

newsletter



Advanced Chromatography Technologies

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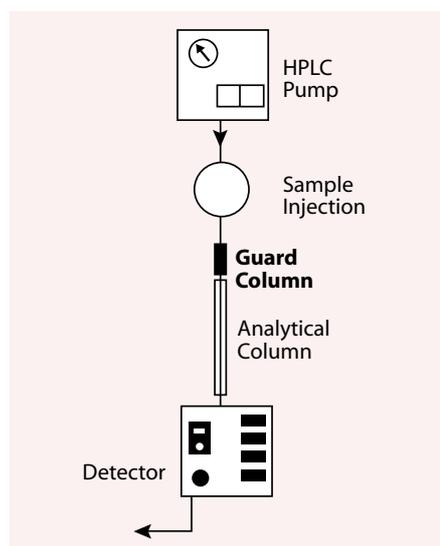
COLUMN TROUBLESHOOTING

When should guard columns be replaced?

Guard columns can reduce analytical column replacement costs and “down time” caused by column failure. They protect the analytical column from becoming “fouled” with non-eluting compounds and act as in-line filters for particulate material.

If a guard column is to provide adequate protection for the analytical column, it must be changed often enough to prevent column fouling material from saturating the guard column and flowing through to the analytical column. In addition, guard columns should be replaced as often as necessary to prevent particulate material build-up on the guard column from adversely affecting chromatographic performance.

FIGURE 1
Using Guard Columns



Guard columns protect the analytical column from any particulate or non-eluting material that may be in the sample or mobile phase.

Monitoring Chromatographic Parameters: A “Quantitative” Way of Knowing When to Replace a Guard Column

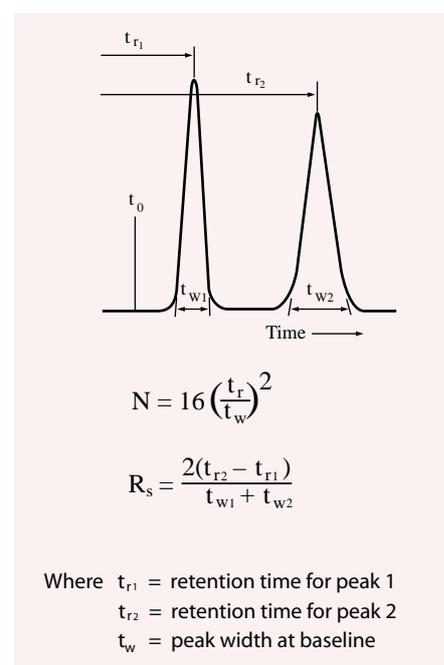
Although the best way to determine the right time to replace a guard column for a specific set of sample and mobile phase conditions is through experience, it is valuable to have some quantitative measure to help make the replacement decision. By monitoring plate number (N), pressure (P), and resolution (R_s), the performance of the guard column, as well as the analytical column, can be closely watched and clues as to when it should be replaced may be found. When any of these parameters, N, P, or R_s , changes by more than 10%, the guard column should be replaced.

When any of these parameters, N, P, or R_s , changes by more than 10%, the guard column should be replaced.

IMPORTANT NOTE: Even though monitoring N, P, and R_s provides clues as to when guard columns should be replaced, you cannot always be certain if the guard column is adequately protecting the analytical column. Fouling of the analytical column can still take place (due to a saturated guard column) long before any change in N, P, or R_s is observed. It is always better to replace the guard column too soon rather than too late.

In the absence of other information, a good rule-of-thumb is to replace the guard column after every 150 sample injections or 1,000 analytical column volumes, whichever comes sooner.

FIGURE 2
Calculation of Plate Number (N) and Resolution (R_s)

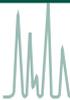


Inside This Issue

- Reversed-Phase HPLC of Basic Compounds: Solving Peak Tailing Problems
- New Ace® Ultra-Inert Base-Deactivated Columns
- 10% Savings on Genuine HP Parts and Supplies
- Special Offer: 20% Discount on New Ace HPLC Columns



Method Development Hints

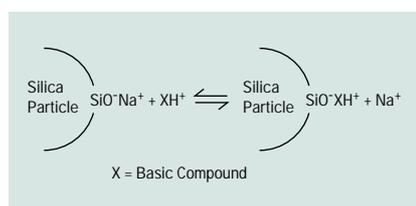


Reversed-Phase HPLC of Basic Compounds: Solving Peak Tailing Problems

What Causes Peak Tailing?

It is generally believed that an ion-exchange interaction between positively charged solutes and acidic silanols on the surface of silica support particles causes peak tailing (Figure 1). Metal impurities on the silica surface can contribute to peak tailing by activating silanol groups.

FIGURE 1
Peak Tailing Interaction



Acidic silanols on the surface of silica stationary phase supports can form ion-exchange sites that interact with basic compounds. This ion-exchange interaction will often contribute to peak retention and cause peak tailing.

How Can Peak Tailing Be Corrected?

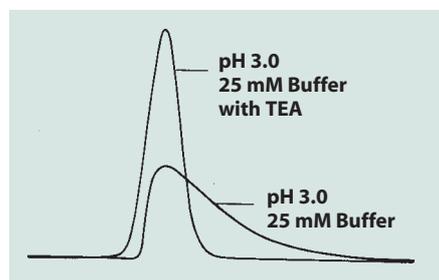
Peak tailing can be corrected by using mobile phase conditions that reduce the solute/silanol ion-exchange interaction, and by choosing a stationary phase that exhibits less silanol activity.

Recommended Mobile Phase Conditions for Reducing Peak Tailing

- Operate at a pH less than 3.0
 - Suppresses the ionization of acidic silanols
- Use a buffer with a concentration between 0.05 and 0.20 M
 - Controls pH and reduces ion-exchange interactions
- And, if necessary, add a competing amine, such as 0.01 M triethylamine (TEA)
 - Blocks silanol sites from interacting with basic solutes

These mobile phase conditions will provide acceptable peak symmetry for basic compounds on most reversed-phase columns, even those packed with stationary phases that have high silanol activity (Figure 2). However, if you would rather avoid using amine additives in the mobile phase, or if you

FIGURE 2
Effect of TEA on Peak Tailing



Adding TEA to the mobile phase will usually produce acceptable peak shapes for basic compounds, even on stationary phases with high silanol activity.

cannot use a mobile phase pH as low as 3.0 because of compound stability problems or unacceptable resolution at low pH, you should select a stationary phase that has low silanol activity. These types of phases are generally referred to as "base-deactivated" phases (Table 1).

Base-Deactivated Phases Reduce Peak Tailing

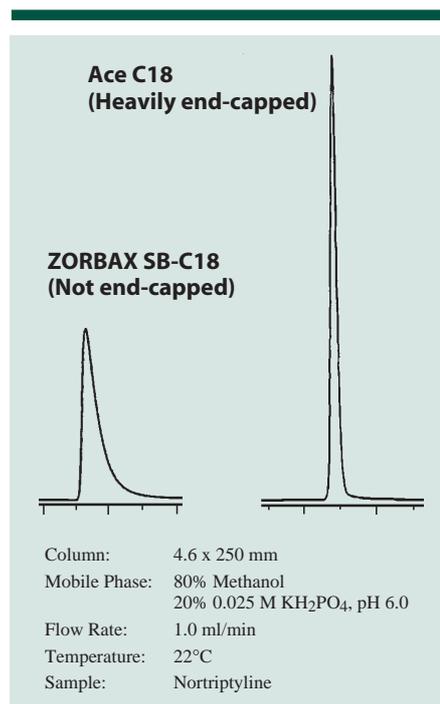
Base-deactivated phases have fewer acidic silanols available to cause peak tailing. These phases are typically made with low activity silica (high purity "Type B" silica) and have their surface highly covered by the bonded phase. Efficient end-capping with trimethylsilane is one of the ways manufacturers use to increase the surface coverage by the bonded phase and further reduce the number of silanols available to cause peak tailing (Figure 3, page 3).

TABLE 1
Ranking of Some Popular C18 Reversed-Phase Columns According to Silanol Activity

High Silanol Activity		
	Hypersil ODS	} Base Deactivated Phases
	Partisil ODS	
	Spherisorb ODS	
	Zorbax ODS	
	LiChrospher RP-18	
	Supelcosil LC-C18	
	Nucleosil C18	
	uBondapak C18	
	Supelcosil LC-C18-DB	
	StableBond SB-C18	
	Alltima C18	
	Kromasil C18	
	Symmetry C18	
	Eclipse XDB-C18	
	Hypersil BDS C18	
	Inertsil ODS-2	
Low Silanol Activity	Ace C18	

Note: The ranking in this table is meant to provide only a relative comparison of silanol activity. Differences between successive listings may not be significant.

FIGURE 3
End-Capping Improves Performance for Basic Compounds



End-capping improves peak shape for basic compounds by further reducing the number of available silanols that can cause peak tailing.

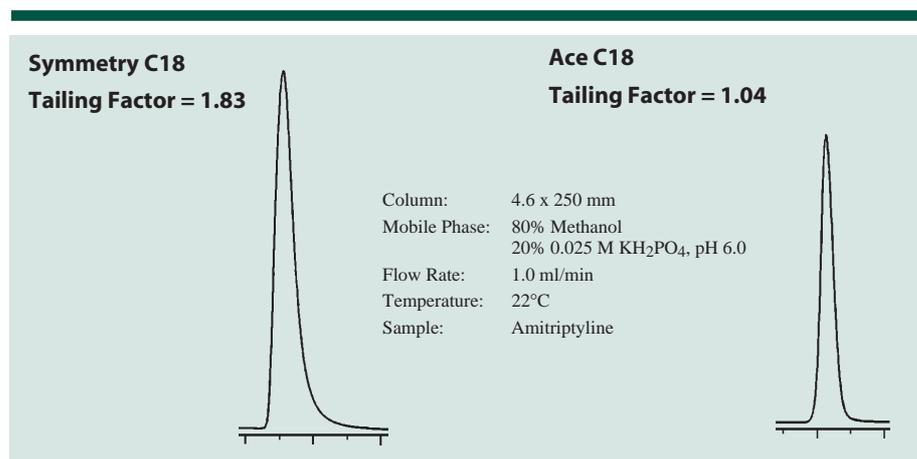
Although most base-deactivated phases will improve peak tailing, they do not eliminate the need for buffering the mobile phase. Column-to-column reproducibility and even injection-to-injection reproducibility can be a problem if the mobile phase is not buffered. In addition, some samples may still require an amine modifier added to the mobile phase to achieve acceptable peak shape. Amine modifiers are more likely to be needed if the mobile phase pH is greater than 4.

Select Base-Deactivated Phases that Have the Lowest Silanol Activity for Particularly Difficult Basic Compounds

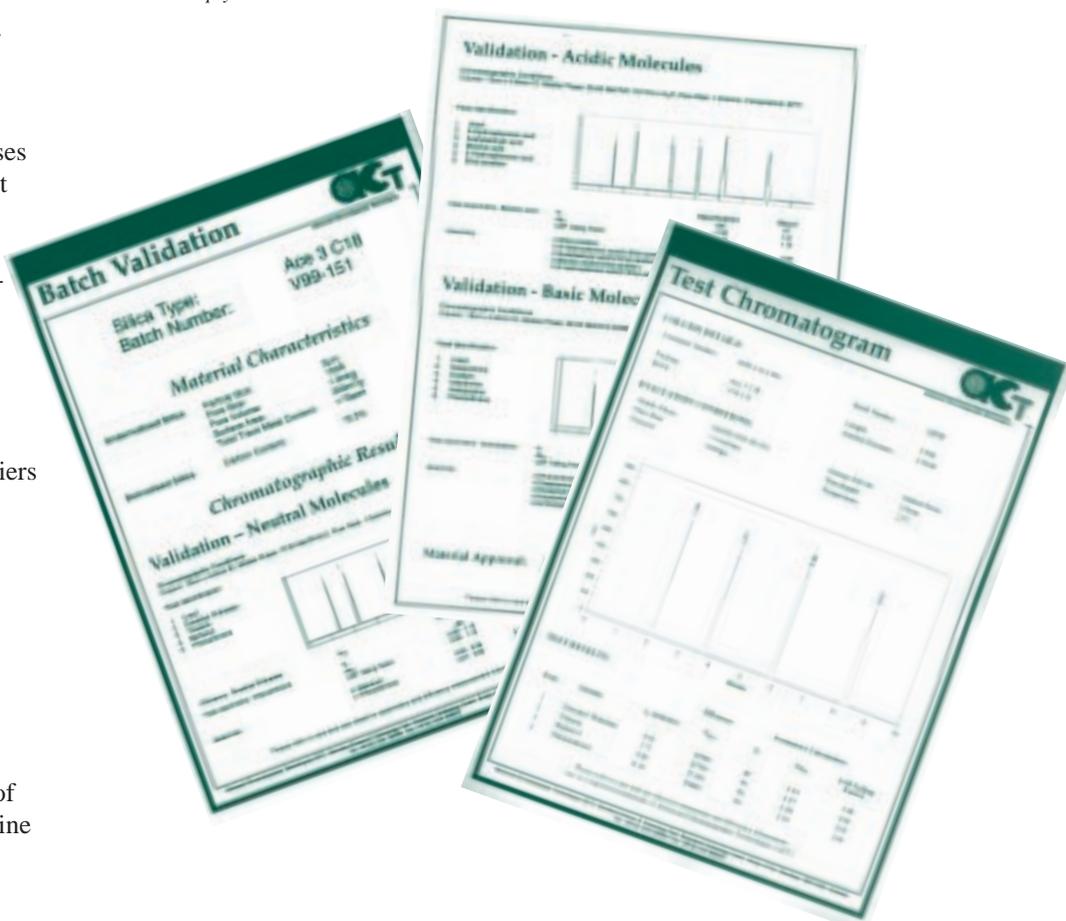
Some basic compounds, such as amitriptyline, will tail even on base-deactivated phases. For these types of difficult basic compounds add an amine

modifier to the mobile phase, or select a column packed with a phase that has extremely low silanol activity (Table 1, page 2). With proper mobile phase buffering, these columns can usually provide acceptable peak shape and dependable reproducibility without having to add an amine modifier (Figure 4).

FIGURE 4
Columns With Extremely Low Silanol Activity Can Provide Acceptable Peak Shape for Even Difficult Basic Compounds



Both Symmetry C18 and Ace C18 are base-deactivated phases with low silanol activity. However, the extremely low silanol activity of Ace C18 provides better peak shape for difficult basic compounds such as amitriptyline.



PRODUCT NEWS



Advanced Chromatography Technologies

Ace Stationary Phases Virtually Eliminate the Negative Effects of Silanols on Reversed-phase Separations



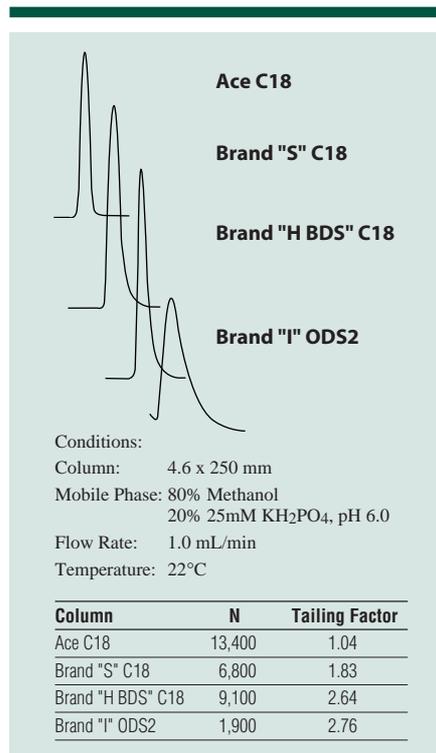
Ace C18 and C8 columns are manufactured using ultra-pure silica that has extremely low silanol activity. This ultra-pure silica is efficiently bonded and exhaustively end-capped using proprietary technology. The result is a silica based stationary phase that has virtually eliminated the negative effects of silanols on reversed-phase HPLC separations.

Excellent Peak Shape for Basic and Acidic Compounds

The ultra-inert characteristics of the Ace columns make them the ideal choice for separating polar basic compounds. When compared to other modern base-deactivated columns, the

New Ace C18 and C8 Ultra-Inert Base-Deactivated HPLC Columns

FIGURE 1
Chromatographic Performance for Amitriptyline



Ace columns consistently produce measurably better peak shape and column efficiency when separating troublesome basic compounds.

Validated Column-to-Column Reproducibility

A series of rigorous quality assurance tests are performed in the manufacturing process to confirm column-to-column reproducibility. This includes three separate chromatographic tests to validate reproducibility on neutral, acidic, and basic compounds. With the tightest specifications in the industry, these columns are guaranteed to provide the best reproducibility of any reversed-phase HPLC columns.

Amitriptyline is commonly used to demonstrate silanol activity on HPLC columns. The Ace columns provide measurably better peak shape and column efficiency compared to other popular base-deactivated columns.

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