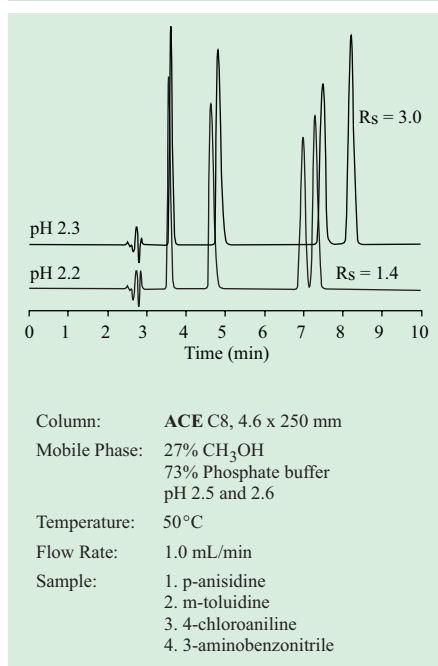


COLUMN TROUBLESHOOTING

Reversed-Phase HPLC Reproducibility Problems Caused by Changes in Mobile Phase pH

Day-to-day reproducibility problems, and even column-to-column reproducibility problems can be caused by changes in mobile phase pH. Sometimes a change of as little as 0.1 pH units can make the difference in an acceptable separation and an unacceptable one (Figure 1). How can you know if your separation is sensitive to mobile phase pH and what can you do to protect yourself from this problem?

FIGURE 1
An Example of the Effect of Changes in Mobile Phase pH on Resolution

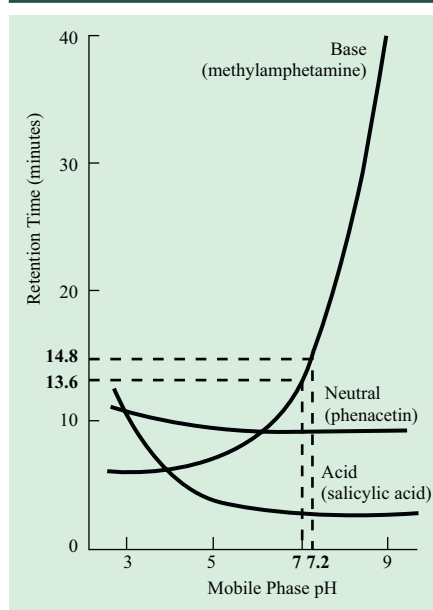


This example shows how resolution can change from an acceptable 3.0 to an unacceptable 1.4 if the mobile phase pH changes by just 0.1.

How Sensitive is My Method to Mobile Phase pH Changes?

Not all compounds and not all methods will be sensitive to mobile phase pH. Figure 1 shows a plot of retention time versus mobile phase pH for a basic, an acidic, and a neutral compound. The retention of neutral compounds is little affected by mobile phase pH. However, the retention of basic compounds can change dramatically in the pH range of 5 to 10 while acidic compounds are sensitive to pH between 2 and 5. An HPLC method may not be as robust as you would like if you are operating at a mobile phase pH where the retention of one or more analytes is sensitive to pH

FIGURE 2
The Effect of Mobile Phase pH on Basic, Acidic, and Neutral Compounds



Neutral compounds are little affected by mobile phase pH. However, the retention of basic and acidic compounds can be significantly affected by changes in pH. Notice how a pH change from 7.0 to 7.2 will increase the retention time of the basic compound by 1.2 minutes.

changes. To test the sensitivity of your method, change the mobile phase pH while keeping all other conditions constant and measure the affect on your separation.

How Can I Avoid Reproducibility Problems Caused by Changes in Mobile Phase pH?

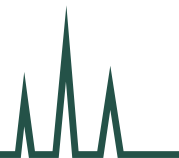
1st Try to develop separations that are not sensitive to changes in mobile phase pH. For example, if you are separating basic compounds use a mobile phase pH below 3 where retention will be little affected by small changes in pH.

2nd Always make sure to buffer the mobile phase properly. This includes using a sufficient amount of buffer, usually 25 to 50 mM, and choosing the right buffer for the pH that you have chosen to use. See the table below for information on buffer selection.

3rd Always prepare the mobile phase the same way and always measure the mobile phase pH the same way. For example, if you usually adjust the pH before adding the organic modifier to the mobile phase, continue to follow that process.

TABLE
Buffer Selection Guidelines

| Buffer | pKa | pH Range |
|-----------------|------|-------------|
| Phosphate | | |
| pK ₁ | 2.1 | 1.1 - 3.1 |
| pK ₂ | 7.2 | 6.2 - 8.2 |
| pK ₃ | 12.3 | 11.3 - 13.3 |
| Acetate | 4.8 | 3.8 - 5.8 |
| Citrate | | |
| pK ₁ | 3.1 | 2.1 - 4.1 |
| pK ₂ | 4.7 | 3.7 - 5.7 |
| pK ₃ | 5.4 | 4.4 - 6.4 |



ACE: A Superior HPLC Column

Ace columns are manufactured using advanced technology that virtually eliminates the negative effects of silanols on reversed phase separations.

The ultra-inert characteristics of the Ace columns make them the ideal choice for separating basic compounds.

Ace columns consistently produce excellent peak shape and high column efficiency, even when separating compounds that tail badly on other so-called inert, base-deactivated columns.

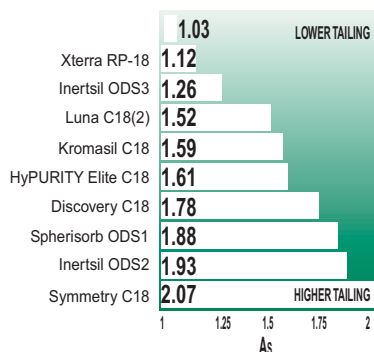
Ace columns are "fully validated" with the tightest specifications in the industry. Column to column reproducibility is guaranteed.

Specifications for ACE® HPLC Columns

| Phase | Functional Group | Endcapped | Particle Size (µm) | Pore Size Å | Surface Area (m ² /g) | Carbon Load (%) |
|---------|------------------|-----------|--------------------|-------------|----------------------------------|-----------------|
| C18 | Octadecyl | Yes | 3, 5, 10 | 100 | 300 | 15.5 |
| C8 | Octyl | Yes | 3, 5, 10 | 100 | 300 | 9.0 |
| C4 | Butyl | Yes | 3, 5, 10 | 100 | 300 | 5.5 |
| CN | Cyano | Yes | 3, 5, 10 | 100 | 300 | 5.5 |
| Phenyl | Phenyl | Yes | 3, 5, 10 | 100 | 300 | 9.5 |
| AQ | Integral Polar | Yes | 3, 5, 10 | 100 | 300 | 14.0 |
| C18-HL* | Octadecyl | Yes | 3, 5, 10, 15 | 90 | 400 | 20.0 |
| C18-300 | Octadecyl | Yes | 3, 5, 10 | 300 | 100 | 9.0 |
| C8-300 | Octyl | Yes | 3, 5, 10 | 300 | 100 | 5.0 |
| C4-300 | Butyl | Yes | 3, 5, 10 | 300 | 100 | 2.6 |

*Hi-Load

Compare Your HPLC Column to Ace



Conditions

Columns: 4.6 x 150 mm, 5 micron

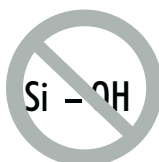
Mobile Phase: 80% Methanol
20% 0.05 M Phosphate buffer, pH 7

Flow Rate: 2.0 mL/min

Temperature: 24° C

Sample: Amitriptyline

Data obtained from the National Institutes of Standards & Technology (NIST) Certificate of Analysis for standard reference material 870, "Column Performance Test Mixture for Liquid Chromatography." To see how your HPLC column compares to Ace C18, get the complete report from the NIST internet site at: <http://ois.nist.gov/srmcatalog/certificates/870.pdf>

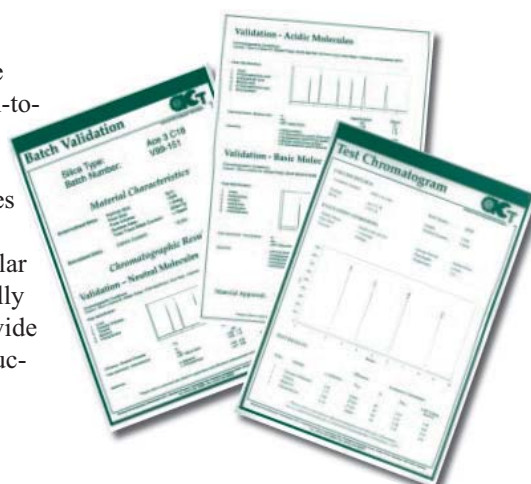


ACE Stationary Phases Virtually Eliminate the Negative Effects of Silanols on HPLC Separations

To confirm column-to-column reproducibility, a series of quality assurance tests are performed in the manufacturing process. Included are three separate chromatographic tests to validate reproducibility on neutral, acidic, and basic compounds.

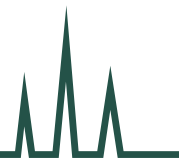
Validated Column-to-Column Reproducibility

Subtle changes in silanol activity are one of the primary causes of column-to-column selectivity changes. Base deactivated columns generally have better reproducibility than other types of columns because they reduce the interactions between silanols and polar solutes. The Ace columns, by virtually eliminating silanol interactions, provide a level of column-to-column reproducibility that has never before been achieved.



" This is the most inert column we have tested. "

- Analytical Chemist,
Industrial Chemical Company



Selectivity

ACE HPLC Columns offer unparalleled performance and guaranteed reproducibility making ACE the finest product available in today's market. But there's more! ACE columns are now available in seven bonded phases (C18, C8, C4, CN, Phenyl, AQ, C18-HL) each offering a unique, and in some cases, dramatically different selectivity. ACE could be the only HPLC column you need to solve any separation problem.

Choosing the Bonded Phase that's Best for Your Application

As a general rule, retention varies inversely with chain length of the bonded phase, that is:

Retention (R):

| | |
|----------------|--------|
| more retentive | C18-HL |
| | C18 |
| | C8, AQ |
| | Phenyl |
| less retentive | C4, CN |

We recommend starting most method development projects with C18 or C8, knowing that if more retention and hence more resolution is needed, your next choice is C18-HL (Hi-Load). Starting with C8 offers the benefit of shorter analysis times and/or lower organic solvent use. The elution order for most compounds will be the same on the aliphatic (C18, C8, C4) phases. If a different elution order is desired for compound verification or to resolve matrix components, changing to a phenyl or CN phase is far simpler than trying to change selectivity by mobile phase changes. In many cases, the ACE CN and ACE Phenyl phases will offer a significant difference in selectivity from the aliphatic phases.

ACE AQ is particularly recommended for applications requiring high aqueous content mobile phases. Improved resistance to phase collapse is seen compared to standard C18 phases.

Need Even More Resolution? Choose 3 micron ACE Columns

With today's increased pace of drug discovery, fast and efficient methods are the rule. Short, narrow-bore columns are replacing the conventional 4.6 x 250 mm versions. ACE HPLC columns are available in both 3 μ m and 5 μ m particle sizes. Although 5 μ m particles are sufficient for most applications, greater efficiency can be obtained by using smaller particles. This increased efficiency is an advantage in shorter (< 150 mm) column lengths. Because of the excellent flow characteristics of ACE silica, you will not experience the high back pressures often encountered with other columns.

Narrow-bore? Rapid Analysis? LC/MS? Preparative? No Problem!

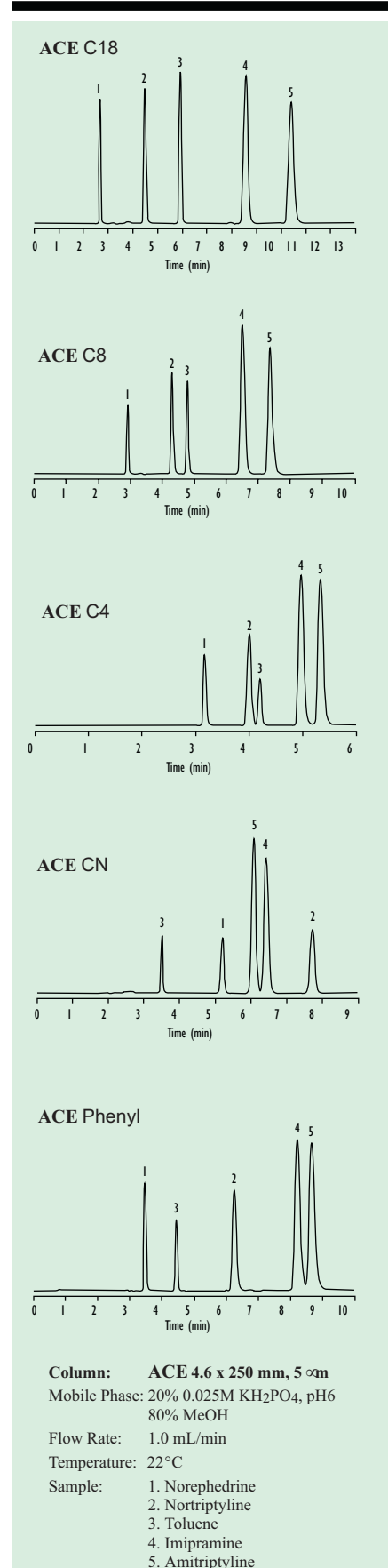
All ACE bonded phases are available in 3 μ m, 5 μ m, and 10 μ m particle sizes so regardless of your application, you can scale up or scale down and be assured of the same selectivity. If fast, high resolution preparative chromatography is needed, ACE 5 μ m materials are available in up to 2" diameter columns!

" You have to try this column to know how good it really is."

*- Analytical Chemist,
Industrial Chemical Company*

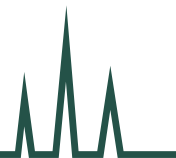


FIGURE 4
Alternate Selectivity Offered with
ACE C18, C8, C4, CN and Phenyl Phases





Product Availability



Increased Column Options

ACE columns are available in more phase and column combinations than any other HPLC column range, allowing complete assay optimization. The following tables provide a brief summary of phases and column dimensions available. In addition, we offer a custom packing facility whereby any non-standard column dimension or hardware can be manufactured to your exact requirements.

ACE Column Kits

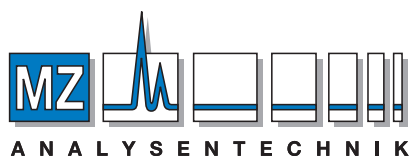
A range of ACE column kits are now available. Method Development (multi-phase kits), Method Validation (multi-batch kits) and Preparative Scale-Up (multi-dimension kits) are among those offered.

| Ace Material | Particle Size (µm) | | |
|------------------|--------------------|--------|---------|
| | 3 | 5 | 10 |
| C1 | ACE-11 | ACE-12 | ACE-131 |
| C8 | CE-112 | CE-122 | CE-132 |
| C3 | CE-113 | CE-123 | CE-133 |
| C4 | CE-114 | E-124 | CE-134 |
| Phenyl | CE-115 | CE-125 | CE-135 |
| AQ | CE-116 | CE-126 | CE-136 |
| C18-300 | CE-211 | CE-221 | CE-231 |
| C8-300 | CE-212 | CE-222 | CE-232 |
| C4-300 | CE-213 | CE-223 | CE-233 |
| C18-HL (Hi-Load) | CE-311 | CE-321 | CE-331 |

| Column Diameter | Column Length | | | | | | | | | Guard Cartridge |
|-----------------|---------------|--------|--------|---------|--------|---------|--------|---------|---------|-----------------|
| | 10 mm | 30 mm | 35 mm | 50 mm | 75 mm | 100 mm | 125 mm | 150 mm | 250mm | |
| 0.3 mm | - | - | - | x-05003 | - | x-10003 | - | x-15003 | x-25003 | - |
| 0.5 mm | - | - | - | x-05005 | - | x-10005 | - | x-15005 | x-25005 | - |
| 1.0 mm | x-0101 | x-0301 | x-3501 | x-0501 | x-7501 | x-1001 | x-1201 | x-1501 | x-2501 | x-0101GD |
| 2.1 mm | x-0102 | x-0301 | x-3502 | x-0502 | x-7502 | x-1002 | x-1202 | x-1502 | x-2502 | x-0102GD |
| 3.0 mm | x-0103 | x-0303 | x-3503 | x-0503 | x-7503 | x-1003 | x-1203 | x-1503 | x-2503 | x-0103GD |
| 4.0 mm | - | - | x-3504 | x-0504 | x-7504 | x-1004 | x-1204 | x-1504 | x-2504 | x-0103GD |
| 4.6 mm | x-0146 | x-0346 | x-3546 | x-0546 | x-7546 | x-1046 | x-1246 | x-1546 | x-2546 | x-0103GD |
| 7.75 mm | - | - | - | x-0508 | x-7508 | x-1008 | x-1208 | x-1508 | x-2508 | x-0110GD |
| 10 mm | - | - | - | x-0510 | x-7510 | x-1010 | x-1210 | x-1510 | x-2510 | x-0110GD |
| 21.2 mm | - | - | - | x-0520 | x-7520 | x-1020 | x-1220 | x-1520 | x-2520 | x-0110GD |
| 35 mm | - | - | - | x-0530 | x-7530 | x-1030 | x-1230 | x-1530 | x-2530 | x-0220GD |
| 50 mm | - | - | - | x-0550 | x-7550 | x-1050 | - | x-1550 | x-2550 | x-0220GD |

Further Information

Please contact your distributor to receive your copy of the latest Ace catalogue, which contains additional information on the full ACE HPLC column range.



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