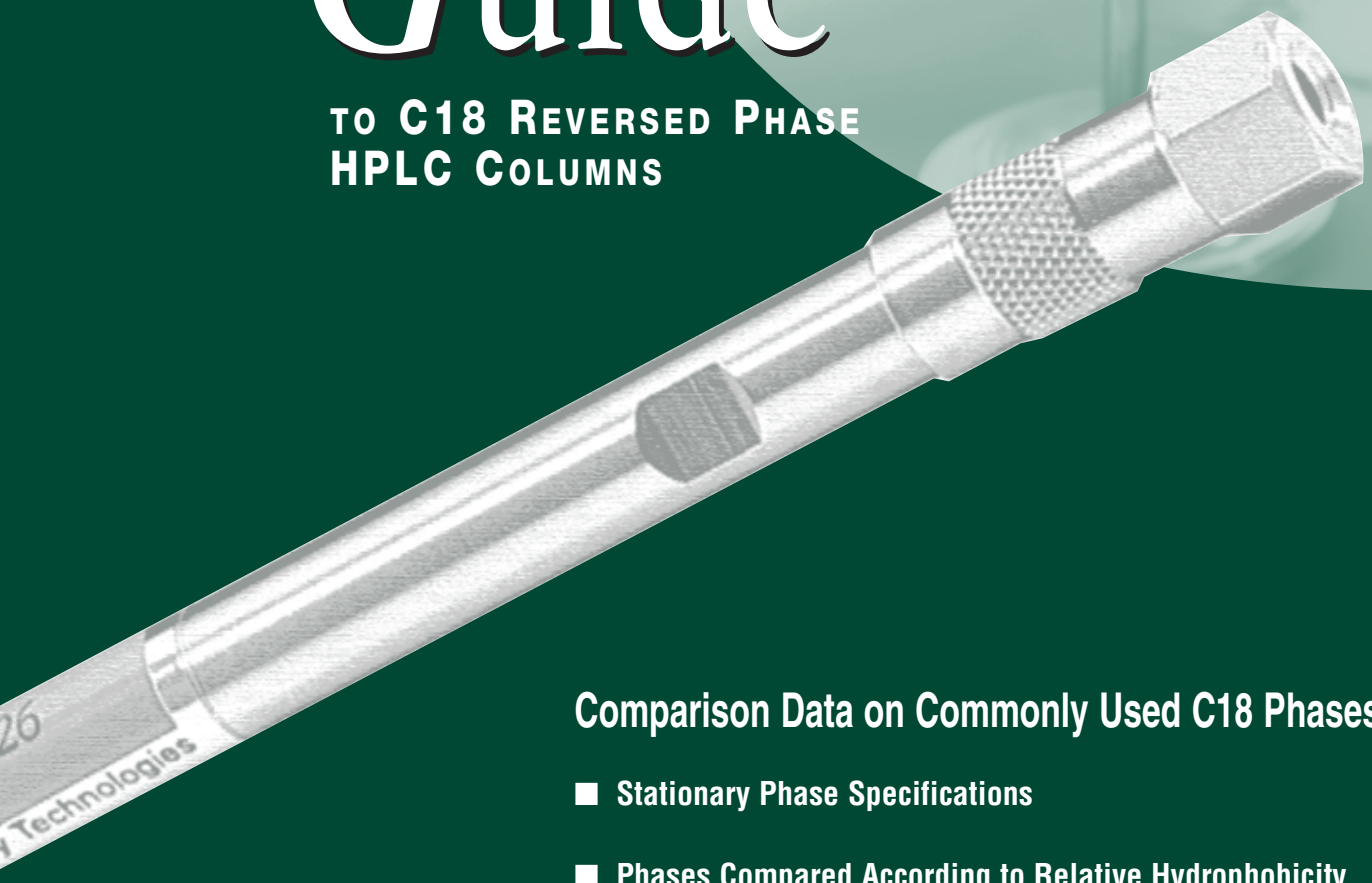
Comparison Guide

TO C18 REVERSED PHASE HPLC COLUMNS



Comparison Data on Commonly Used C18 Phases

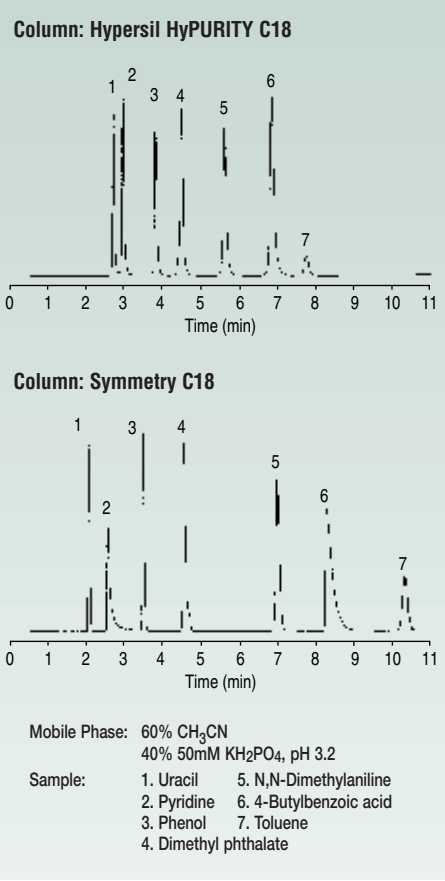
- Stationary Phase Specifications
- Phases Compared According to Relative Hydrophobicity
- Comparison of Column Efficiency for a Neutral Compound
- Phases Compared According to Metal Activity
- Phases Grouped According to Silanol Activity
- Comparison of Column Efficiency for Basic Compounds
- Categorization of Phases According to Silanol Activity and Inertness

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



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.

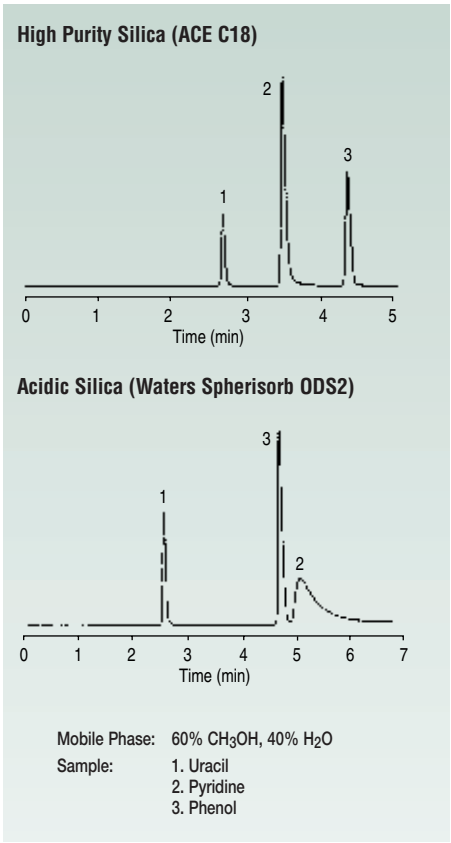
This Guide provides the following comparison data on commonly used C18 phases:

Stationary Phase Specifications

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



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

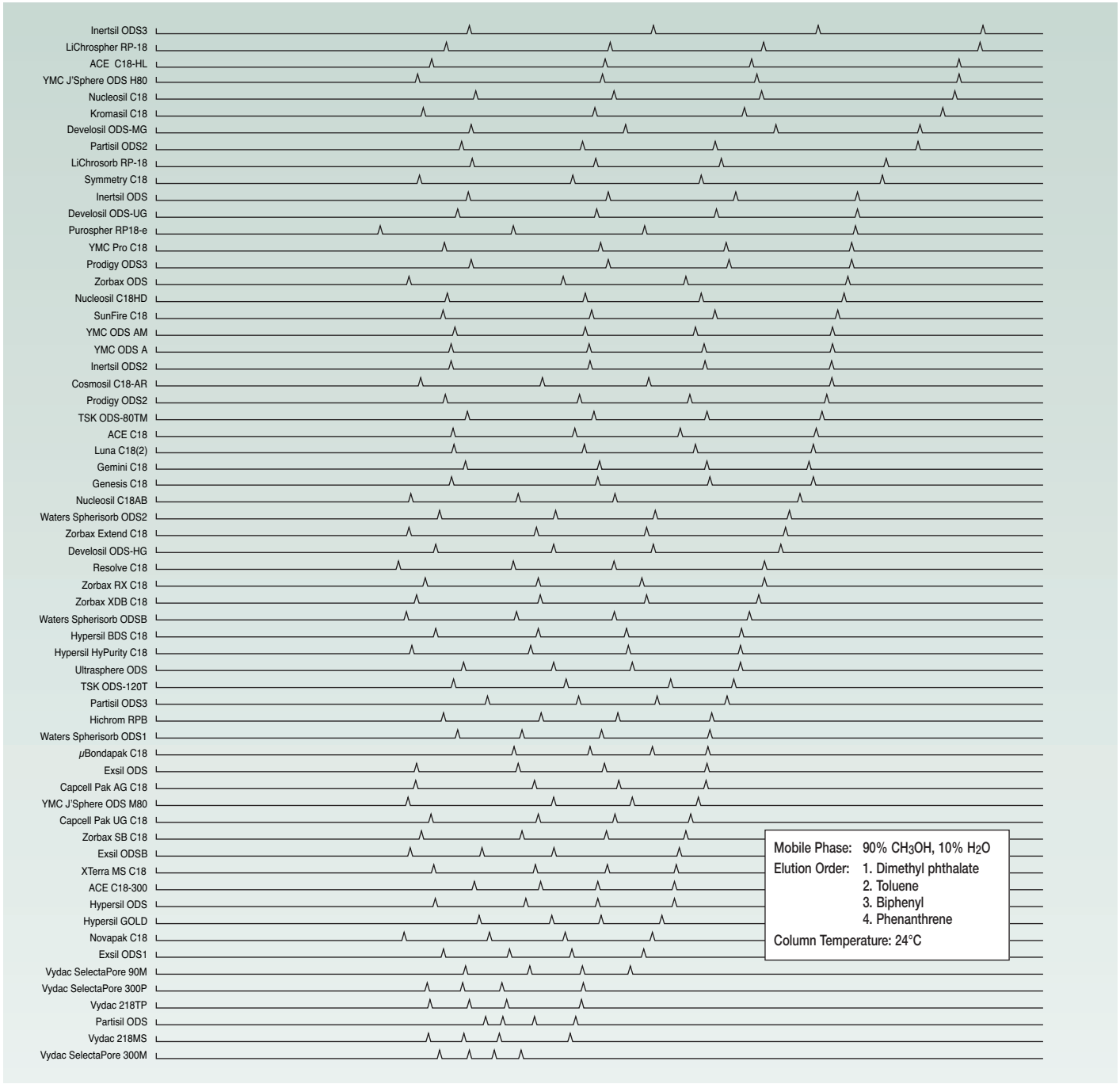
Specifications of C18 Stationary Phases

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
ACE C18-300	5	300	100	9.0	yes	yes
ACE C18-HL	5	90	400	20.0	yes	yes
µBondapak C18	10	125	330	10	yes	no
Capcell Pak AG C18	5	120	300	15	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
Gemini C18	5	110	375	14	yes	-
Genesis C18	4	120	300	-	yes	yes
Hichrom RPB	5	110	340	14	yes	yes
Hypersil BDS C18	5	130	170	11	yes	no
Hypersil GOLD	5	180	200	10	yes	yes
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 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
SunFire C18	5	100	340	16	yes	yes
Symmetry C18	5	100	335	19	yes	yes
TSK ODS-120T	5	120	-	22	yes	no
TSK ODS-80TM	5	80	-	15	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.

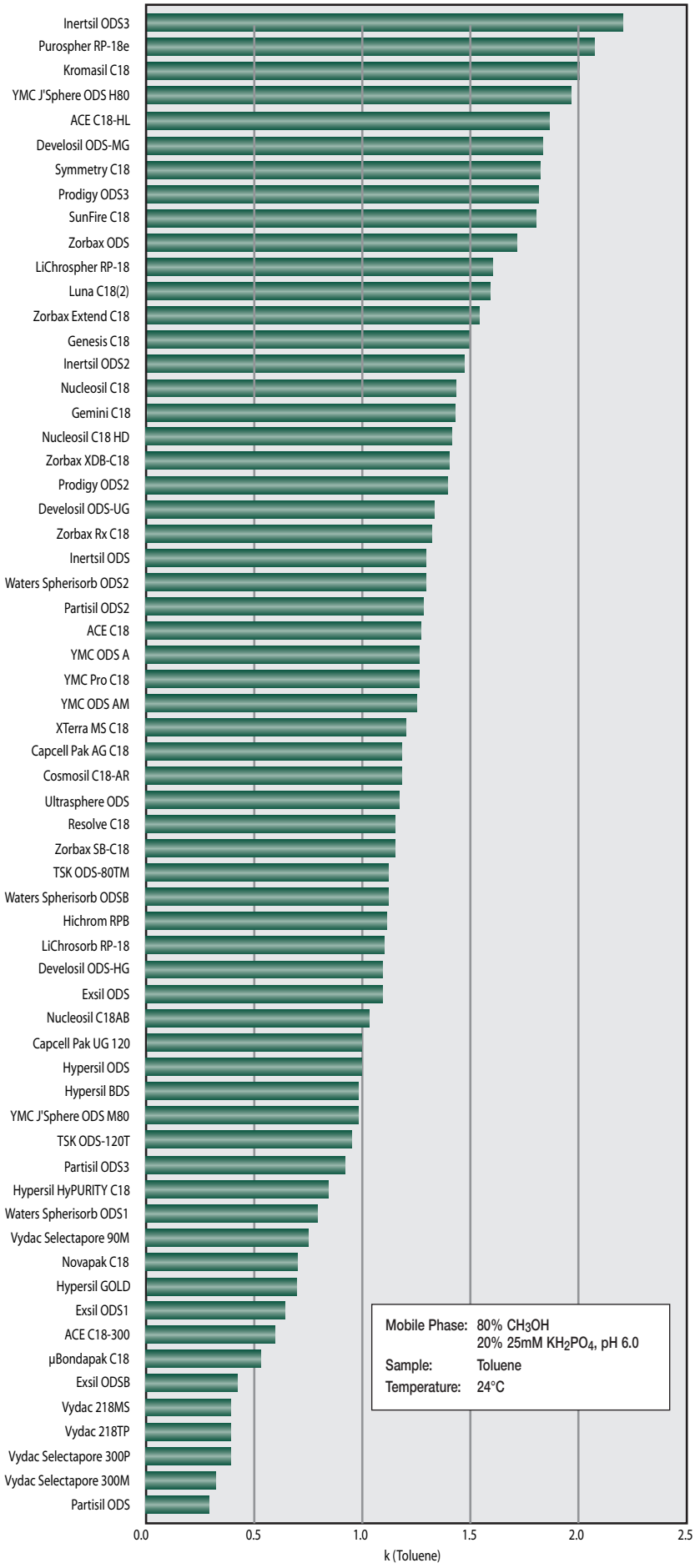


Figure 5

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).

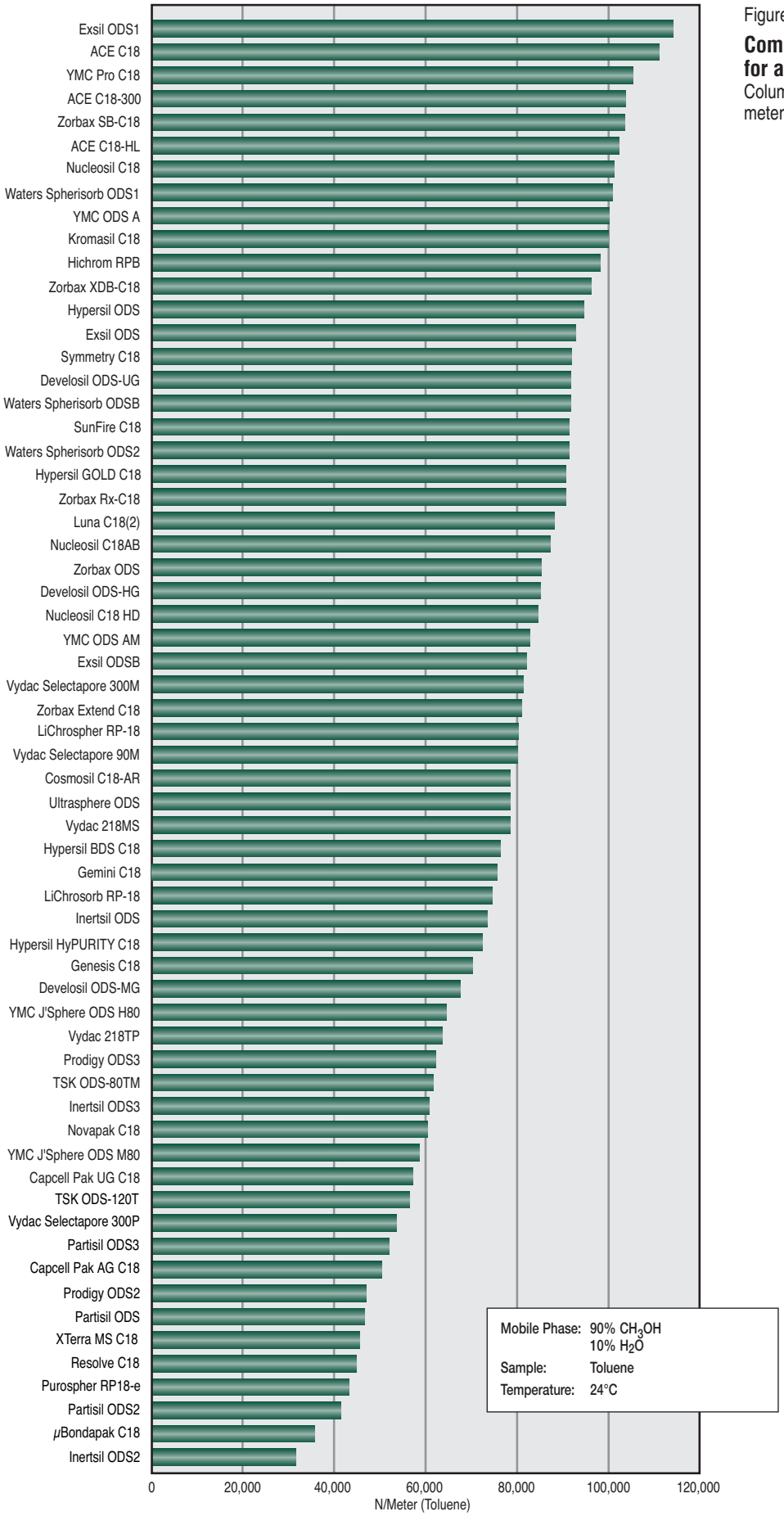


Figure 6

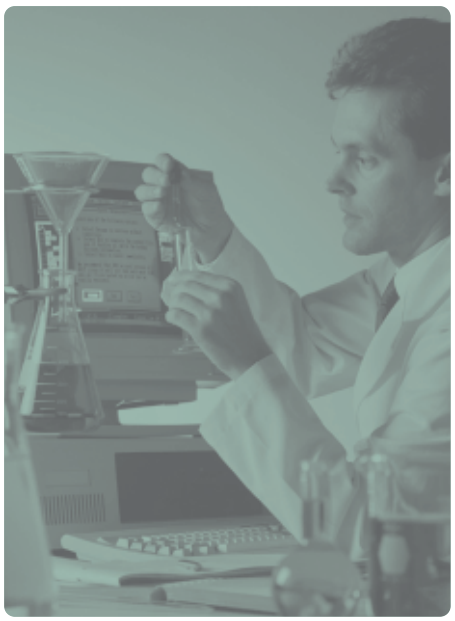
Comparison of Column Efficiency for a Neutral Compound

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



Independently Performed Comparison Tests

There are numerous suggestions from different scientific groups about how to best characterize stationary phases. Most of these tests have merit, but the fact that the ranking of columns will often differ among the different tests shows the difficulty in devising a definitive test that will predict column behavior in all, or even most circumstances. The National Institute of Standards & Technology (NIST) has developed test conditions (Standard Reference Material 870) that do a particularly good job of characterizing stationary phases according to metal activity and silanol 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 NIST test uses peak asymmetry of quinizarin, a strong metal chelating agent, to measure metal activity. Figure 7 ranks stationary phases according to metal activity using the NIST test.

To test silanol activity, the NIST test uses amitriptyline, as does the test used to generate the data in Figure 11. However, the NIST test specifies a mobile phase pH of 7.0 rather than 6.0, and measures peak asymmetry rather than plate count to determine silanol activity. The lower the asymmetry value of the amitriptyline peak (less tailing) the less silanol activity. Figure 8 ranks stationary phases according to silanol activity using the NIST test.

Figure 7

Comparison of Metal Activity Using the NIST Test: Asymmetry for Quinizarin

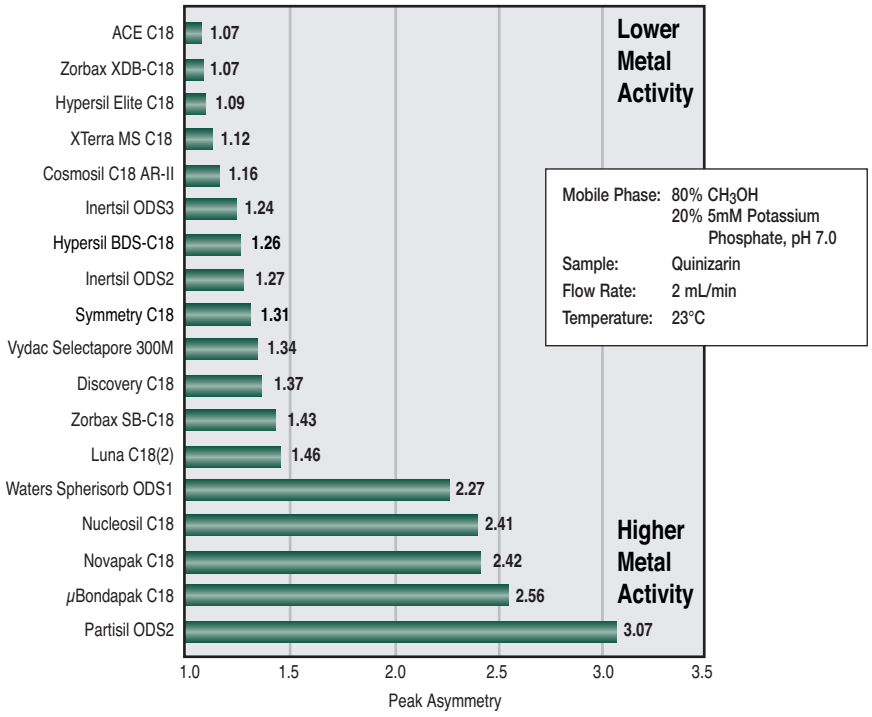
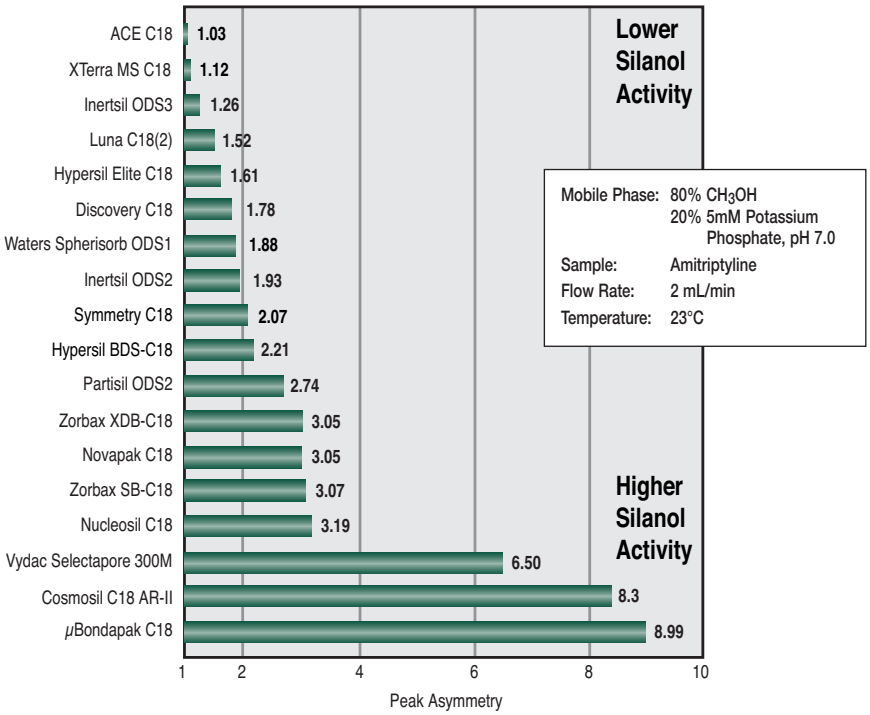


Figure 8

Comparison of Silanol Activity Using the NIST Test: Asymmetry for Amitriptyline



Data obtained from the National Institute of Standards and Technology (NIST), USA. Certificate of Analysis for Standard Reference Material SRM 870, "Column Performance Test Mixture for Liquid Chromatography".

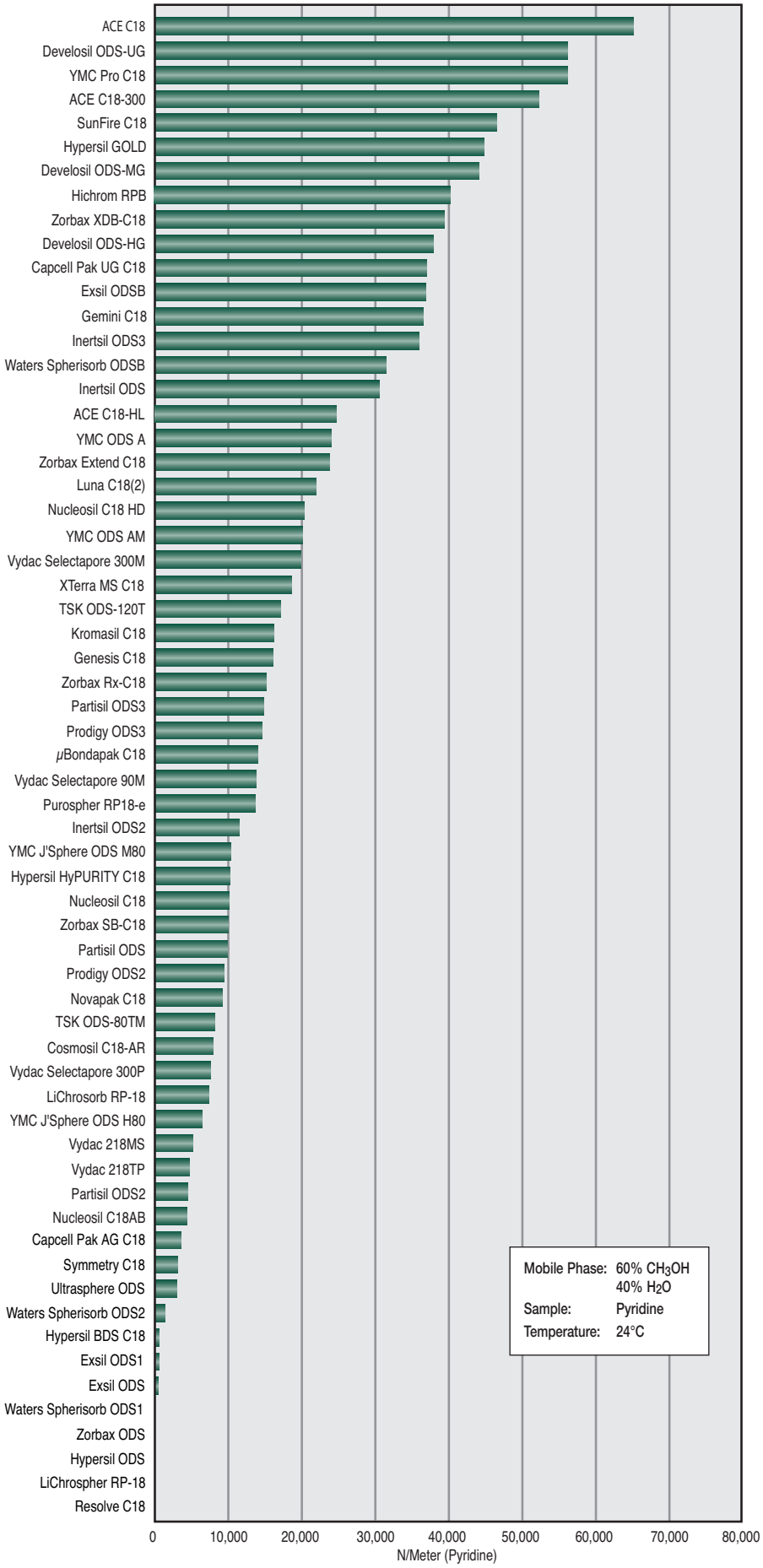


Figure 9

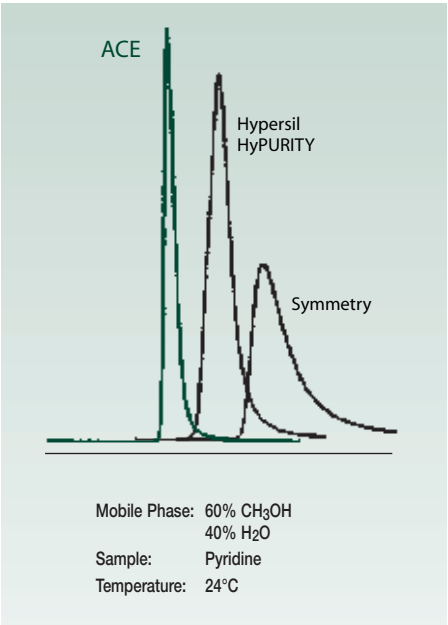
Comparison of Column Efficiency for a Basic Compound: Pyridine

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 10

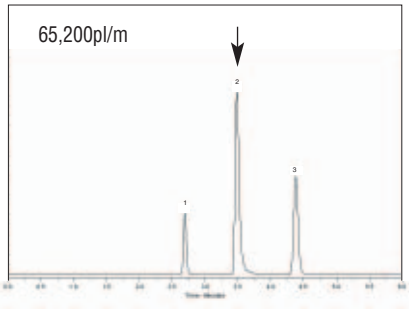
Comparison of Peak Shape



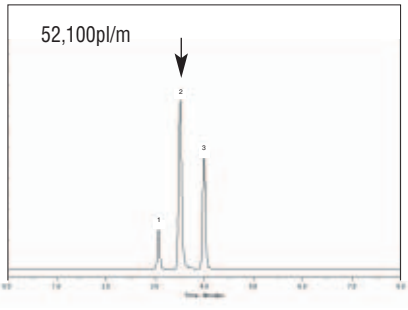
ACE C18 gave the best peak shape and highest column efficiency for pyridine.

Comparison of Column Efficiency For a Basic Compound: Pyridine

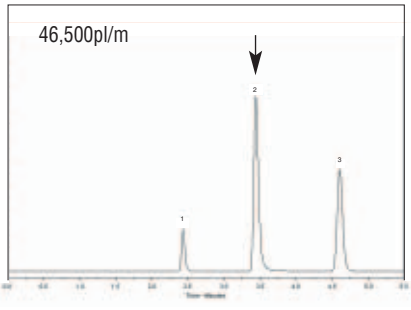
ACE C18



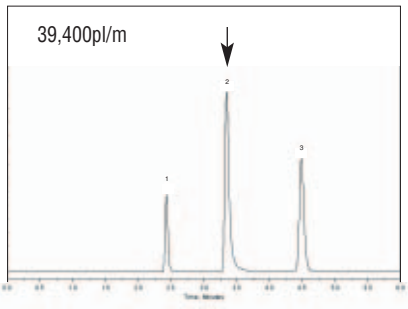
ACE C18-300



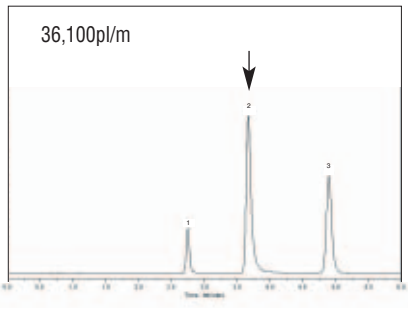
SunFire C18



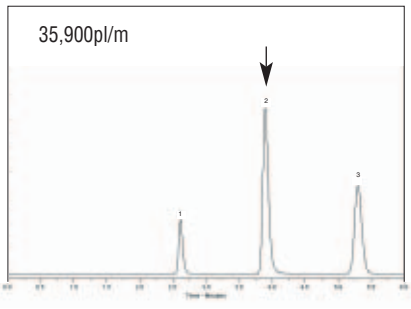
Zorbax XDB C18



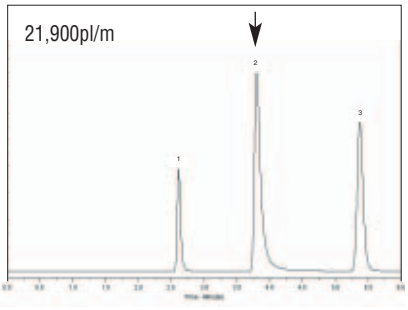
Gemini C18



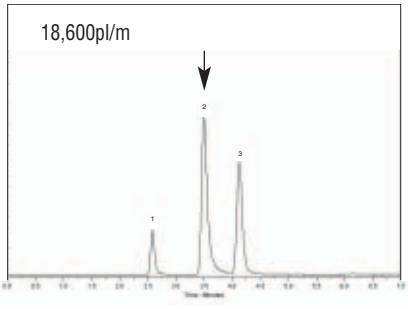
Inertsil ODS3V



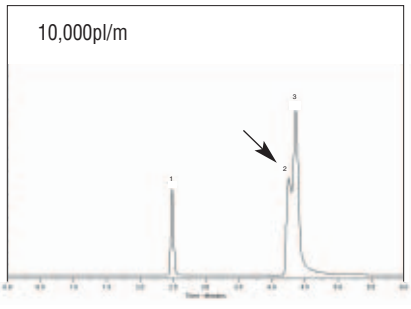
Luna C18(2)



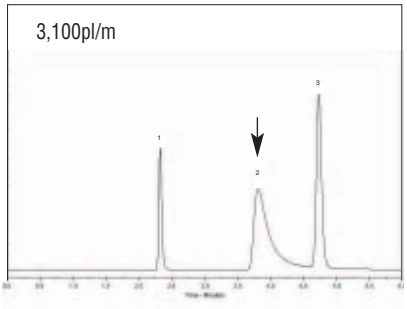
XTerra MS C18



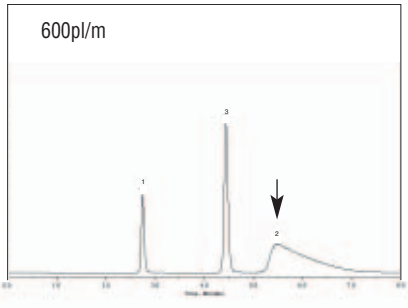
Zorbax SB C18



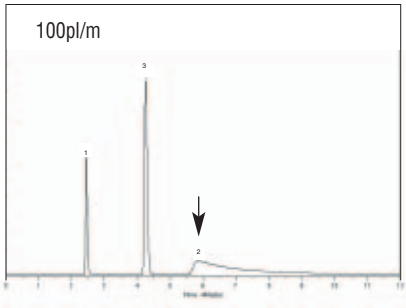
Symmetry C18



Hypersil BDS C18



Zorbax ODS



Column efficiency measured at 10% pyridine peak height to account for peak tailing effects

Column Dimensions: 250 x 4.6mm, 5µm Sample: 1) uracil 2) pyridine 3) phenol
Mobile Phase: 60:40 MeOH/H_2O Temperature: 24°C Flow 1.0ml/min

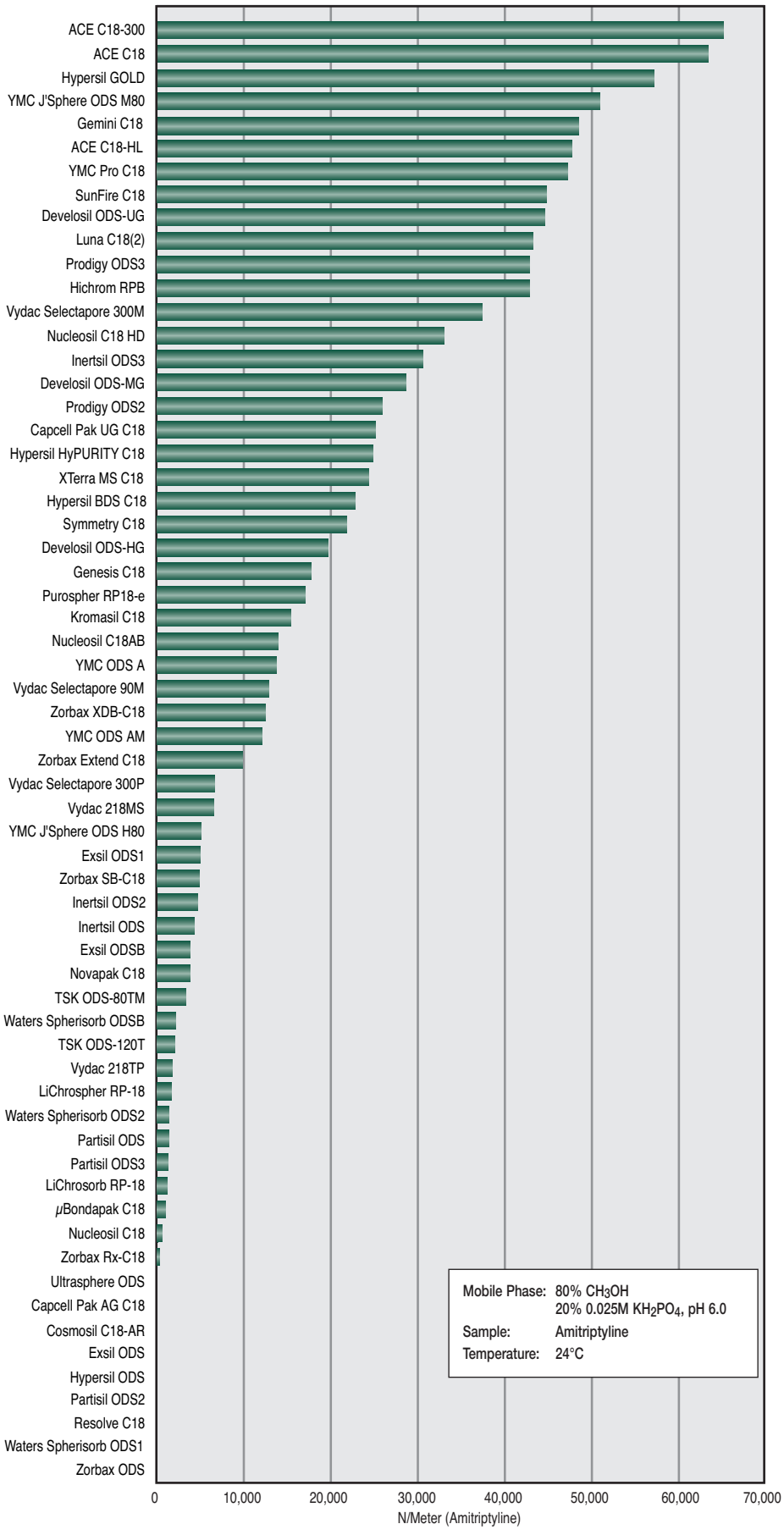


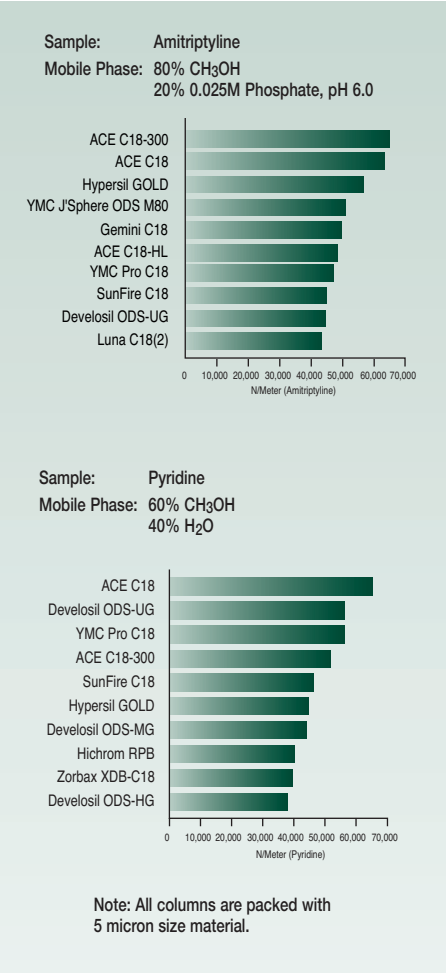
Figure 11

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 12

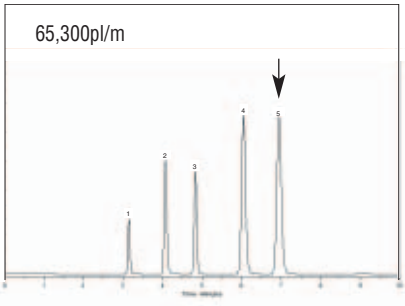
Top 10 Columns Ranked According to Peak Shape and Efficiency



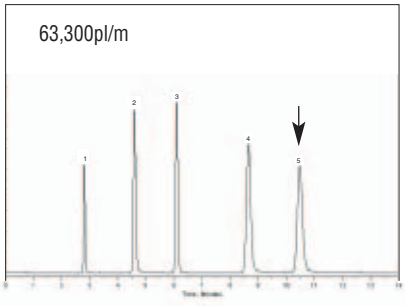
Column ranking does differ in the two tests of column efficiency for a basic compound (Figures 9 and 11). However, of the 14 columns that ranked in the top 10 on at least one of the tests, 6 ranked in the top 10 on both tests.

Comparison of Column Efficiency For a Basic Compound: Amitriptyline

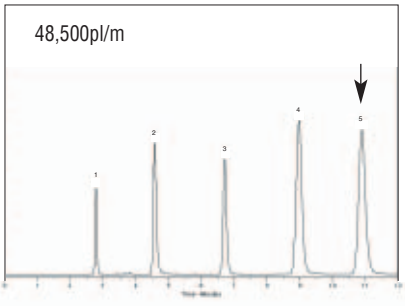
ACE C18-300



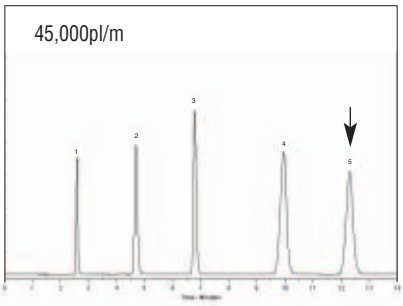
ACE C18



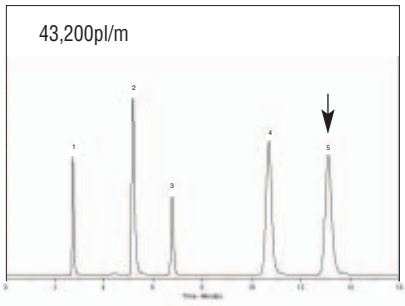
Gemini C18



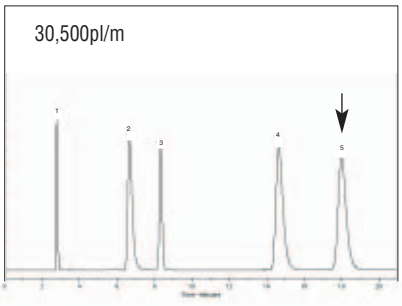
SunFire C18



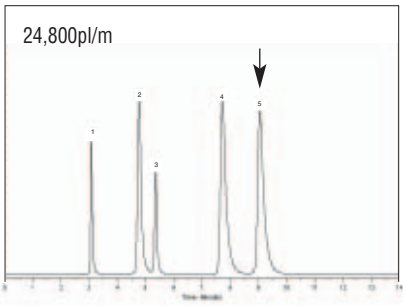
Luna C18(2)



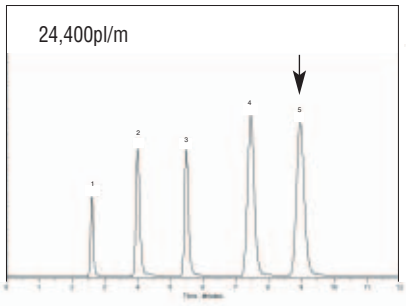
Inertsil ODS3V



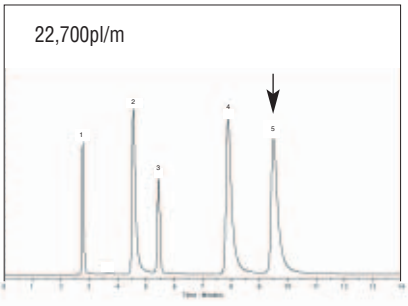
Hypersil HyPURITY C18



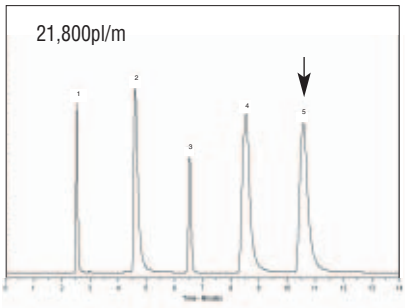
XTerra MS C18



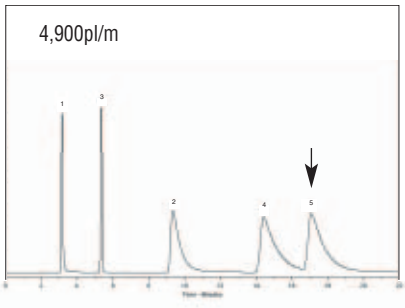
Hypersil BDS C18



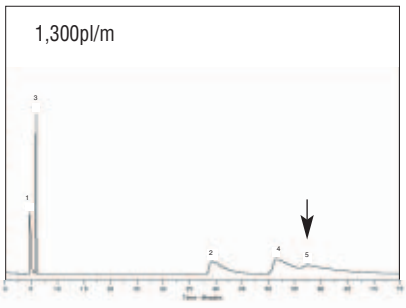
Symmetry C18



Zorbax SB C18



Waters Spherisorb S5ODS2



Column efficiency measured at 10% amitriptyline peak height to account for peak tailing effects

Column Dimensions: 250 x 4.6mm, 5µm Sample: 1) norephedrine 2) nortriptyline 3) toluene 4) imipramine 5) amitriptyline
Mobile Phase: 80:20 MeOH/0.025M KH_2PO_4 Temperature: 24°C Flow 1.0ml/min

Figure 13

Grouping of C18 Columns According to Silanol Activity

Material	Silanol Activity
ACE C18 ACE C18-300 Develosil ODS-UG Hypersil GOLD SunFire C18 YMC Pro C18	**Very Low**
ACE C18-HL Capcell Pak UG C18 Develosil ODS-HG Develosil ODS-MG Genesis C18 Gemini C18 Hichrom RPB Hypersil HyPURITY C18 Inertsil ODS3 Kromasil C18 Luna C18(2) Nucleosil C18 HD Prodigy ODS2 Prodigy ODS3 Purospher RP18-e Symmetry C18 XTerra MS C18 YMC ODS A YMC ODS AM Zorbax Extend C18 Zorbax XDB-C18	**Low**
Capcell Pak C18 SG Cosmosil C18-AR Exsil ODSB Hypersil BDS C18 Inertsil ODS Inertsil ODS2 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	**Moderate**
Capcell Pak C18 AG 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	**High**

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 and peak asymmetry deterioration 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 (Figure 13).

Columns classified as having "very low" silanol activity appear in the Top 10 rankings on *both* the pyridine and amitriptyline tests (Figure 12).

In general, phases identified as having "very low" silanol activity will give the highest column efficiency in the pyridine and amitriptyline tests (Figures 8, 9 & 10).

Benefits of Using Ultra-Inert Stationary Phases

Phases identified as having "very low" silanol activity may also be identified as "ultra-inert" or "ultra-pure" due to the elimination of undesirable secondary interactions between the sample and silanol groups on the silica.

In addition to the previously highlighted benefits of improved peak efficiency and asymmetry, the elimination of unpredictable silanol interactions significantly improves stationary phase reproducibility.

For further technical information on ultra-inert HPLC columns please contact your local distributor;