A new range of highly retentive reversed-phase packings for LC and LC/MS

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Introduction

Developing an analytical method for HPLC can be time consuming, and having phases with differing degrees of hydrophobicity can be beneficial to enable retention of more polar compounds. A new range of highly retentive reverse phase columns has been developed for LC and LC/MS. The high retention is obtained by having a higher surface area, generated by having smaller pores. The surface area is 320m^2/g, compared to 200m^2/g from a typical silica based material. This greater surface area ensures good retention of analytes with a range of hydrophobicites and also better sample loading.

To allow the surface chemistry of these phases to be fully characterized, they have been extensively tested using a series of diagnostic chromatographic tests . These tests characterize analyte / stationary phase interactions and combine probes to measure hydrophobicity, shape selectivity and secondary interactions with bases, acids and chelators. These test probes have been designed to allow comparisons with other phases, in particular the less retentive Thermo Scientific Hypersil GOLD. Another benefit of the testing regime is that it allows for substantially better column to column and batch to batch reproducibility.

Explanation of Interactions

The retention properties of a reversed-phase packing material can be categorized into hydrophobic retentivity, which is a measure of the hydrophobicity of the ligand and its density, steric or shape selectivity and secondary interactions such as silanol and surface metal activity. The impact of silanol / analyte interaction on the chromatographic performance depends on the pH of the mobile phase. Silanols on the silica surface can hydrogen bond (both as a donor and acceptor) and dissociated silanols can ion exchange with protonated bases.

1) Hydrophobicity
Hydrophobic retention (HR) – the capacity factor of a hydrophobic hydrocarbon, pentylbenzene, give a broad measure of hydrophobicity.
Hydrophobic selectivity (HS) - The selectivity factor between pentylbenzene and butylbenzene 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.

2) Steric selectivity (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.

3) Hydrogen bonding capacity (HBC)
Selectivity factor between caffeine and phenol, which provides a measure of the number of available silanol groups and the degree of endcapping.

4) Ion-exchange capacity at pH 2.7 (IEC2)
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. The latter contribute to the retention of the protonated amines. The contribution of the free silanols to retention can be estimated by the selectivity factor between benzylamine and phenol, at pH 2.7.

5) Ion-exchange capacity at pH 7.6 (IEC7)
The selectivity factor between benzylamine and phenol is used to estimate 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.

6) Activity towards bases
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.

7) Activity towards chelators
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.

8) Activity towards acids
The capacity factor and tailing factor of chlorocinnamic acid are also measured to test the applicability of the stationary phase to a range of different types of analytes.

9) Efficiency and peak area
The efficiency measured in plates/m and peak area of triphenylene area used as a measure of column performance and column-to-column reproducibility.

Methods

Instrumentation:
Thermo Scientific Surveyor HPLC system fitted with MayLab Column Switcher

Columns:
Hypersil GOLD™ 5 µm, 150 x 4.6 mm and 100 x 4.6mm
Experimental C18 column 5 µm, 100 x 4.6 mm

TABLE 1. Experimental conditions and description of diagnostic chromatographic tests.

	Test 1	Test 2	Test 3
Mobile phase	H_2O / Methanol (35:65)	K_2HPO_4 10 mM pH 7.6 / Methanol (20:80)	KH_2PO_4 10 mM pH 2.7 / Methanol (55:45)
Flow rate (mL/min)	1 (or 1.5)	1	1
Temperature (°C)	40	40	40
Detection (nm)	254	254	254
Injection volume (µL)	10	5	5

	Variable	Probe	Test #
Primary interactions	Hydrophobic retention	k' pentylbezene	1
	Hydrophobic Selectivity	α (pentylbenzene, butylbenzene)	1
	Steric selectivity	α (triphenylene, o-terphenyl)	1
Secondary interactions	H-bonding capacity	α (caffeine, phenol)	1
	IEX capacity pH 7.6	α (benzylamine, phenol)	2
	IEX capacity pH 2.7	α (benzylamine, phenol)	3
	Activity towards bases	k' and tailing factor of amitriptyline	2
	Activity towards chelators	k' and tailing factor of quinizarin	2
	Activity towards acids	k' and tailing factor of chlorocinnamic acid	3
Other	Efficiency	Plates/meter for triphenylene	1
	Peak area	Peak area for triphenylene	1

Results

The tests were initially developed on Hypersil GOLD, which is a L1 phase based on a highly pure silica support. The chromatographic data obtained on this phase for each test is displayed in Figures 1-3. Figures 4 to 6 illustrate the chromatograms obtained for each test on the new C18 material.

FIGURE 1. Test 1 – Primary interactions on Hypersil GOLD.

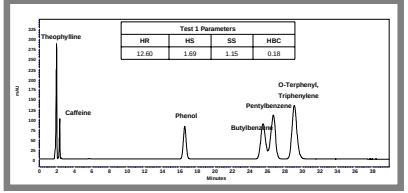


FIGURE 2. Test 2 on Hypersil GOLD.

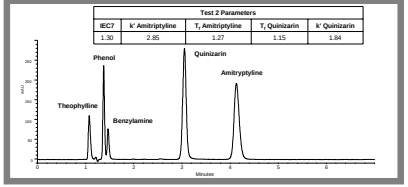


FIGURE 3. Test 3 on Hypersil GOLD.

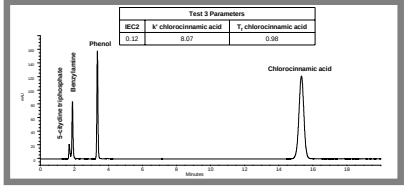


FIGURE 4. Test 1 – Primary interactions on high surface area C18 material.

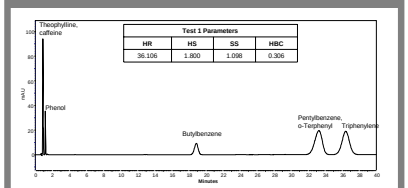


FIGURE 5. Test 2 on high surface area C18 material.

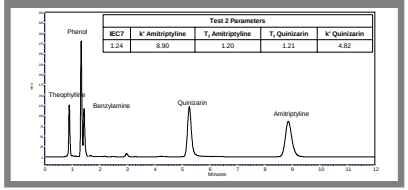
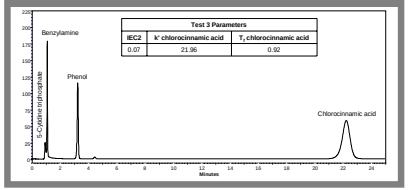


FIGURE 6. Test 3 on high surface area C18 material.



Comparison of the hydrophobicity parameters on Hypersil GOLD and the new high surface area C18 material reveals an approximate 3 fold increase in the hydrophobic retention for the latter column. Ion exchange capacity values are similar on both materials, which demonstrates that the high density proprietary endcapping on the new phase is effective in reducing interactions with the surface of the silica. Tailing factors for bases, chelators and acids are also comparable on both phases.
Table 2 and 3 demonstrate column to column and batch to batch reproducibility, respectively, for the new C18 material using test 1 and test 2 chromatographic testing.

TABLE 2. Intra-batch reproducibility for Test 1 and Test 2 .

Test 1 Parameters - Batch A (10 columns)							
	HR	HS	SS	HBC	Efficiency	Tf	Area
Average	36.22	1.80	1.10	0.31	71536	0.96	1153254
%RSD	**0.99**	**0.24**	**0.18**	**0.71**	**8.99**	**2.08**	**0.41**

Test 2 Parameters - Batch A (10 columns)					
	IEC7	k' Amitriptyline	Tf Amitriptyline	Tf Quinizarin	k' Quinizarin
Average	1.24	8.96	1.18	1.23	4.91
%RSD	**2.84**	**3.75**	**3.86**	**8.08**	**1.30**

TABLE 3. Inter-batch reproducibility for Test 1 and Test 2 .

Test 1 Parameters							
	HR	HS	SS	HBC	Efficiency	Tf	Area
Batch A	36.22	1.80	1.10	0.31	71536	0.96	1153254
Batch B	34.50	1.80	1.11	0.28	76144	1.02	1111401
Batch C	35.44	1.79	1.12	0.30	68804	0.96	1110317
Average	35.39	1.80	1.11	0.30	72161	0.98	1124991
%RSD	**2.44**	**0.27**	**1.12**	**4.16**	**5.14**	**3.33**	**2.18**

Test 2 Parameters - Batch-to-batch Reproducibility					
	IEC7	k' Amitriptyline	Tf Amitriptyline	Tf Quinizarin	k' Quinizarin
Batch A	1.24	8.96	1.18	1.23	4.91
Batch B	1.34	8.55	1.40	1.31	4.45
Batch C	1.27	8.37	1.22	1.24	4.57
Average	1.28	8.63	1.26	1.26	4.64
%RSD	**3.69**	**3.50**	**9.11**	**3.54**	**5.13**

Conclusions

- A new regime of chromatographic testing which probes for primary and secondary interactions has been described.
- These probes are used to fully characterize a new high surface area C18 material and to compare it with the existing Hypersil GOLD.
- Extensive sets of data derived from the same chromatographic probes demonstrate that the new high surface area material is highly robust and reproducible.

References

(1) K. Kimata, K. Iawguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki and N. Tanaka, Journal of Chromatographic Science, 27 (1989) 721-728

For additional information, please visit our Chromatography Resource Centre which can be found at:
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