# *Liquid Chromatography Problem Solving and Troubleshooting*

### *Question*

I am a newcomer to HPLC and I am wondering whether to buy an endcapped or a nonendcapped C18 column. What is the difference and what difference will this mean to me when doing HPLC separations?

### *Answer*

To answer your questions I will need to present some general guidelines. First, let me discuss the difference between an endcapped and nonendcapped column. The reversed-phase column you are considering purchasing is usually made by using a primary reaction of a monochloro-octadecyldimethylsilane with surface hydroxyl groups on the silica. This produces a hydrophobic surface—a bonded, oil-like layer of hydrocarbon on the polar silica surface. However, as a result of the long C18 chain on the silane, not all of the surface hydroxyl groups will be reacted during this primary bonding because of steric blocking of the surface. Therefore, to bond the remaining unreacted silane groups, a secondary reaction of a small alkylsilane (e.g., a monochloro-trimethoxysilane) is run to "cap" the unreacted surface hydroxyl groups. This secondary reaction is referred to as endcapping the stationary phase. However, even with exhaustive endcapping, not all of the surface silanols are reacted. In fact, it has been estimated that as high as 50% of the surface silanols remain on the surface after the primary and secondary bonding reactions. Also, it has been reported that with regard to the negative influence of unreacted silanol groups upon chromatographic behavior, it is not the number of silanols remaining on the surface that is important but their accessibility to analytes (1). That is, if the silanols that remain are freely accessible, there is no deleterious effect upon peak shape and tailing. So, in summary, endcapping is an attempt to improve chromatographic peak shape by bonding more silanols groups with the hydrophobic phase, and, in some circumstances, endcapping may be good (in others endcapping may not make a difference).

To understand the role of endcapping and decide whether to buy an endcapped or nonendcapped column, focus should be on the application(s) that might be used. If your application will be the separation of acidic compounds, you would choose a column that can be operated at low pH because acids are not ionized at low pH (< 3) and as such have appropriate retention. If your application will be analyzing basic compounds, you will be again most likely using a low pH mobile phase (2). In these two example applications, when using a low pH mobile phase, the type of column will initially not make a significant difference. Either column type, endcapped or nonendcapped, would be an appropriate choice. An example of this is shown in Figure 1. Here you see a comparison of the separation of seven compounds on the two types of C8 columns. The chromatographic behavior on both types of columns at low pH (the lower half of the figure) is quite reasonable for the two basic compounds propranolol (P) and amitryptiline (A), which exhibit appropriate retention and good peak shape.

However, one point to remember is that it has been reported and is widely believed that the low pH mobile phases will cleave the short alkylsilaxone bonds of the endcapping reagent. Therefore, during use cleavage of the siloxane bond occurs, and this results in the loss of the hydrophobic phase (the endcapping reagent) causing a change in the retention characteristics of the column for both neutral and charged molecules.

The difference between nonendcapped and endcapped columns is most apparent when operating at intermediate pH (~7), in which the silanols become ionized and take on a negative charge, which can interact with basic analytes. This electrostatic interaction will influence retention and peak shape. If you look at the top half of the figure, you can see the differences between the two columns in which the nonendcapped column exhibits long retention and tailed peaks of the basic analytes compared with that on the endcapped column. It should be noted that the neutral compounds do not show much difference in retention on either column and at both pH values.

Operation at intermediate pH (≥ 7) also has a downside in that silica will slowly dissolve. This may be very slowly over many months or quite quickly depending upon the column manufacturer (3). When silica dissolves three things will eventually

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happen. First, more silanol groups are formed resulting in longer retention of basic analytes and probably more peak tailing (3). Second, with the loss of silica, bonded phase loss, will follow and retention of neutrals will be reduced. Third, eventually with silica dissolved, the column bed will diminish resulting in a "voided" column. If you dedicate a column to use at intermediate pH, the best column is one that is exhaustively bonded and exhaustively endcapped so that it will minimize the dissolution of silica and thus minimize the loss of stationary phase (3).

However, if you use that column at low and intermediate pH, the behavior may be very different at the intermediate pH after operation at low pH because the endcapped column will change its retention behavior as the endcapping is cleaved when used at low pH as mentioned.

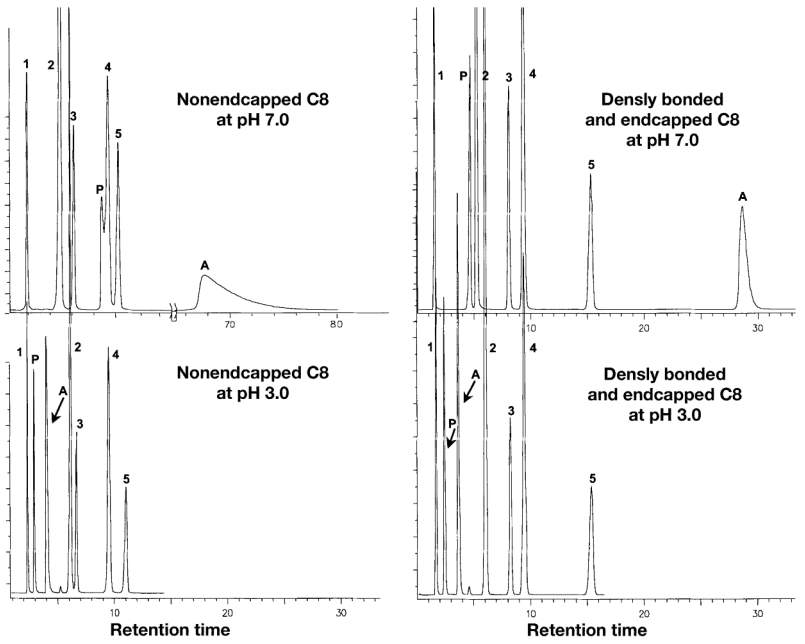


**Figure 1.** Effect of pH upon peak retention and shape. Peaks are: 1, uracil; P, propranolol; A, amitryptiline; 2, butyl paraben; 3, napthalene; 4, dipropyl phthlalate; and 5, acenaphthene. The mobile phase was methanol–water (65:35) with 7mM phosphate (pH 7.0). The endcapped C8 column was a Zorbax Eclipse XDB C8, and the nonendcapped column was a Zorbax StableBond C8.

In summary, the preferred pH of the mobile phase for most separations is pH < 3. Therefore, using a nonendcapped column will be the most stable and useful. If, however, you will be using many different mobile phases with different pH values, it may make sense to buy a column and dedicate it to a single application if consistent retention time and peak shape is important. Thus, for low pH use a nonendcapped column and for intermediate pH use a well-coated and endcapped column.

As I have said many times in these troubleshooting articles, "Chromatography is a lot like life—a choice of trade-offs. What you choose depends upon what you value." And with regard to column choices I will be quick to echo something one of my early teachers said, "pay your money and take your choice."

## References

1. B.A. Bidlingmeyer, J. DelRios, and J. Korpi. Separations of organic amine compounds on silica gel with reversed-phase eluents. *Anal. Chem.* **54:** 442–47 (1982).
2. B.A. Bidlingmeyer. Liquid chromatography problem solving and troubleshooting. *J. Chromatogr. Sci.* **33:** 666–67 (1995).
3. B.A. Bidlingmeyer. Liquid chromatography problem solving and troubleshooting. *J. Chromatogr. Sci.* **35:** 405–406 (1997).