

Waters

Lab Highlights

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COMMENTS CONCERNING END-CAPPING

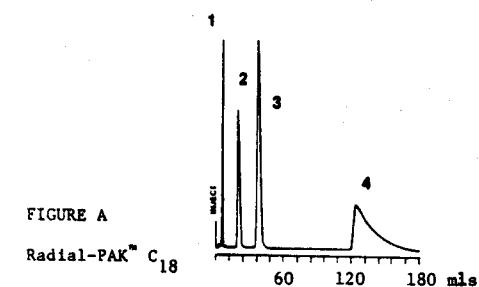
How often have you heard "Are your columns end-capped?" The assumption is that end-capped columns are better, especially when trying to separate basic compounds. The μ BONDAPAK™ C₁₈ column is end-capped and can be used successfully for the separation of bases (minimum tailing). Radial-PAK™ C₁₈ is not end-capped and results in excessive retention of bases with badly tailed peaks. Therefore, end-capping is the key to good peak symmetry. Right? Not entirely.

Additional confusion results from the fact that much effort has been directed toward the chemistry of preparing a bonded phase where residual silanols are eliminated. Secondary bonding reactions (i.e. end-capping), after the primary reaction, have been claimed in the literature to reduce the surface silanols (1). On the other hand, it has been shown that if "complete" coverage is obtained with the primary reaction, no additional bonding from a secondary reaction occurs and this end-capping does not reduce the concentration of surface silanols (2).

Furthermore, when using aqueous/organic eluents containing an inorganic salt, amines have badly tailed peaks when separated on most C₁₈ reversed-phase packings, both those which have and have not been end-capped. That is why people add amine modifiers ("competing bases") to improve peak symmetry on most bonded C₁₈ columns.

Obviously, there is a difference of opinion - all based upon experience.

Let's look at some recent experiments (3) where columns were evaluated using the same mobile phase.



Name	pKa	Structure
1. BENZOCAINE	2.8	<chem>CC(=O)Oc1ccc(N)cc1</chem>
2. LIDOCAINE	7.9	<chem>CC1=CC=C(C=C1)N(C)C(=O)CCN(CC)CC</chem>
3. ETHIDOCAINE	7.9	<chem>CC1=CC=C(C=C1)N(C)C(=O)CCN(CC)CC</chem>
4. TETRACAINE	8.5	<chem>CC1=CC=C(C=C1)N(C)C(=O)CCN(CC)CC</chem>

Organic amines have been traditionally separated on μ BONDAPAK™ C₁₈ columns using an inorganic salt in the eluent in order to reduce the tailing which would occur if the salt were not present. An example of such a separation is shown in Figure A for a mixture of anesthetics on a Radial-PAK™ C₁₈ cartridge with dibasic ammonium phosphate in the eluent. Tetracaine (Peak 4) which is the most basic amine was retained the longest with poor peak symmetry.

To understand the role of the surface silanols and their contribution to retention, we analyzed these anesthetics on a column which was purposely coated to one half of the C₁₈ level of the original Radial-PAK™ C₁₈. If peak tailing is due solely to the silanol interaction, the peak symmetry should be worse than on the fully coated column.

(See Figure C for conditions)

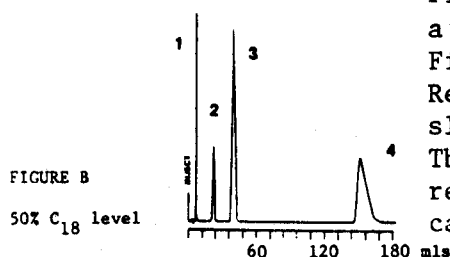
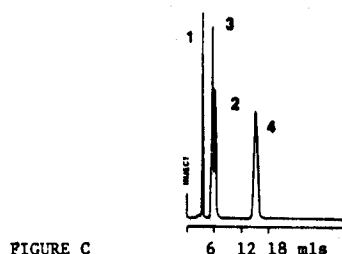


Figure B shows the separation of the C₁₈ column with a coating level of one half of that of the column in Figure A. There was no decrease in peak symmetry. Retention of the tetracaine (Peak 4) increased slightly while the peak symmetry, in fact, improved. Thus, it appears that silanols do contribute to retention but the amount of silanols is not the main cause of peak asymmetry.



The logical extension of this approach is to separate the compounds on unbonded silica gel as shown in Figure C. The chromatogram indicates that the surface silanols by themselves are not deleterious to the retention of organic amines when using organic, aqueous eluents containing an inorganic salt. In fact, silica gel itself appears to be the preferred adsorbent for the separation of these bases.

The key to good peak symmetry is not the presence or absence of residual silanols, but more probably the accessibility of the surface groups. If surface silanols are freely accessible, there is good symmetry. After bonding it is possible that the residual silanols are in micropores which would affect mass transfer due to reduced accessibility of the surface silanols. This would result in the type of skewed peak which is shown in Figure A.

In conclusion, seemingly conflicting observations concerning end-capping can be considered consistent if one realizes that the key to symmetrical peaks on bonded phase columns is accessibility of the residual silanols.

Unfortunately, the accessibility of silanols is a complex relationship concerning the nature (source) of the silica, surface morphology, silanol concentration (density), and bonding. Therein lies the proprietary knowledge which will differentiate Waters.

There is no easy answer as to what column is "best" for separating bases. Remember that many bonded C₁₈ columns from various suppliers are end-capped and exhibit excessive peak tailing of amine samples when compared against μ BONDAPAK™ C₁₈. Some people use bare silica gel (see Lab Highlight "Bare Silica Gel as the Preferred Reversed-Phase Support for Organic Amine Samples").

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1. N. Cooke, K. Olsen, *J. Chromatogr. Sci.*, 18, 512 (1981).
2. G. Berendsen et al., *J. Liq. Chromatogr.*, 3, 1437 (1980).
3. B. A. Bidlingmeyer et al., *Anal. Chem.*, 54, 442 (1982).