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Quaternary amines are notoriously difficult compounds for chromatographic analysis. They are non volatile, and cannot be analyzed by GC. Since most of these compounds are water and polar organic solvents soluble, they are good candidates for reverse phase (RP) HPLC. However there are two problems:

1. If quaternary amines are hydrophilic, they are not retained on RP columns (Fig. 1). Typically for primary, secondary, or tertiary amines, switching to high pH (8-11) significantly decreases their polarity, making them suitable for RP separation. Unfortunately, this is not true for quaternary amines -- the pH of the mobile phase has no effect on charge state of quaternary amine functionality.
2. If quaternary amines are hydrophobic, then retention in RP mode can be easily obtained, but the peak shape is usually a problem. Even the use of the end-capped or base-deactivated stationary phases does not offer a symmetrical peak nor high efficiency (Fig. 2).

Mixed-mode columns with additional strong ionic interaction allow to retain and separate quaternary amines (Fig. 3). Depending on hydrophobic properties of these compounds, different methods and different columns can be selected (Fig. 4).

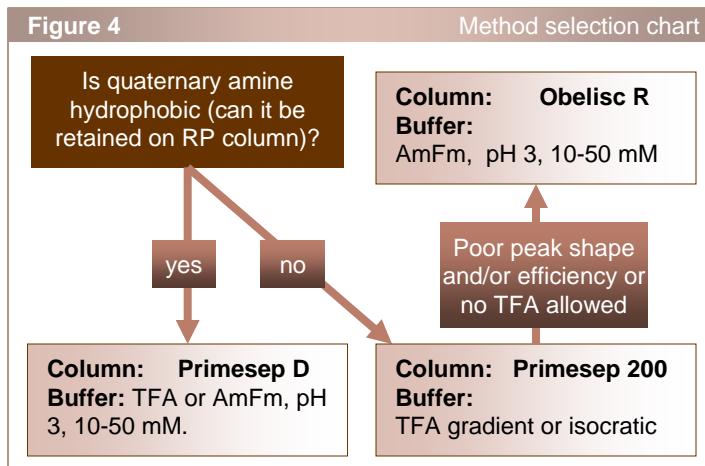
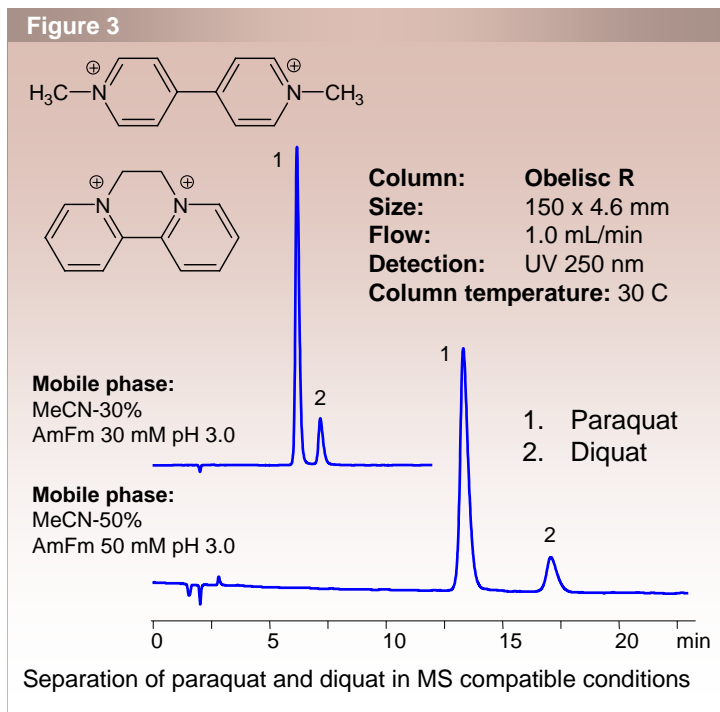
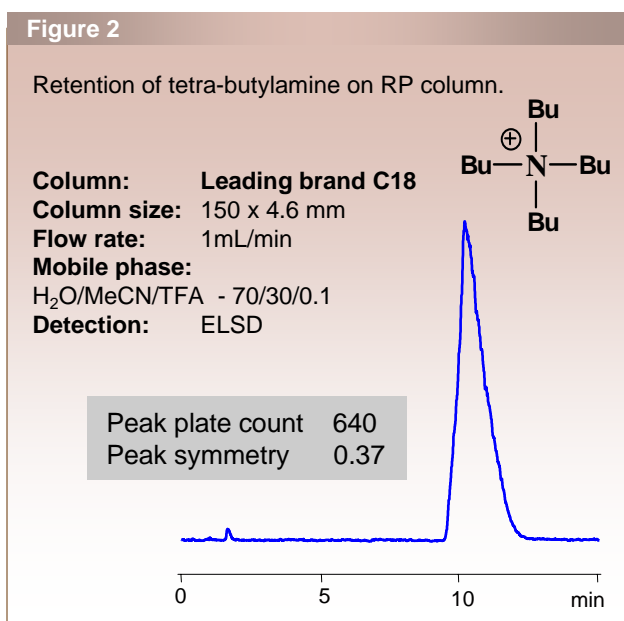
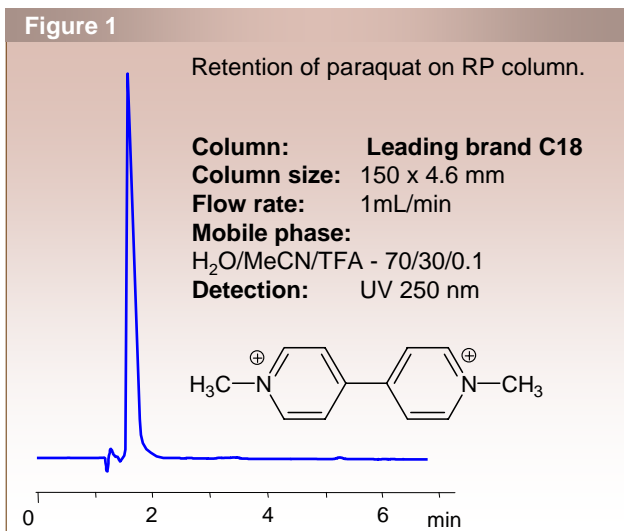
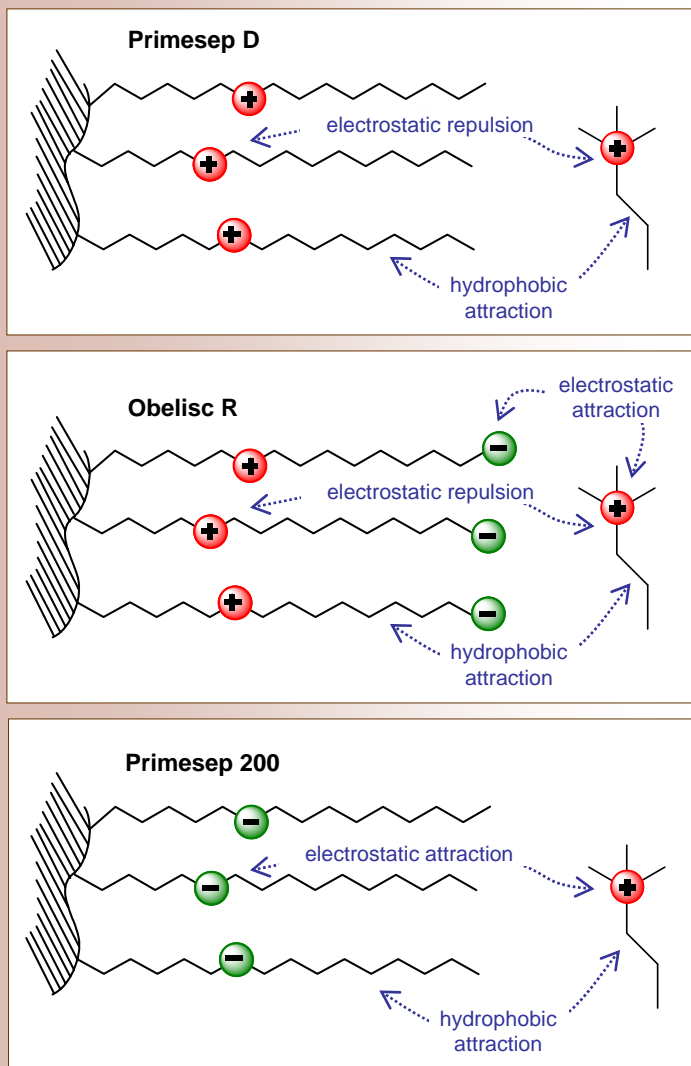
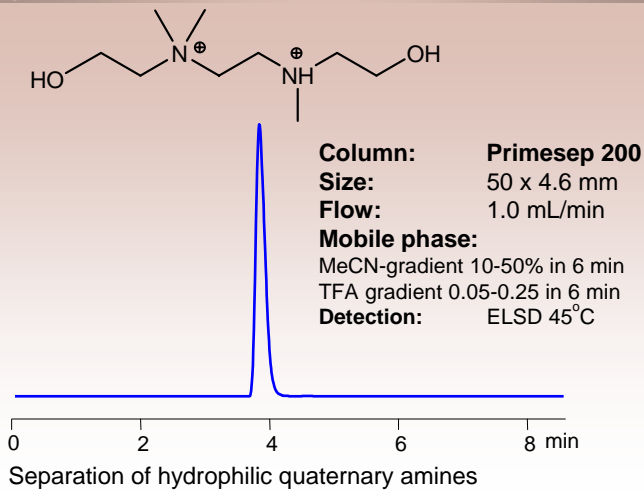


Figure 5



Type of interactions of quaternary amines with Primesep D, Primesep 200, and Obelisc R stationary phases

Figure 6



Types of interactions observed with each column are summarized in Fig 5. Strong ionic functionality embedded in the stationary phase prevents the quaternary amine from interacting with silica surface, and offers improved peak shape efficiency and retention (Fig. 6-8). Different acidic buffer types can be used. Many quaternary amines are not UV active, conveniently LC-MS and LC-ELSD compatible mobile phase can be used with mixed-mode columns.

Figure 7

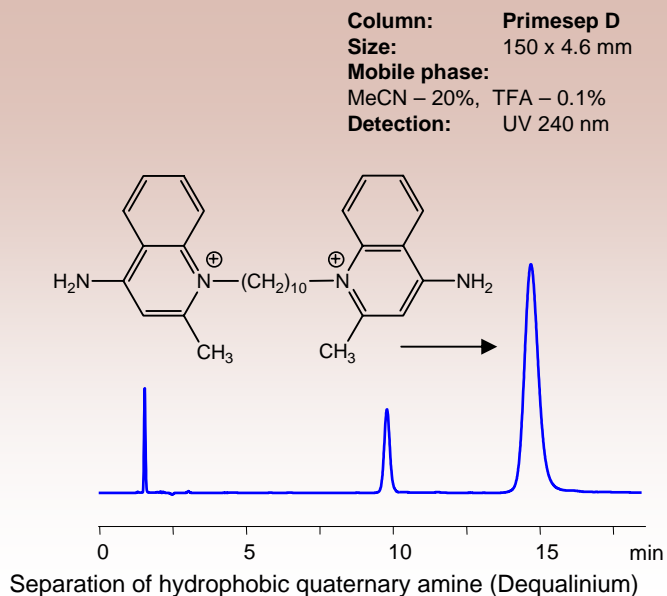


Figure 7

