Selectivity Choices in Reversed-Phase Fast LC

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Key Words
Accucore, Hypersil GOLD, Syncronis, Column Chemistry, Fast LC, Radar Plots, Resolution, Solid Core, UHPLC

Abstract
The effect that different chemistries have on the selectivity of reversed-phase (RP) packing materials is investigated. 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). Full characterization of the surface interactions of Thermo Scientific™ Accucore™, Thermo Scientific Hypersil GOLD™, and Thermo Scientific Syncronis™ stationary phases was achieved.

Introduction
The primary goal of developing a chromatographic separation is to resolve a mixture of analytes. From the graphical representation of the general resolution equation (Equation 1) in Figure 1 it is evident that the selectivity parameter has the greatest impact on resolution. Selectivity can be changed by modification of the mobile phase composition, column chemistry or temperature.

Equation 1: $$R_s = \frac{1}{4}\sqrt{N}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{1 + k'}\right)$$

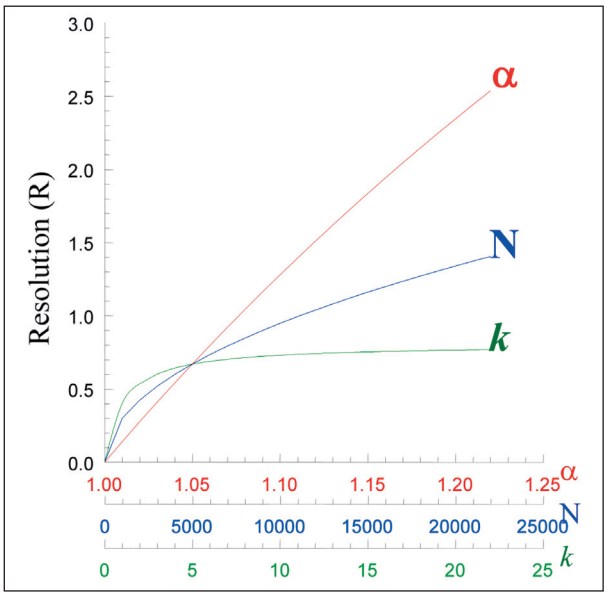

Figure 1: Graphical representation of the general resolution equation

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. These tests are described as follows:

- **Hydrophobic retention (HR) –** is the retention factor of a hydrophobic hydrocarbon, pentylbenzene, which gives a broad measure of hydrophobicity of the ligand and its density.
- **Hydrophobic selectivity (HS) –** is the selectivity factor between pentylbenzene and butylbenzene and provides a measure of the surface coverage of the phase; these two alkylbenzenes differ by one methylene group and their selectivity is dependent on ligand density.

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- **Steric selectivity (SS) –** 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 can be retained quite differently.
- **Hydrogen bonding capacity (HBC) –** is the selectivity factor between caffeine and phenol, which provides a measure of the number of available silanol groups and the degree of endcapping.
- **Ion-exchange capacity at pH 2.7 (IEX2.7) –** is estimated by the selectivity factor between benzylamine and phenol, at pH 2.7. Tanaka 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. The latter contribute to the retention of the protonated amines.
- **Ion-exchange capacity at pH 7.6 (IEX7.6) –** 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 dissociated and combine with the ion exchange sites to influence the retention of benzylamine.
- **Base activity (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 with regard to potential secondary interactions involving charged bases.
- **Secondary metal interactions (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 metal interactions.
- **Acid interactions (AI) –** the capacity factor and tailing factor of chlorocinnamic acid are also measured to test the applicability of the stationary phases to a range of different types of acidic analytes.

The phase characterization data obtained was summarized in radar plots, which allow visual comparison of the overall selectivity of the different stationary phase chemistries. Radar charts, 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.

Selectivity of Accucore Solid Core Range of Phases

Accucore columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. Their 2.6 µm diameter particles have a solid core and a porous outer layer. The tightly controlled diameter provides much lower backpressure than typically seen with comparable size materials. The use of high purity silica combined with optimized phase bonding in Accucore columns creates a series of high coverage, robust phases. Accucore columns are available in a series of chemistries to provide a wide range of RP selectivities for method development:

- Optimized alkyl chain (RP-MS)
- C18
- C8
- Polar endcapped C18 (aQ)
- Phenyl-Hexyl
- Pentafluorophenyl (PFP)

The hydrophobic retention (HR) and selectivity (HS) of the C18, RP-MS and aQ are comparable and significantly higher than those of the PFP and Phenyl-Hexyl phases. The steric selectivity (SS) of the aQ phase is slightly higher than that of the C18 or RP-MS phase but considerable lower than that of the PFP phase, which shows the highest steric selectivity. The introduction of fluorine groups into the stationary phase causes significant changes in analyte-stationary phase interactions, which can produce high selectivity for positional isomers of halogenated compounds. The Phenyl-Hexyl phase offers a mixed mode separation mechanism, with the C6 chain responsible for hydrophobic interactions and the phenyl ring responsible for π–π interactions. Accucore RP-MS uses an optimized alkyl chain length for more effective coverage of the silica surface. This coverage results in a significant reduction in secondary interactions and thus highly efficient peaks with very low tailing, as evident from the low values for activity towards bases (BA) and chelators (C). The C8 phase has a similar radar plot shape to the C18 and RP-MS phases but with lower hydrophobic retention.

These differences can be observed in the radar plots (Figure 2).

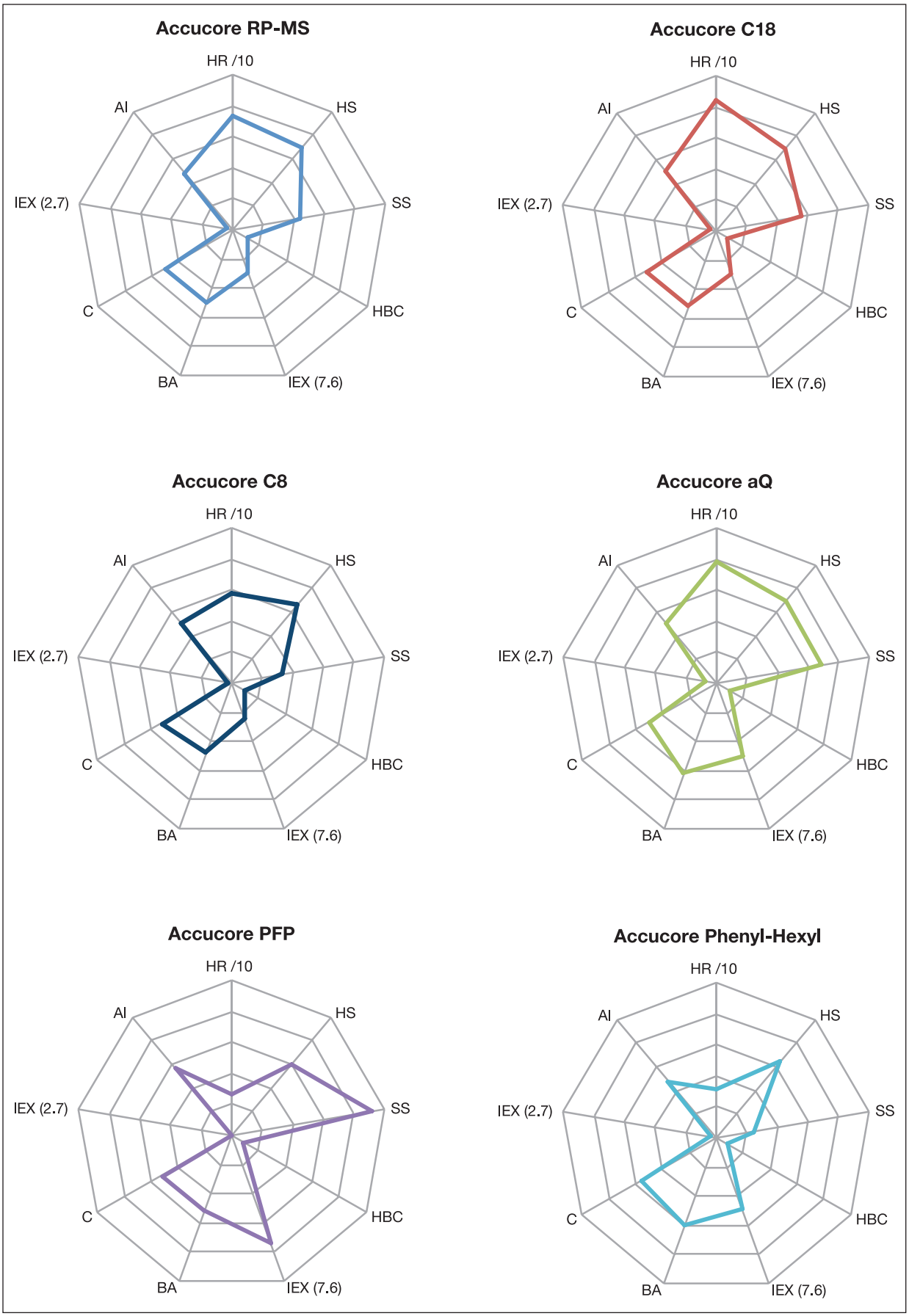


Figure 2: Summary of differences in selectivity offered by Accucore columns. HR= hydrophobic retention; HS= hydrophobic selectivity; SS= steric selectivity; HBC= hydrogen bonding capacity; IEX (7.6)= ion exchange capacity at pH 7.6; BA= activity towards bases; C= activity towards chelators; IEX (2.7)= ion exchange capacity at pH 2.7; AI= activity towards acids.

Selectivity of Hypersil GOLD 1.9 µm Range of Phases

Based on highly pure silica, Hypersil GOLD columns provide very symmetrical peaks, even when analyzing compounds that give notoriously poor peak shape on traditional silica-based chemistries. Hypersil GOLD media provides a stationary phase with C18 selectivity and a predictable elution order, but can provide new capabilities such as improved peak shape, increased peak capacity, and greater sensitivity, especially for trace compound analysis. In addition to the C18 selectivity phase, Hypersil GOLD columns are available in a range of chemistries to provide a wide range of RP options for method development:

- C8 and C4
- Polar endcapped C18 (aQ)
- Phenyl
- Pentafluorophenyl (PFP)

As demonstrated in Figure 3, all phases show low values for BA (activity towards bases), and C (activity towards chelators), as expected from the very high purity silica support and the proprietary bonding and end-capping procedures used in the manufacturing of these range of phases. The Hypersil PFP phase shows the highest steric selectivity of all phases in the range and also slightly higher than Accucore PFP. It is interesting to note that although the C4 phase is the least hydrophobic, it has hydrophobic selectivity comparable to the other phases investigated here (for instance Phenyl and PFP).

Selectivity of Syncronis 1.7 µm Range of Phases

Syncronis columns are based on a highly pure 100 Å silica, with a surface area of 320 m^2/g, compared to 175 Å and 220 m^2/g for the Hypersil GOLD material. This greater surface area ensures greater retention of analytes by a hydrophobic mechanism. The high surface area also allows for higher sample loading. The Syncronis RP stationary phases investigated in the current study are:

- C18
- C8
- Polar endcapped C18 (aQ)
- Phenyl

Syncronis C18 and Syncronis aQ provide very high hydrophobic retention (HR), as shown in the radar plots in Figure 4. Ion exchange capacity (7.6) is higher on Syncronis aQ than on the other phases. The highest steric selectivity is observed for Syncronis aQ.

The chart in Figure 5 provides an overview of the relative hydrophobic retention and steric selectivity of all the phases compared in this document. Syncronis C18 has the highest hydrophobic retention, Hypersil GOLD PFP has the highest steric selectivity. For phases that show a value of steric selectivity below 1 there is a reversal of the elution order of o-terpheyl and triphenylene, the probes used to measure that parameter.

Conclusion

When working with fast LC it is important to have a wide choice of column chemistries to be explored during method development . The following guidelines can be used to make the correct column selection:

- The Syncronis range of columns having a high surface area provides very retentive phases for polar and moderately polar analytes
- The phases in the Hypersil GOLD range exhibit the lowest secondary interactions, providing the most symmetrical peaks for difficult analytes such as bases and chelators
- The Accucore solid core columns have a good range of chemistries with a wide spread of selectivity
- Syncronis aQ and Syncronis C18 exhibit the highest hydrophobic retention
- The perfluorinated phases (Hypersil GOLD PFP and Accucore PFP) exhibit the highest steric selectivity
- From the phases with aromatic groups, the Phenyls and Pheny-Hexyl exhibit inverse steric selectivity to the PFPs

References

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

Appendix

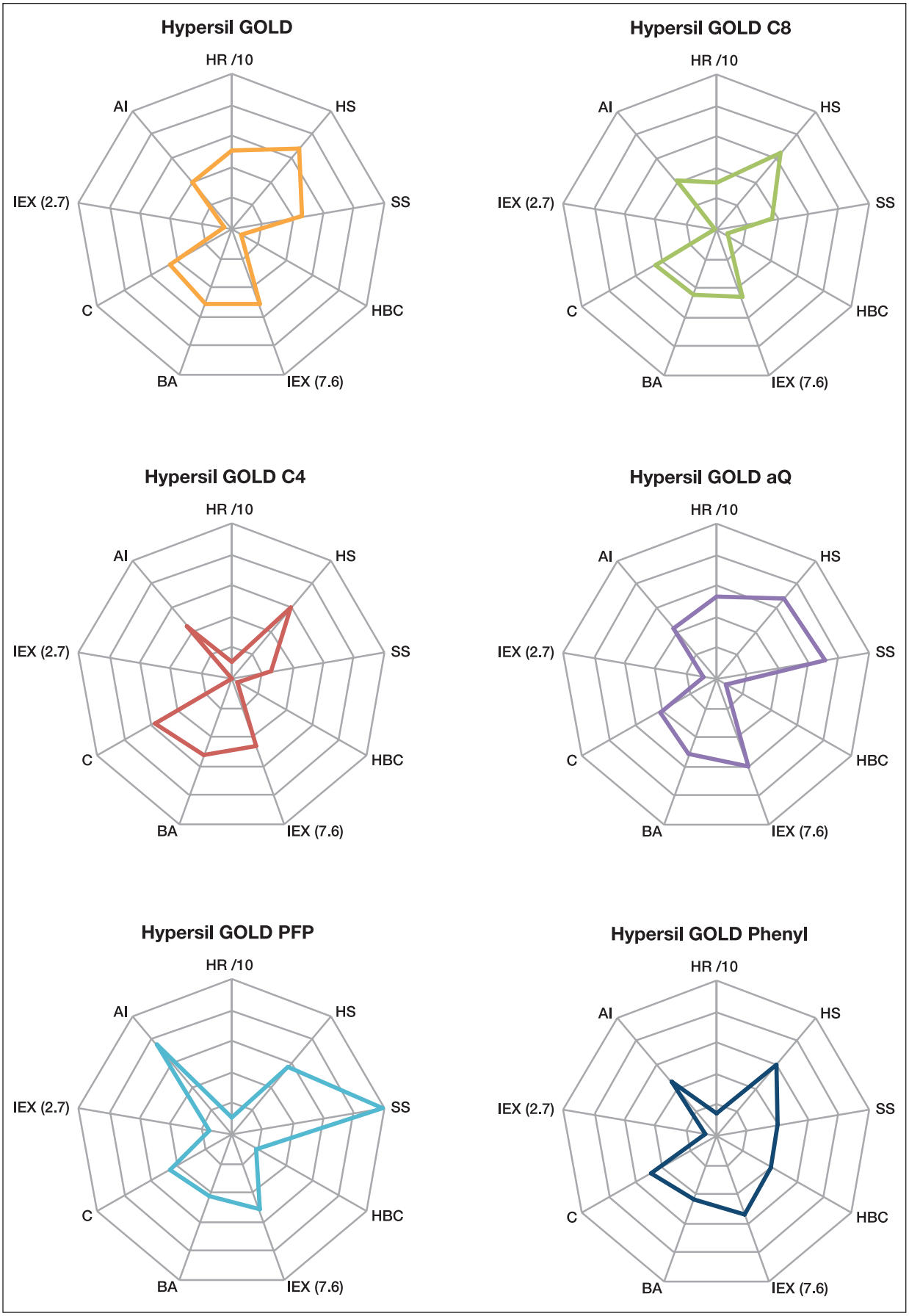


Figure 3: Summary of differences in selectivity offered by Hypersil GOLD columns. HR= hydrophobic retention; HS= hydrophobic selectivity; SS= steric selectivity; HBC= hydrogen bonding capacity; IEX (7.6)= ion exchange capacity at pH 7.6; BA= activity towards bases; C= activity towards chelators; IEX (2.7)= ion exchange capacity at pH 2.7; AI= activity towards acids.

Figure 4: Summary of differences in selectivity offered by Syncronis columns. 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.

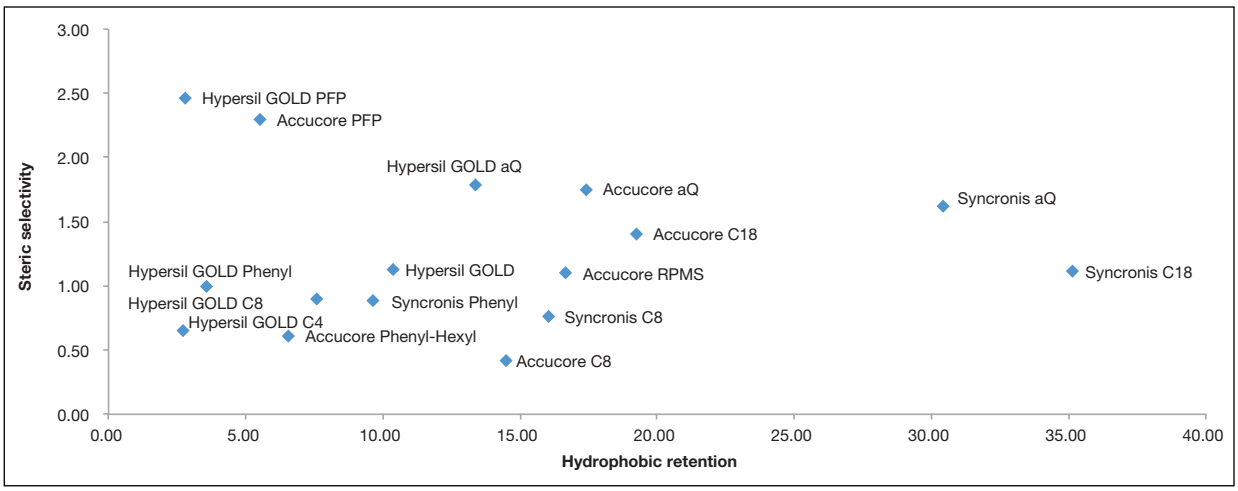


Figure 5: Comparison of the steric selectivity and hydrophobic retention of Accucore, Hypersil GOLD and Syncronis ranges.

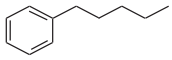
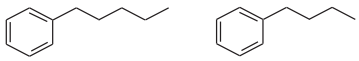
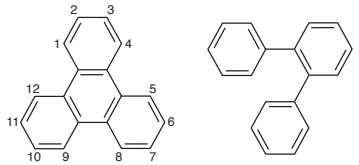
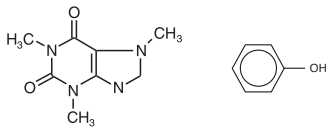
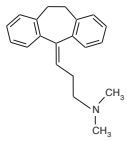
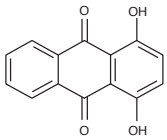
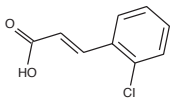
	Variable	Probe	Structure	Test #
Primary Interactions	Hydrophobic retention (HR)	k′ pentylbenzene		1
	Hydrophobic selectivity (HS)	α (Pentylbenzene/butylbenzene)		1
	Steric selectivity (SS)	α (Triphenylene/o-terphenyl)		1
	H-bonding capacity (HBC)	α (caffeine/phenol)		1
Secondary Interactions	IEX capacity pH 7.6 (IEX 7.6)	α (benzylamine/phenol)		2
	IEX capacity pH 2.7 (IEX 2.7)	α (benzylamine/phenol)		3
	Activity towards bases (BA)	Tailing factor of amitriptyline		2
	Activity towards chelators (C)	Tailing factor of quinizarin		2
	Activity towards acids (AI)	Tailing factor of chlorocinnamic acid		3

Table 1: Summary of test probes and structures.

	Test 1	Test 2	Test 3
Mobile Phase	H_2O/CH_3OH (35:65)	K_2HPO_4 10 mM pH 7.6/CH_3OH (20:80)	KH_2PO_4 10 mM pH 2.7/CH_3OH (55:45)
Flow Rate (mL/min)	0.5–0.6	0.5–0.6	0.5–0.6
Temperature (°C)	40	40	40
Detection (nm)	254	254	254
Injection Volume (µL)	10	5	5

Table 2: Experimental conditions of diagnostic tests.

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