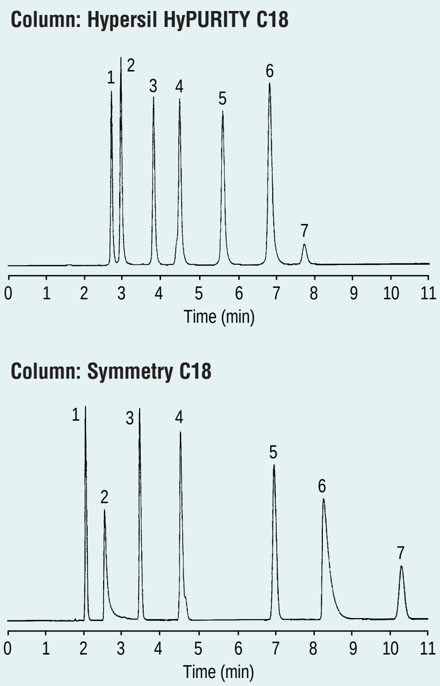
Introduction

There are so many different C18 columns to choose from, that finding the right column for a particular separation can be very time consuming and expensive. Two apparently similar C18 phases can give very different results. For example, Figure 1 compares the separation of the same sample mixture on a Hypersil HyPURITY C18 and a Symmetry C18 column under identical mobile phase conditions. Even though both columns are packed with "new generation" C18 stationary phases, the band spacing (selectivity) between peaks is very different on the two columns. Without more information, it is impossible to predict how the performance of different stationary phases will compare.

Figure 1

Apparently Similar C18 Phases Can Give Very Different Chromatographic Results



Mobile Phase: 60% CH_3CN
40% 50mM KH_2PO_4, pH 3.2

Sample: 1. Uracil 5. N,N-Dimethylaniline
2. Pyridine 6. 4-Butylbenzoic acid
3. Phenol 7. Toluene
4. Dimethyl phthalate

Both Symmetry C18 and Hypersil HyPURITY C18 are new generation phases. You would expect them to provide similar performance, and in some cases they do. However, in the example given here you can see significant differences in peak retention times, selectivity, and even peak shape.

This *Comparison Guide to C18 Reversed Phase HPLC Columns* provides basic comparison information on commonly used C18 columns to help you more easily identify similarities and differences before investing time and money in chromatographic testing. Hopefully, this information will help you find the right column for your application quicker.

Only silica based C18 bonded phases are evaluated in this Guide. Other bonded phases, such as C8, CN, Phenyl and polar embedded phases, are excluded.

This Guide does not identify an overall "best" column. The column that works best for one application will not necessarily be the column that will work best for other applications. And, there certainly is not a single column that will work best for all applications. However, this Guide can help you identify columns that are likely to perform well so that at least you can narrow the number of columns for chromatographic testing. You may find that this Guide helps you identify several columns that provide good separations and performance. It is always desirable to have more than one column identified for an application, especially if you are running routine assays.

Increasingly, chromatographers are seeking to identify alternate brands of HPLC columns suitable for their assays. Having an alternate column choice for a method reduces the risk of "down time" due to column problems such as a change in selectivity from one manufactured lot to another or slow supplier delivery. Finding an alternate or back-up column that will provide acceptable selectivity and performance when substituted into a method can be as expensive and time consuming as finding the right column for developing an initial separation. It is our hope that this Guide will make that job easier by identifying columns with similar chromatographic characteristics.

This Guide provides the following comparison data on commonly used C18 phases:

Stationary Phase Specifications
Specifications provided by column manufacturers

Phases Compared According to Relative Hydrophobicity
Retention data for hydrophobic and neutral compounds

Phases Compared According to Relative Polarity

Categorization of Phases According to Hydrophobicity and Polarity

Comparison of Column Efficiency for a Neutral Compound

Comparison of Column Efficiency for Basic Compounds
Also measures peak tailing

Phases Grouped According to Silanol Activity

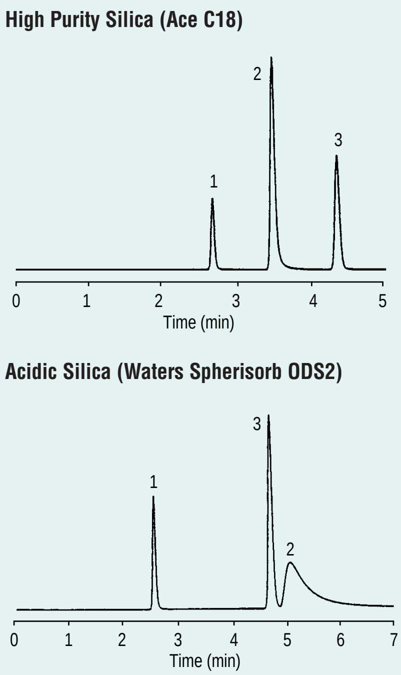
Phases Compared According to Metal Activity

Stationary Phase Specifications

Stationary phase specifications provide basic information that can be helpful in deciding which phases to select for evaluation. For example, phases with high surface area and high carbon load will generally retain hydrophobic compounds longer than phases with low surface area and low carbon load. If you are analyzing macromolecules, such as peptides and proteins, a wider pore (200 — 300 Å) phase usually provides better performance than a phase with small pores. New high purity silicas usually provide better peak shape for basic compounds than older, more acidic silicas (see Figure 2). Stationary phase specifications, however, will not give you enough information to accurately predict retention or band spacing (selectivity). This is especially true when separating polar compounds.

Figure 2

High Purity Silicas Provide Better Peak Shape for Basic Compounds



Mobile Phase: 60% CH_3OH, 40% H_2O
Sample: 1. Uracil
2. Pyridine
3. Phenol

Interaction between cationic compounds and acidic silanol sites on the surface of silica stationary phase supports can contribute to retention and peak tailing. Phases made with high purity silica (less acidic silica) generally can be expected to provide better peak shape for basic compounds.

Figure 3

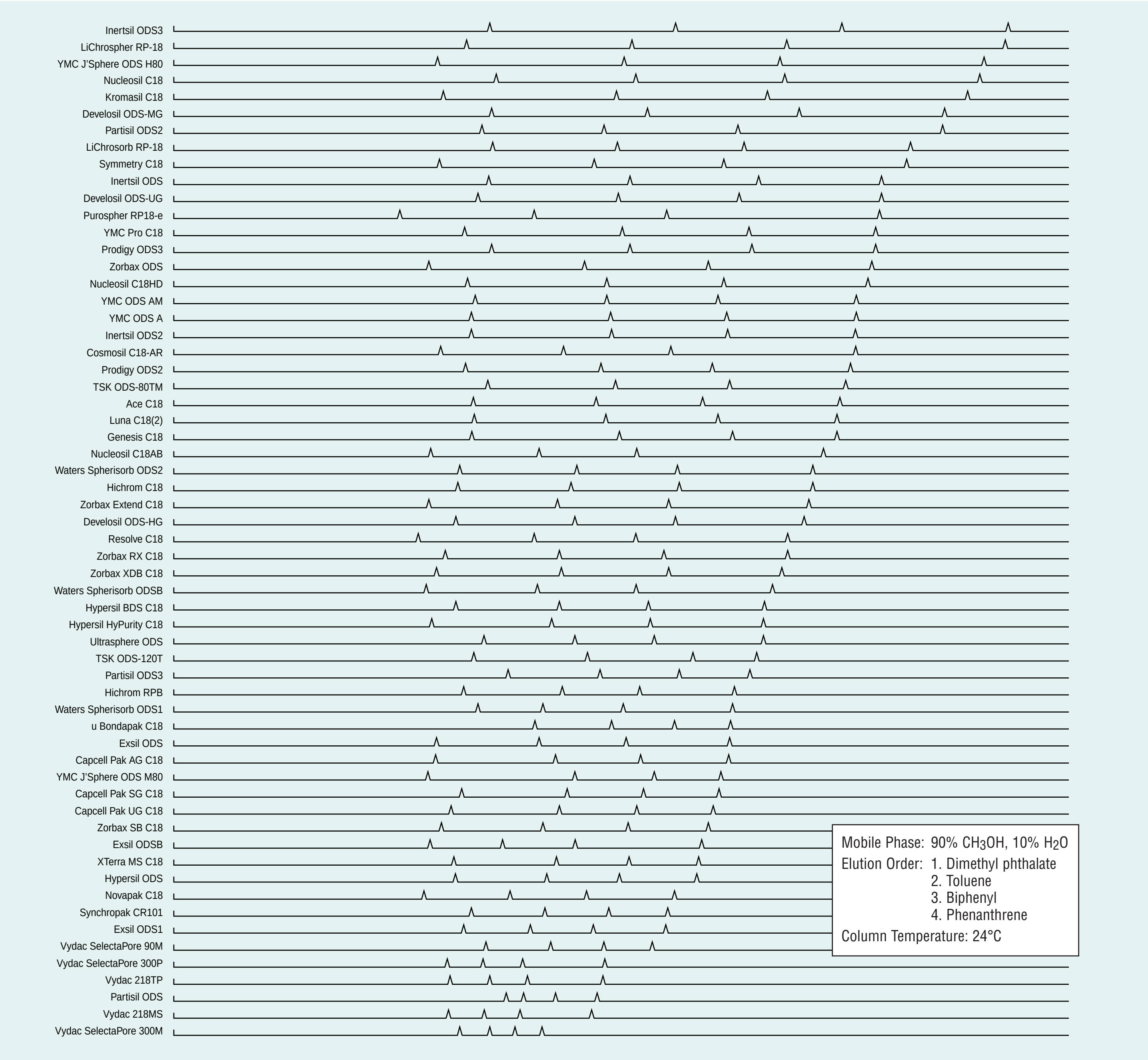
Specifications of C18 Stationary Phases

Stationary Phase	Particle Size (µm)	Pore Size (Å)	Surface Area (m^2/g)	Carbon Load (%)	Endcapped	High Purity Silica
Ace C18	5	100	300	15.5	yes	yes
Capcell Pak AG C18	5	120	300	15	yes	no
Capcell Pak SG C18	5	120	300	14	yes	no
Capcell Pak UG C18	5	120	300	15	yes	yes
Cosmosil C18-AR	5	120	300	17	yes	yes
Develosil ODS-HG	5	140	300	18	yes	yes
Develosil ODS-MG	5	100	450	15	yes	yes
Develosil ODS-UG	5	140	300	18	yes	yes
Exsil ODS	5	100	200	11	yes	no
Exsil ODS1	5	100	200	11	yes	no
Exsil ODSB	5	100	200	12	yes	no
Genesis C18	4	120	300	?	yes	yes
Hichrom C18	5	150	250	15	yes	yes
Hichrom RPB	5	110	340	14	yes	yes
Hypersil BDS C18	5	130	170	11	yes	no
Hypersil HyPURITY C18	5	180	200	13	yes	yes
Hypersil ODS	5	120	170	10	yes	no
Inertsil ODS	5	100	350	14	yes	no
Inertsil ODS3	5	100	450	15	yes	yes
Inertsil ODS2	5	150	320	18.5	yes	yes
Kromasil C18	5	100	340	19	yes	yes
LiChrosorb RP-18	10	100	300	17	no	no
LiChrospher RP-18	5	100	350	21.6	no	no
Luna 5 C18(2)	5	100	400	17.5	yes	yes
Novapak C18	4	60	120	7.3	yes	no
Nucleosil C18	5	100	350	15	yes	no
Nucleosil C18 HD	5	100	?	20	yes	yes
Nucleosil C18AB	5	100	350	24	yes	no
Partisil ODS	10	85	350	5	no	no
Partisil ODS2	10	85	350	15	yes	no
Partisil ODS3	10	85	350	10.5	yes	no
Prodigy ODS2	5	150	310	18.4	yes	yes
Prodigy ODS3	5	100	450	15.5	yes	yes
Purospher RP18-e	5	80	500	?	yes	yes
Resolve C18	5	90	200	10	no	no
Symmetry C18	5	100	335	19	yes	yes
Synchropak CR101	5	100	?	?	no	no
TSK ODS-120T	5	120	?	22	yes	no
TSK ODS-80TM	5	80	?	15	yes	no
µBondapak C18	10	125	330	10	yes	no
Ultrasphere ODS	5	80	?	12	yes	no
Vydac 218MS	5	300	70	?	yes	no
Vydac 218TP	5	300	70	8	yes	no
Vydac Selectapore 300M	5	300	70	?	yes	yes
Vydac Selectapore 300P	5	300	70	?	yes	yes
Vydac Selectapore 90M	5	90	250	?	yes	yes
Waters Spherisorb ODS1	5	80	220	6.2	no	no
Waters Spherisorb ODS2	5	80	220	11.5	yes	no
Waters Spherisorb ODSB	5	80	220	11.5	yes	no
XTerra MS C18	5	125	?	15.5	yes	—
YMC J'Sphere ODS H80	4	80	510	22	yes	no
YMC J'Sphere ODS M80	4	80	510	14	yes	no
YMC ODS A	5	120	300	17	yes	no
YMC ODS AM	5	120	300	17	yes	no
YMC Pro C18	5	120	335	16	yes	yes
ZORBAX Extend C18	5	80	180	12.5	yes	yes
ZORBAX ODS	5	70	330	20	yes	no
ZORBAX Rx-C18	5	80	180	12	no	yes
ZORBAX SB-C18	5	80	180	10	no	yes
ZORBAX XDB-C18	5	80	180	10	yes	yes

Specifications were obtained from manufacturer's literature.

Figure 4

C18 Phase Compared According to Relative Hydrophobicity

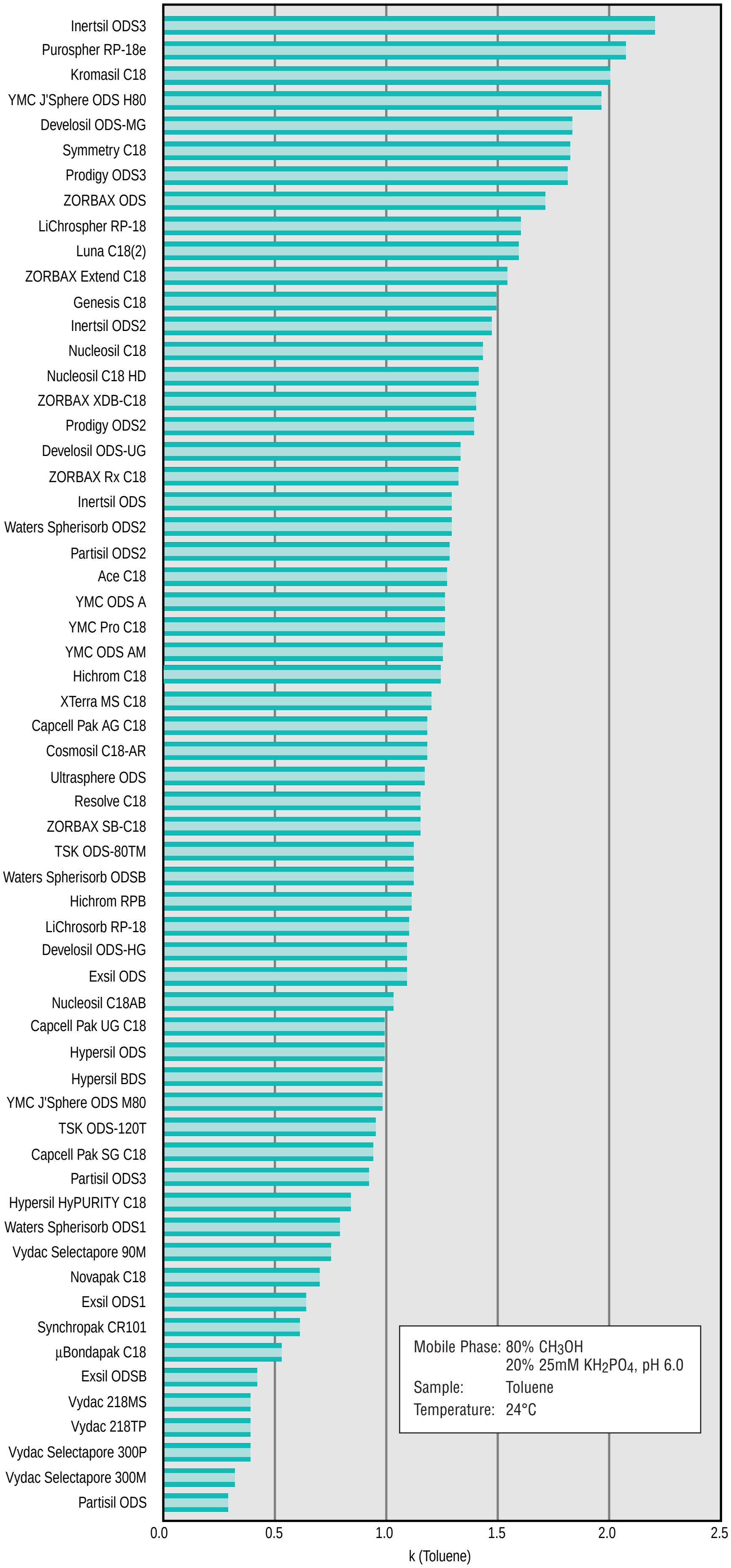


Phases Compared According to Relative Hydrophobicity

Hydrophobicity is measured as the retention of a hydrophobic solute, phenanthrene. Figure 4 gives a comparison of hydrophobicity with the C18 phases listed according to hydrophobicity. Notice, however, that the retention for dimethyl phthalate, the least hydrophobic solute in the mixture, cannot always be predicted from the hydrophobicity ranking. Some low hydrophobicity phases actually have greater retention for dimethyl phthalate than some high hydrophobicity phases. We find that this is not unusual when separating polar compounds. Phases that are significantly more retentive for hydrophobic analytes may show only slightly more retention for polar compounds than low hydrophobicity phases, and sometimes they show less.

Figure 5

C18 Phases Ranked According to Retention for Toluene



Alternative Test for Hydrophobicity

Toluene can also be used as a probe to measure hydrophobicity. Notice that the ranking of C18 phases according to retention for toluene (Figure 5) is slightly different from the ranking according to retention for phenanthrene (Figure 4).

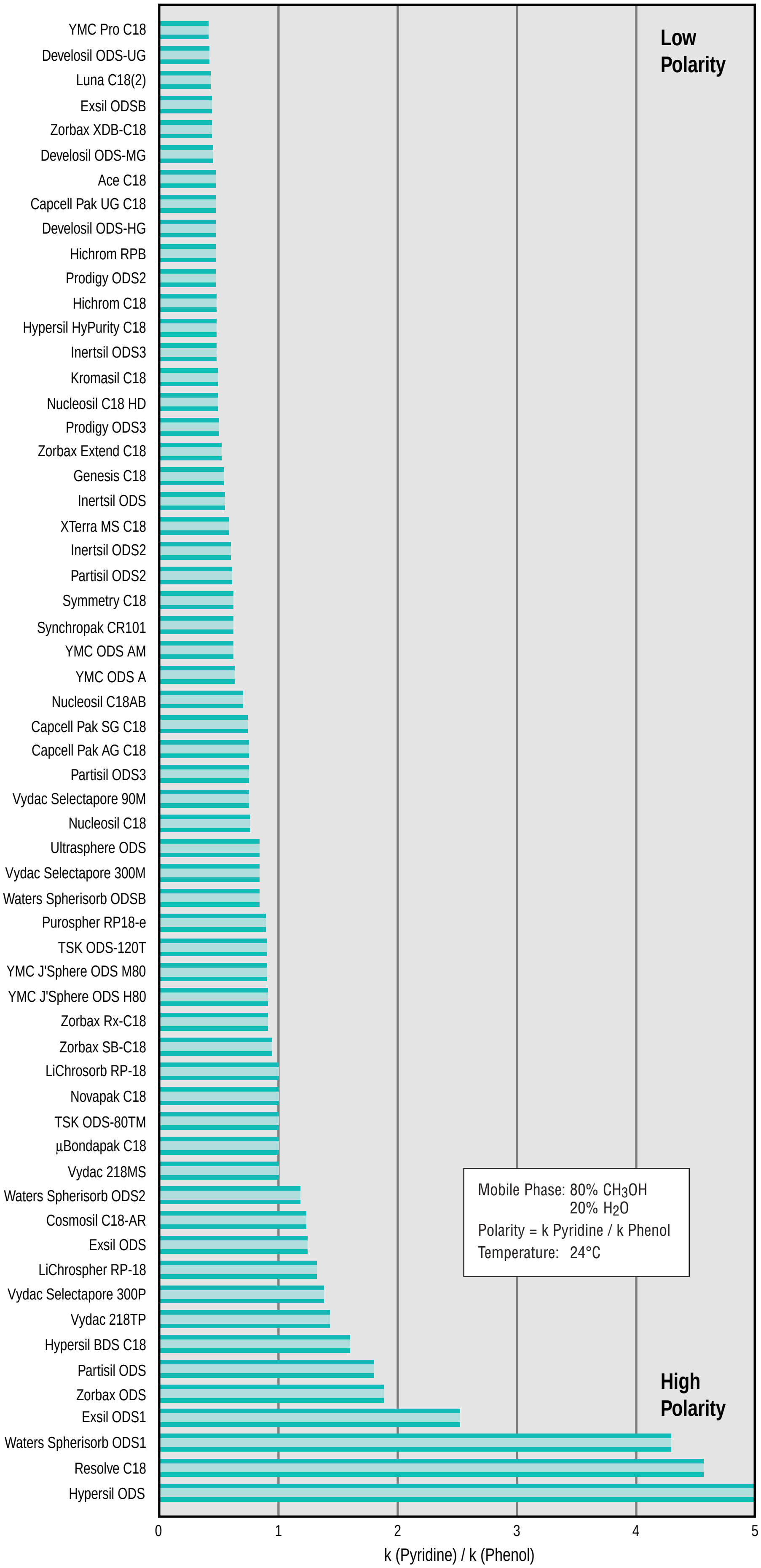


Figure 6
C18 Phases Ranked According to Polarity

Phases Ranked According to Relative Polarity

There have been several chromatographic tests suggested for measuring polarity of stationary phases. Although there is no one test that we think provides a definitive measurement, we have chosen to use the ratio of k values for pyridine and phenol as our measure of relative polarity for these C18 phases.

Figure 6 ranks stationary phases according to relative polarity using the test conditions given. In this ranking, there is not necessarily a significant difference between consecutive listings. If a different mobile phase condition was used for the test, e g., a lower mobile phase pH, or if different probes were used, the ranking may be somewhat different. However, phases at the high polarity end of the ranking and phases at the low polarity end of the ranking are likely to test that way under most polarity tests conditions. Therefore, this ranking can be used to identify relative differences and similarities in polarity that can affect selectivity for polar compounds

Since silanol activity is a major contributor to phase polarity, the test conditions used here to measure polarity have also been used by some chromatographers as an indication of silanol activity. This seems consistent with the fact that most phases at the high polarity end of the ranking use more acidic silicas as stationary phase supports where phases at the low polarity end of the ranking use less acidic (high purity) silicas. However, there are other factors that contribute to the retention of pyridine and phenol that prevent us from using their relative retention as a reliable measure of silanol activity. For example, Inertsil ODS has moderate polarity but shows significant silanol activity in other tests. Also, we see that Prodigy ODS2 tests with similar polarity as the Ace C18, but in tests for silanol activity, the Ace C18 shows significantly less silanol activity (see Figure 14).

Figure 7

C18 Phases Grouped According to Hydrophobicity and Polarity

Phases are listed in alphabetical order by category.

High Polarity/ Low Hydrophobicity	High Polarity/ Moderate Hydrophobicity	High Polarity/ High Hydrophobicity
Exsil ODS1	Cosmosil C18-AR	LiChrosorb RP-18
Hypersil ODS	Exsil ODS	LiChrospher RP-18
Novapak C18	Hypersil BDS C18	
Partisil ODS	Resolve C18	
Vydac 218MS	TSK ODS-80TM	
Vydac 218TP	μBondapak C18	
Vydac Selectapore 300P	Waters Spherisorb ODS1	
Waters Spherisorb ODS2		
Zorbax ODS		

Moderate Polarity/ Low Hydrophobicity	Moderate Polarity/ Moderate Hydrophobicity	Moderate Polarity/ High Hydrophobicity
Synchropak CR101	Capcell Pak AG C18	Nucleosil C18
Vydac Selectapore 300M	Capcell Pak SG C18	Partisil ODS2
Vydac Selectapore 90M	Genesis C18	Symmetry C18
Xterra MS C18	Inertsil ODS	YMC J'Sphere ODS H80
	Inertsil ODS2	
	Nucleosil C18 AB	
	Partisil ODS3	
	Prodigy ODS3	
	Purospher RP18-e	
	TSK ODS-120T	
	Ultrasphere ODS	
	Waters Spherisorb ODSB	
	YMC J'Sphere ODS M80	
	YMC ODS A	
	YMC ODS AM	
	Zorbax Extend C18	
	Zorbax Rx-C18	
	Zorbax SB-C18	

Low Polarity/ Low Hydrophobicity	Low Polarity/ Moderate Hydrophobicity	Low Polarity/ High Hydrophobicity
Exsil ODSB	Ace C18	Develosil ODS-MG
	Capcell Pak UG C18	Inertsil ODS3
	Develosil ODS-HG	Kromasil C18
	Develosil ODS-UG	
	Hichrom C18	
	Hichrom RPB	
	Hypersil HyPURITY C18	
	Luna C18(2)	
	Nucleosil C18 HD	
	Prodigy ODS2	
	YMC Pro C18	
	Zorbax XDB-C18	

Categorization of phases according to hydrophobicity and polarity.

The hydrophobicity and polarity data can be used to group phases with similar characteristics into categories. The following criteria was used for the categories:

Hydrophobicity

k for phenanthrene

High	> 7.50
Moderate	5.50 to 7.49
Low	< 5.50

Polarity

$$\frac{k\ \text{pyridine}}{k\ \text{phenol}}$$

High	> 1.00
Moderate	0.50 to 0.99
Low	< 0.50

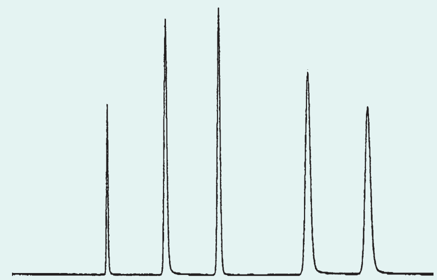
Figure 8 provides an example of how columns from different polarity/hydrophobicity categories will compare. In this separation of antidepressants, Symmetry C18 (high hydrophobicity) is slightly more retentive than Hypersil BDS C18 (moderate hydrophobicity), and the band spacing of Ace C18 (low polarity) is more similar to Symmetry C18 (moderate polarity) than it is to Hypersil BDS C18 (high polarity).

Figure 8

Chromatographic Comparison of Stationary Phases from Different Categories

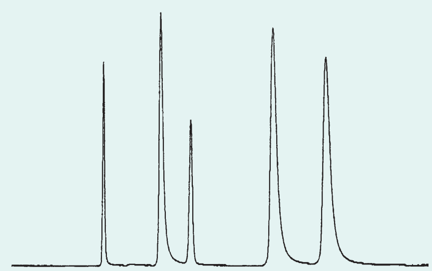
Ace C18

Low Polarity/Moderate Hydrophobicity



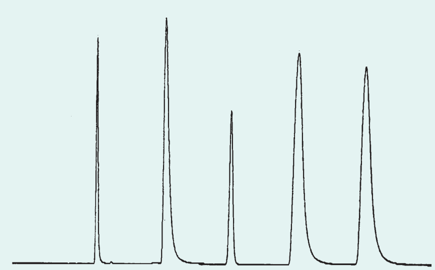
Hypersil BDS C18

High Polarity/Moderate Hydrophobicity



Symmetry C18

Moderate Polarity/High Hydrophobicity



Mobile Phase: 80% CH_3OH,
20% 25mM KH_2PO_4, pH 6.0

Sample:
1. Norephedrine
2. Nortriptyline
3. Toluene
4. Imipramine
5. Amitriptyline

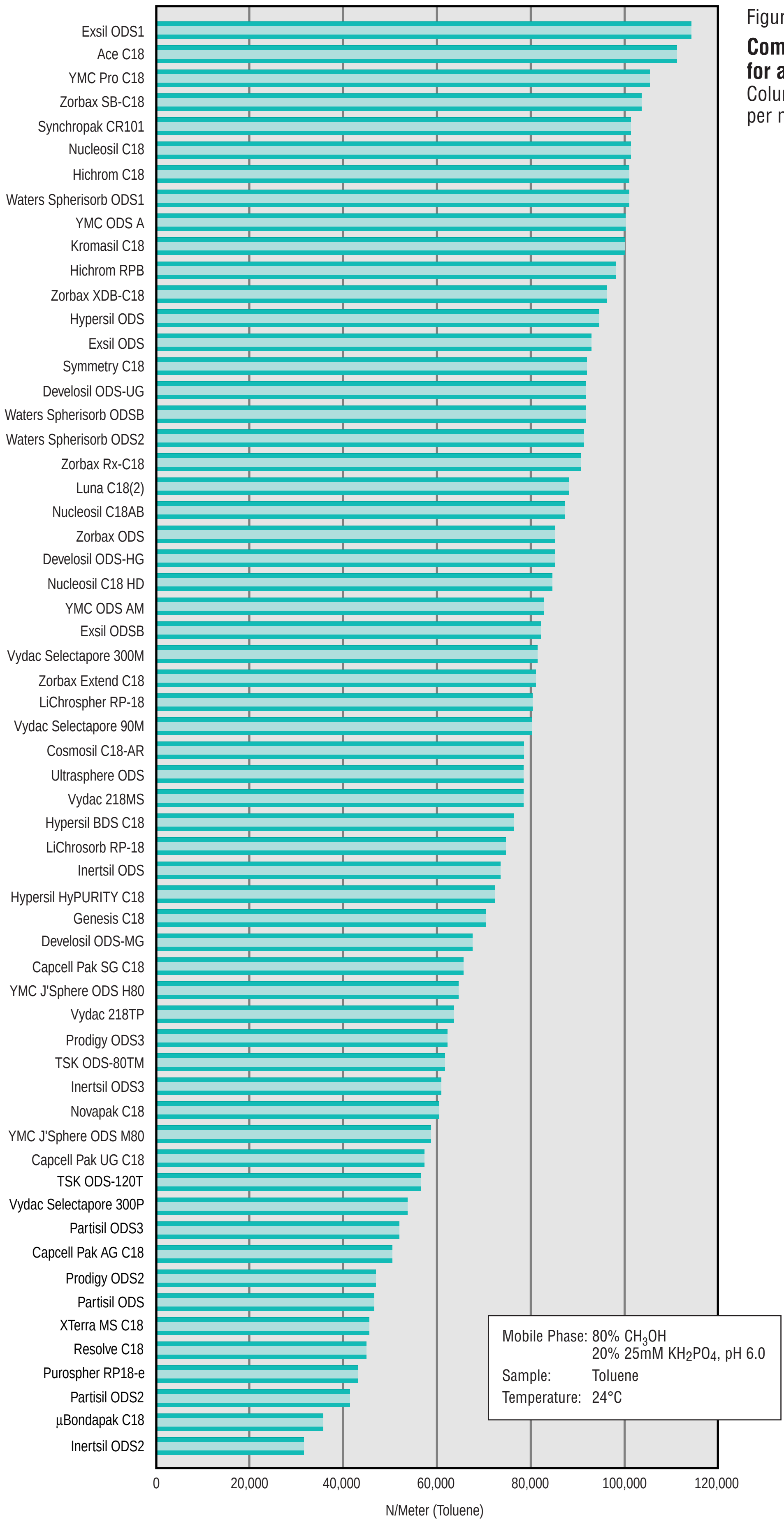


Figure 9

Comparison of Column Efficiency for a Neutral Compound

Column efficiency reported as Plates per meter (N/Meter)

Comparison of Column Efficiency for a Neutral Compound

Column efficiency is reported as plates per meter (N/Meter). Using a neutral compound (toluene) for the measurement greatly reduces the effects of secondary retention on the measurement of N and allows us to obtain data that is a better indication of the following factors:

- Particle size
  Smaller average packing particle size = Larger N
- Particle size distribution
  Broader particle size distribution = Smaller N
- Packing efficiency
  Better packing procedures = Larger N

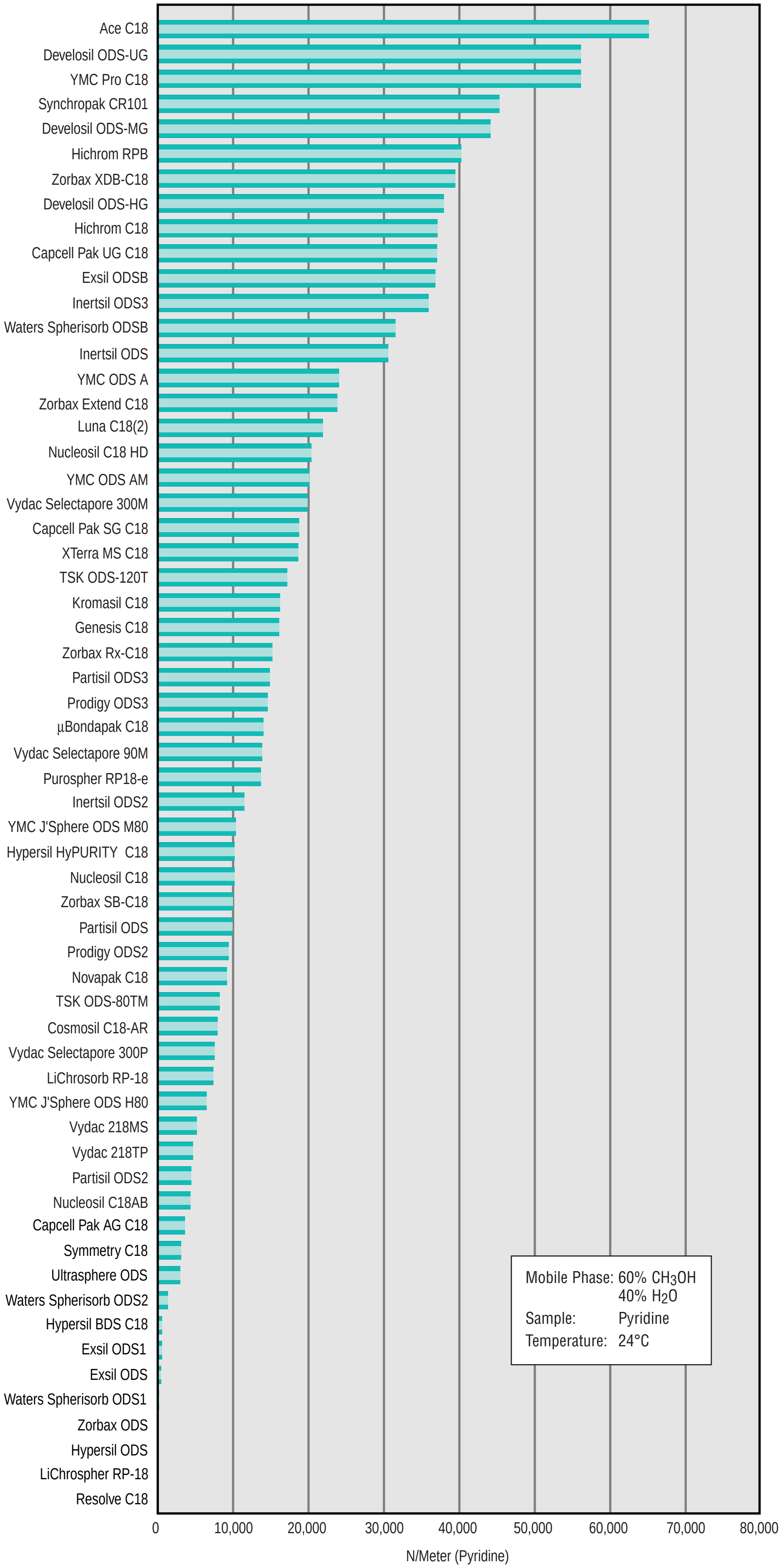


Figure 10

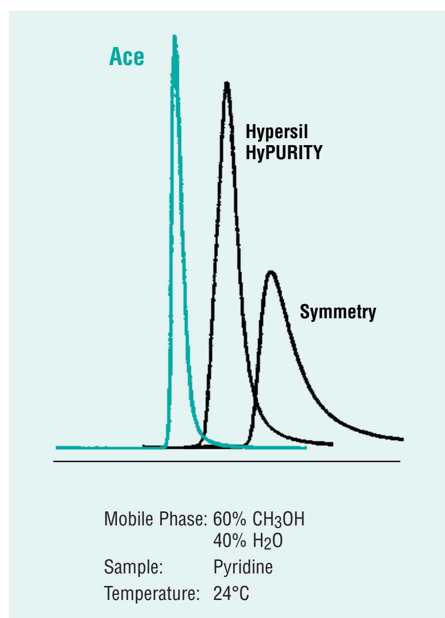
Comparison of Column Efficiency for a Basic Compound: Pyridine

Comparison of Column Efficiency for a Basic Compound

Measuring column efficiency using a neutral compound is not very useful in predicting column performance when separating ionic compounds. Interaction between polar solutes and silanol sites on the stationary phase can cause tailing peaks and poor column efficiency. To gain a better understanding of column performance with basic compounds columns were tested using pyridine and amitriptyline as probes. Although columns are ranked somewhat differently on the two tests, phases at the higher end of the ranking scale can be expected to give better peak shape and higher resolution for basic compounds than phases at the lower end of the scale. Not surprisingly, stationary phases that use high purity silicas exhibit better peak shape and higher column efficiency than stationary phases that use more acidic silicas as their stationary phase supports.

Figure 11

Comparison of Peak Shape



Ace C18 gave the best peak shape and highest column efficiency for pyridine.

Figure 12

Comparison of Column Efficiency for a Basic Compound: Amitriptyline

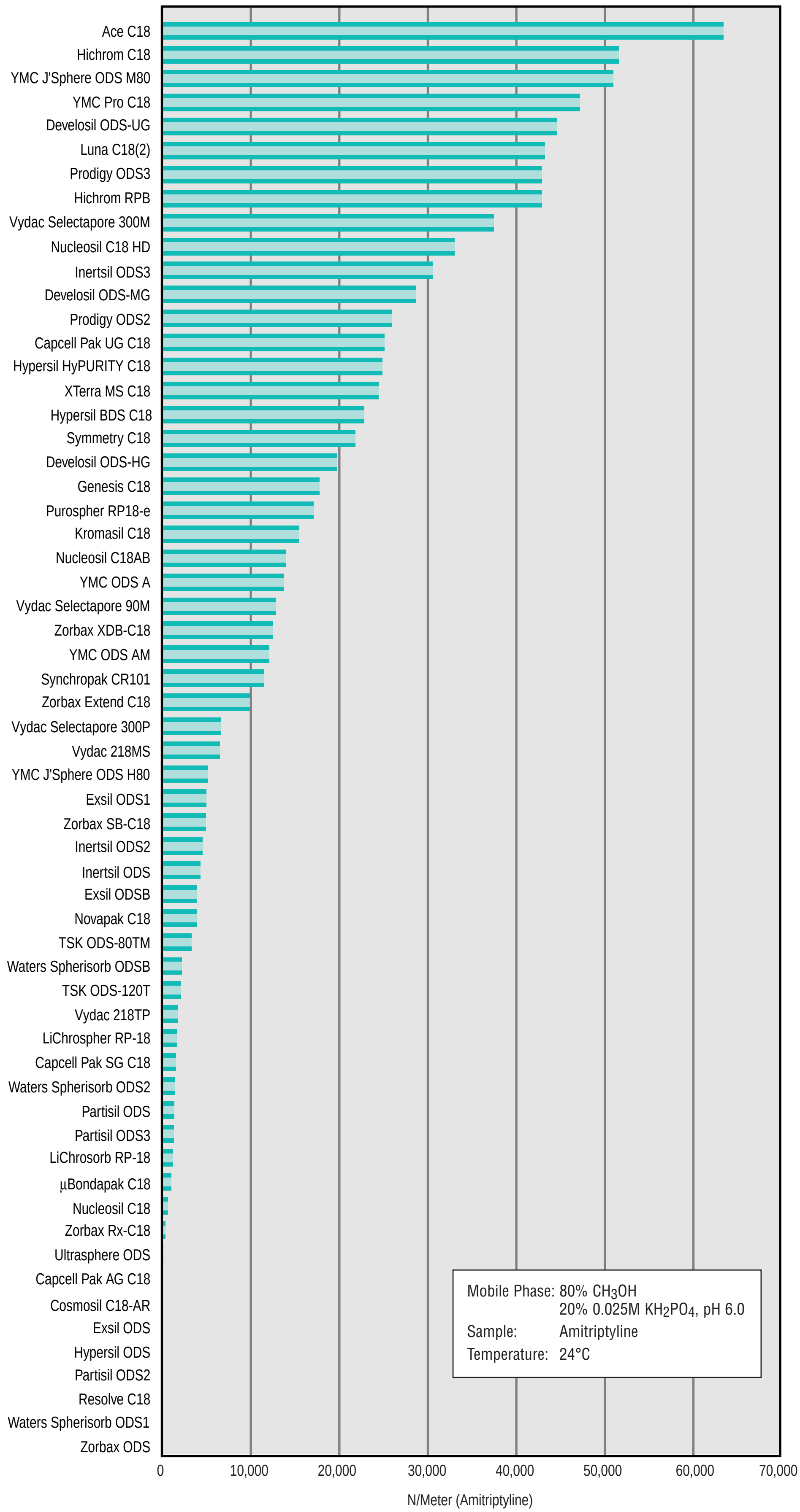
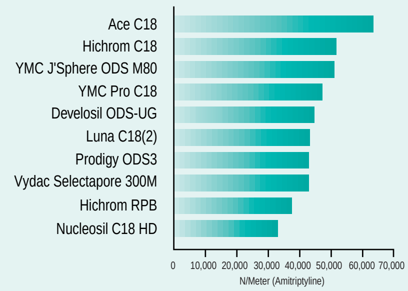


Plate count is measured at 10% of peak height to include peak tailing in the calculation. Both tests use mobile phases at neutral pH to encourage interaction between the basic probes and silanols on the stationary phase.

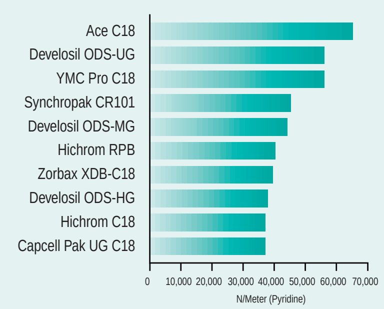
Figure 13

Top 10 Columns Ranked According to Peak Shape and Efficiency

Sample: Amitriptyline
Mobile Phase: 80% CH_3OH
20% 0.025M Phosphate, pH 6.0



Sample: Pyridine
Mobile Phase: 60% CH_3OH
40% H_2O



Note: All columns are packed with 5 micron size material.

Column ranking does differ in the two tests of column efficiency for a basic compound (Figures 10 and 12). However, of the 15 columns that ranked in the top 10 on at least one of the tests, 5 ranked in the top 10 on both tests.

Figure 14

Grouping of C18 Columns According to Silanol Activity

Material	Silanol Activity
Ace C18	Very Low
Develosil ODS-MG	
Hichrom C18	
Hypersil HyPURITY C18	
Inertsil ODS3	
Luna C18(2)	
Nucleosil C18 HD	
XTerra MS C18	
YMC Pro C18	
Capcell Pak UG C18	Low
Develosil ODS-HG	
Develosil ODS-UG	
Genesis C1	
Hichrom RPB	
Inertsil ODS2	
Kromasil C18	
Prodigy ODS2	
Prodigy ODS3	
Purospher RP18-e	
Symmetry C18	
YMC ODS A	
YMC ODS AM	
Zorbax Extend C18	
Zorbax XDB-C18	
Capcell Pak C18 SG	Moderate
Cosmosil C18-AR	
Exsil ODSB	
Hypersil BDS C18	
Inertsil ODS	
Nova-Pak C18	
Nucleosil C18AB	
Partisil ODS3	
Synchropak CR101	
TSK ODS-120T	
TSK ODS-80TM	
µBondapak C18	
Vydac 218MS	
Vydac 218TP	
Vydac Selectapore 300M	
Vydac Selectapore 300P	
Vydac Selectapore 90M	
Waters Spherisorb ODSB	
YMC J'Sphere ODS H80	
YMC J'Sphere ODS M80	
Zorbax Rx-C18	
Zorbax SB-C18	
Capcell Pak C18 AG	High
Exsil ODS	
Exsil ODS1	
Hypersil ODS	
LiChrosorb RP-18	
LiChrospher RP-18	
Nucleosil C18	
Partisil ODS	
Partisil ODS2	
Resolve C18	
Ultrasphere ODS	
Waters Spherisorb ODS1	
Waters Spherisorb ODS2	
Zorbax ODS	

Phases Grouped According to Silanol Activity

Amitriptyline and pyridine are both good test probes to use for measuring silanol activity of stationary phases. Even a small amount of silanol exposure by the stationary phase can cause measurable peak broadening on one or both of these compounds. Chromatographic tests using these two probes are the primary measurements used to group these C18 phases according to silanol activity. In general, phases identified as having "very low" silanol activity will give the highest column efficiency in the pyridine and amitriptyline tests (Figures 10 and 12).

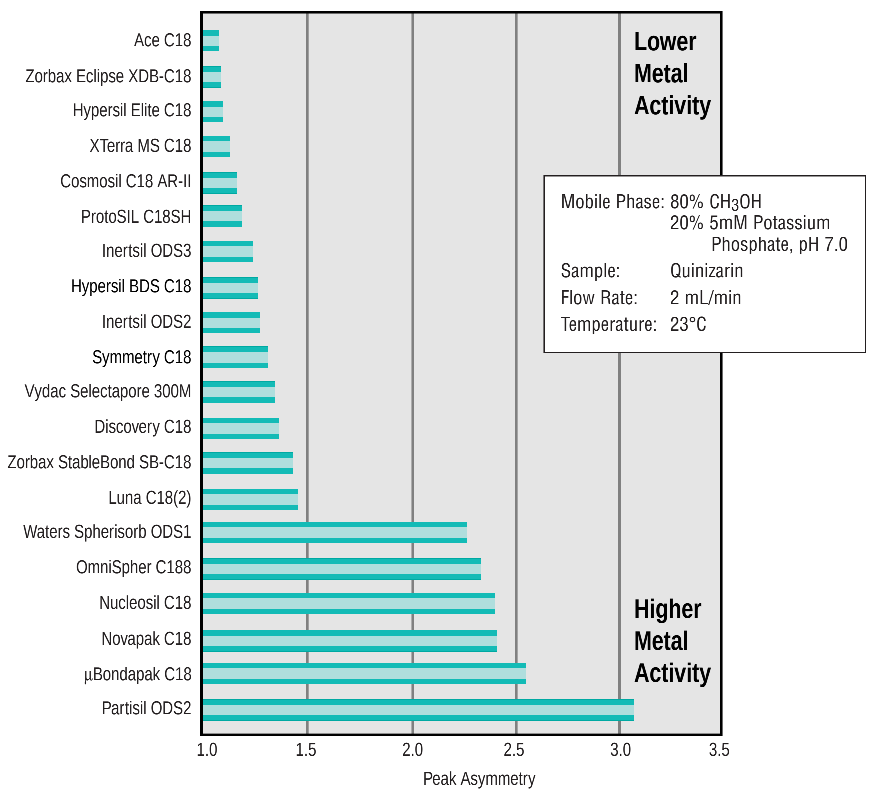
Phases Compared According to Metal Activity

The presence of metals on the surface of stationary phases can have a significant effect on chromatographic performance. Even trace levels of metal impurities can contribute to peak tailing of some compounds. In addition, subtle lot-to-lot variations in the amount of trace metals are another cause of poor column reproducibility.

The National Institute of Standards & Technology (NIST) uses peak asymmetry of quinizarin, a strong metal chelating agent, in their column performance test mixture SRM 870 to measure metal activity of stationary phases. Their internet site (http://ois.nist.gov/srmcatalog/certificates/870.pdf) has data for 41 C18 stationary phases evaluated with test mixture SRM 870. Figure 15 was produced using the quinizarin asymmetry data obtained from the NIST internet site. In this Figure, the 20 columns with the lowest metal activity are ranked according to peak asymmetry for quinizarin. The columns with lower asymmetry values have lower metal activity.

Figure 15

Comparison of Peak Asymmetry for Quinizarin: A Measure of Metal Activity



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