exchange capacity at pH 7.6** is estimated by the selectivity factor between benzylamine and phenol and is a measure of the total silanol activity on the surface of the silica. At pH > 7 the silanol groups are fully dissociated and combine with the ion exchange sites to influence the retention of benzylamine.	Benzylamine  Phenol 
C	Silica surface metal interactions can cause changes in selectivity and peak shape for analytes which are able to chelate. Changes in the capacity factor and **tailing factor of quinizarin**, which is a chelator, are indicative of secondary metal interactions.	Quinizarin 
BA	The presence of dissociated silanols at pH > 7 can cause poor peak shapes of protonated basic compounds such as amitriptyline. Secondary ion exchange and silanolic interactions can cause shifts in retention and asymmetrical peaks. The capacity factor and **tailing factor of amitriptyline** are indicative of the overall performance of the column.	Amitriptyline 

Table 3: Secondary interactions and ion exchange tests at neutral pH

Characterization tests

Material	Particle Diameter	Pore Diameter	Bonded Phase	Dimensions
Accucore	2.6 µm	80 Å	RP-MS	100 × 2.1 mm
Accucore	2.6 µm	80 Å	C18	100 × 2.1 mm
Kinetex®	2.6 µm	100 Å	C18	100 × 2.1 mm
Poroshell® 120	2.7 µm	120 Å	SB-C18	100 × 2.1 mm
Ascentis® Express	2.7 µm	90 Å	C18	100 × 2.1 mm
Halo®	2.7 µm	90 Å	C18	100 × 2.1 mm
Nucleoshell®	2.7 µm	90 Å	RP C18	100 × 2.0 mm
SunShell®	2.6 µm	90 Å	C18	100 × 2.1 mm

Table 4: Columns tested

Experimental Conditions	Hydrophobic Test	Low pH Test	Neutral pH Test
Mobile phase	65:35 (v/v) methanol /water	45:55 (v/v) methanol/ 10 mM phosphate buffer pH 2.7	80:20 (v/v) methanol/ 10 mM phosphate buffer pH 7.6
Flow rate (mL/min)	0.55	0.55	0.55
Column temperature (°C)	40	40	40
Detection	UV at 254 nm	UV at 254 nm	UV at 254 nm
Injection volume (µL)	1	1	1

Table 5: Experimental conditions

Selectivity comparison

Figures 1 to 3 give examples of the chromatograms from the three tests on the Accucore RP-MS column, demonstrating typical elution order for the test probes in each test. Figure 4 provides an overview of the relative hydrophobic retention (HR) and steric selectivity (SS) of all the phases compared in this document. Accucore RP-MS, Poroshell 120 SB-C18, and Kinetex C18 phases all exhibit similar HR. HR is significantly higher on SunShell C18, Nucleoshell RP-C18, and Halo C18 phases. The latter exhibits the highest hydrophobic retention of all the phases tested. The phase that exhibits the highest steric selectivity is Accucore C18 (SS 1.4). Accucore RP-MS, Poroshell 120 SB-C18, and Kinetex C18 phases all exhibit similar steric selectivity (SS around 1.1); for the other four phases, SS varies between 1.17 and 1.30. Therefore, out of all the phases tested, Accucore C18 provides the best balance between hydrophobic retention, and thus retention time, and steric selectivity, which is a measure of the ability of the phase to separate compounds with similar structures.

From Figure 5, we can observe that the hydrophobic selectivity (HS) is comparable for all the materials. The hydrogen bonding capacity (HBC) is very low for all the materials assessed in this study, providing evidence that all the phases are effectively endcapped.

The activity towards bases (BA, tailing factor of amitriptyline) is comparable for most materials (average of 1.3), but slightly higher for SunShell C18 (1.6), which is evidence of the presence of dissociated silanols, which interact with protonated bases. The activity towards chelators (C) is relatively low, reflected by an average tailing factor for quinizarin of 1.5; however, the quinizarin tailing factor values for Poroshell 120 SB-C18 and SunShell C18 phases are 2.0 and 1.9, respectively, which demonstrate higher metal contents on the silica supports in these phases. The ion exchange capacity at pH 7.6 is very low for Accucore C18 and RP-MS, Halo C18, Ascentis Express C18, and SunShell C18 columns (IEX 7.6 of 1.0) but high for Poroshell 120 SB-C18 (IEX 7.6 of 1.6), highlighting a higher silanolic activity on the surface of the silica. In contrast, there is no evidence of dissociated acidic silanols on any of the phases tested, since the values of IEX 2.7 are very low for all phases. The activity towards acids (AI, tailing factor for chlorocinnamic acid) is similarly low for all the columns (average value 1.2), with the exception of SunShell, which has a value of 1.5.

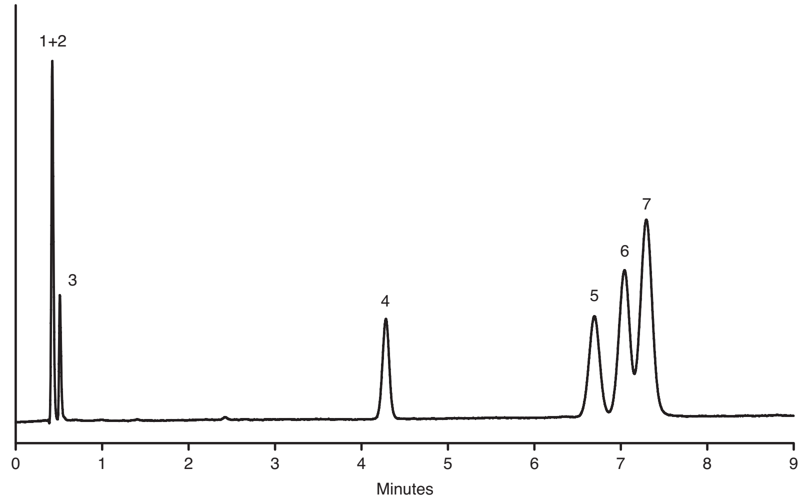


Figure 1: Example chromatogram for the hydrophobic interactions test on the Accucore RP-MS column
1. Theophylline (t_0 marker); 2. Caffeine; 3. Phenol; 4. Butylbenzene; 5. o-Terphenyl; 6. Pentylbenzene; 7. Triphenylene

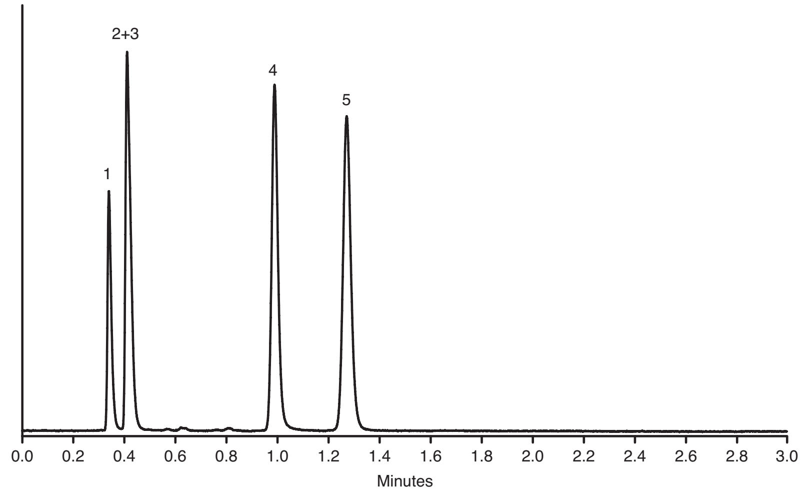


Figure 2: Example chromatogram for the neutral pH test on the Accucore RP-MS column
1. Theophylline (t_0 marker); 2. Phenol; 3. Benzylamine; 4. Quinizarine; 5. Amitriptyline

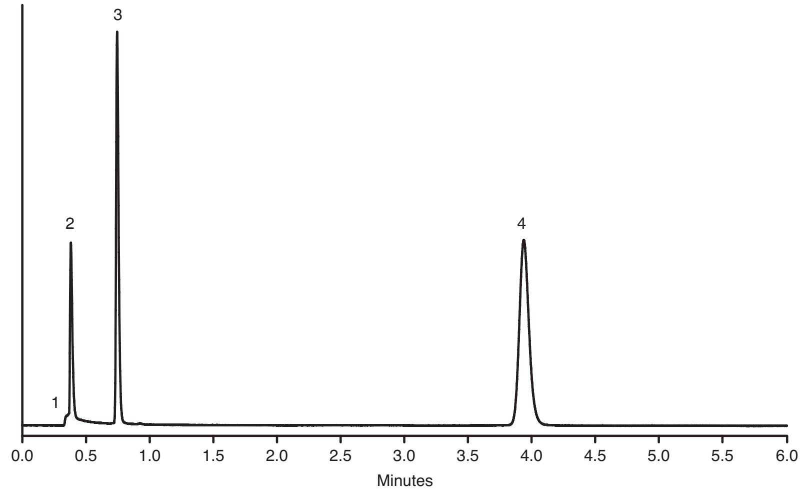


Figure 3: Example chromatogram for the lower pH test on the Accucore RP-MS column
1. Cytidine triphosphate (t_0 marker); 2. Benzylamine; 3. Phenol; 4. Chlorocinnamic acid

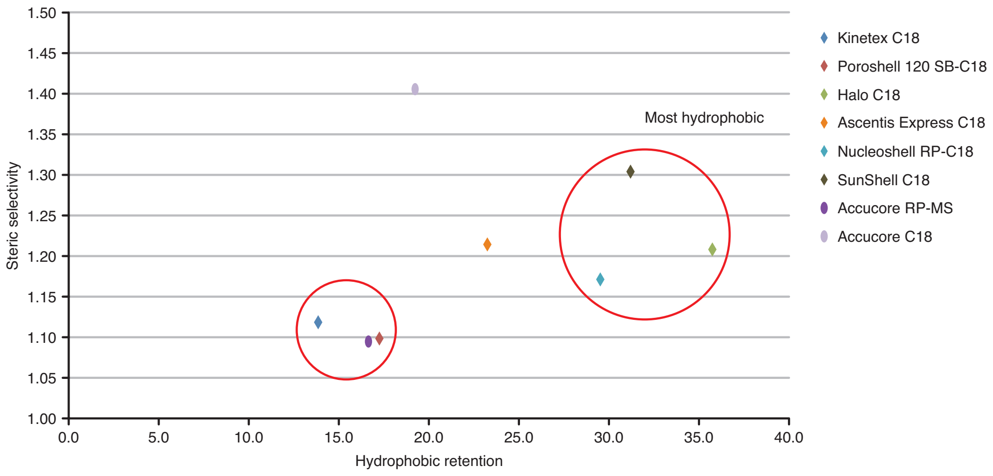
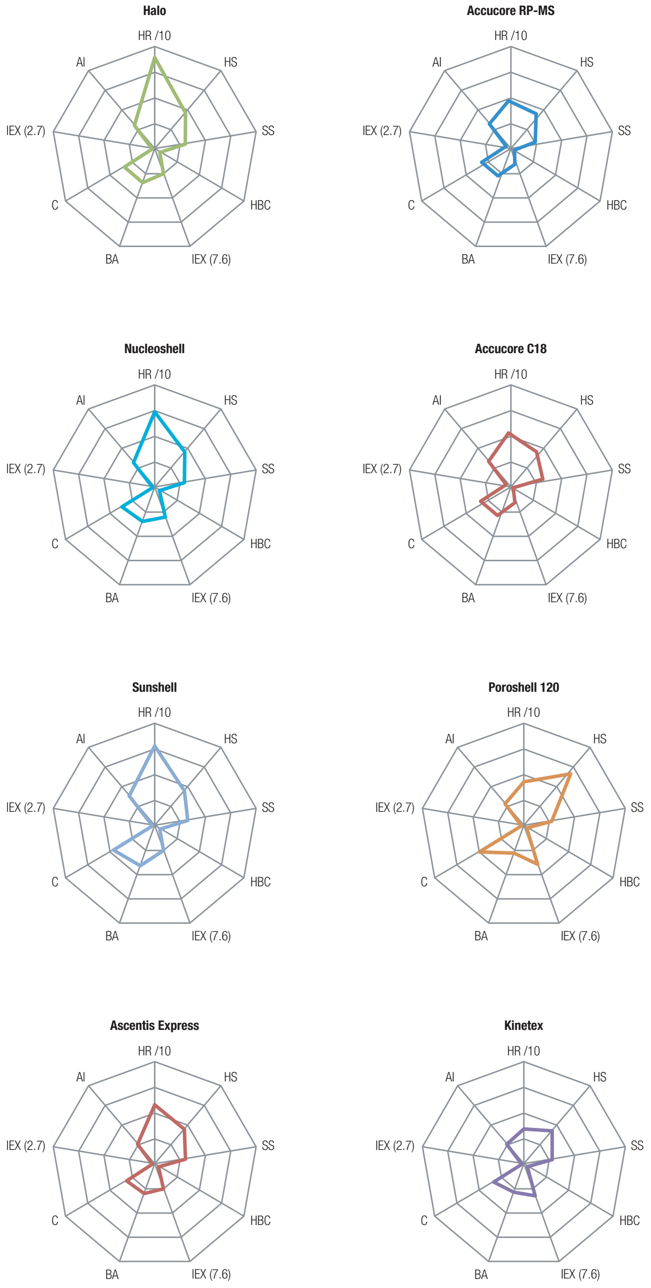


Figure 4: Comparison of the steric selectivity and hydrophobic retention of the tested stationary phases


Halo

Accucore RP-MS

Nucleoshell

Accucore C18

Sunshell

Poroshell 120

Ascentis Express

Kinetex

Figure 5: Radar plots for the phases tested, showing the differences in selectivity. HR = hydrophobic retention; HS = hydrophobic selectivity; SS = steric selectivity; HBC = hydrogen bonding capacity; IEX (7.6) = ion exchange capacity at pH 7.6; BA = activity towards bases; C = activity towards chelators; IEX (2.7) = ion exchange capacity at pH 2.7; AI = activity towards acids

Conclusion

The selectivities of the Accucore C18 and RP-MS phases were assessed against six other comparable solid core reversed-phase stationary phases. The retention properties of the stationary phases were categorized by analyzing primary modes of interaction (hydrophobicity, steric selectivity, and hydrogen bonding) and secondary or unwanted modes of interaction (ion exchange and chelation). The results were summarized in radar plots, which identified the following main differences:

1) Primary interactions
  - Halo C18, SunShell C18, and Nucleoshell RP-C18 are the most hydrophobic phases and therefore the most retentive towards hydrophobic solutes. A varying degree of hydrophobicity was observed throughout the materials, reflecting different degrees of surface area and coverage of this silica surface.
  - Accucore C18 shows the highest steric selectivity, achieved without excessive retention. This attribute of the phase facilitates good separation of solutes that have similar hydrophobicity but dissimilar steric structures.

2) Secondary interactions
  - All phases show evidence of efficient endcapping and the absence of dissociated acidic silanols.
  - Poroshell 120 SB-C18 and SunShell C18 have the highest levels of chelation, indicating the highest metals content; analysis of solutes which can chelate on these phases may result in asymmetrical peaks.
  - Poroshell 120 has the highest silanolic activity at neutral pH; this can cause tailing of solutes capable of ion exchanging with silanols under these pH conditions.
  - SunShell C18 has the highest activity towards acids and protonated bases, and therefore asymmetrical peaks can be expected for these types of compounds on this phase.

References

[1] Tanaka, N. et al., *Journal of Chromatographic Science*, **1989**, *27*, 721-728.

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