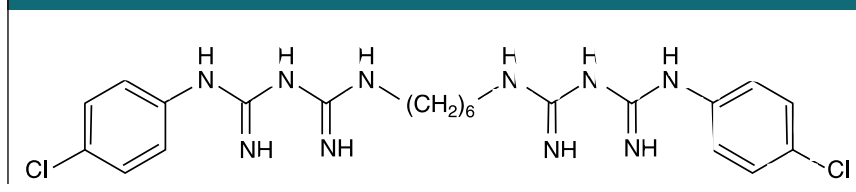


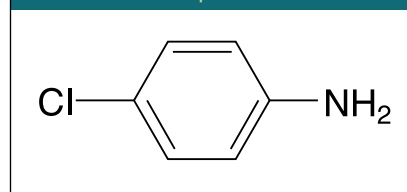
HPLC METHOD DEVELOPMENT ...AN EXAMPLE

TARGET COMPOUNDS

Structure of chlorhexidine



Structure of *p*-chloroaniline



Chlorhexidine is found in many pharmaceutical formulations and *p*-chloroaniline is a common impurity. Both these compounds have basic amine groups and are quite polar, so a 250mm long high purity silica C18 column is the column of choice in order to minimize tailing and maximize retention.

In this case a 250 x 4mm ID Wakosil II™ 5C18 RS column (Part No. 207026) has been used.

THE METHOD

The first step is to examine the chromatography of Chlorhexidine as this is the compound most likely to tail due to the large number of amine functional groups. (The chromatogram should be run at a pH that is at least 1.5 lower than the pKa)

Figure 1 illustrates the tailing that occurs when the mobile phase is buffered to an incorrect pH. Acetonitrile is always the first choice for organic solvent due to low viscosity of Water/Acetonitrile mixes.

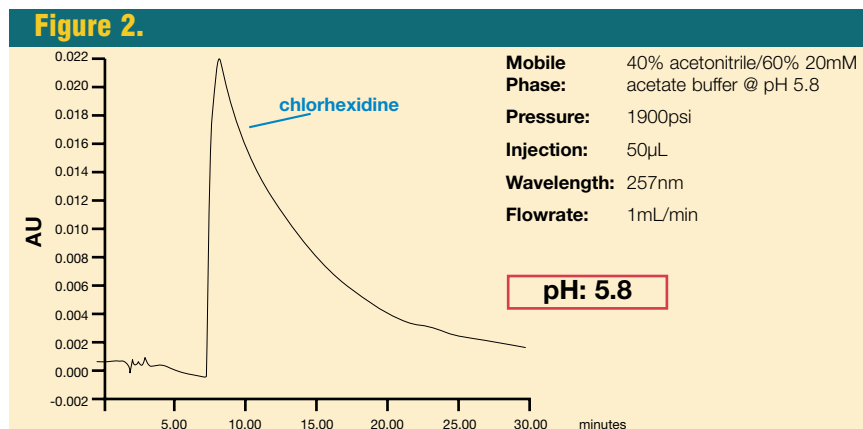
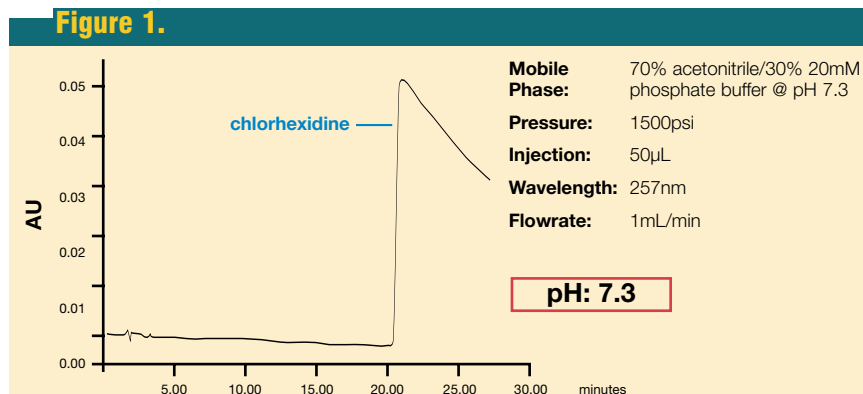


Figure 2 shows that by lowering the pH of the buffer, the peak is significantly sharpened although it still tails badly. (The retention characteristics have also been affected by the pH modification.)

By reducing the pH of the buffer to 3.8, a more suitable peak shape is obtained (Figure 3). Once an appropriate peak shape is achieved, modification of the percentage of organic solvent in the mobile phase can be used to adjust the retention characteristics. (Note: Increasing the amount of organic solvent will decrease the retention time in a reverse phase separation. (Figure 4))

The next step (Figure 5) is to look at any other peaks of interest, in this case ρ -chloroaniline, under the same conditions. In many cases, further adjustment of pH and organic percentage of the mobile phase are required to achieve a suitable separation. (Note: To optimize the sensitivity of p-chloroaniline, a wavelength of 241nm is used.)

In Figure 6, conditions are optimized for the separation of all compounds of interest. The wavelength has been returned to 254nm – the optimum for chlorhexidine which is the primary compound of interest. (Some detectors may be programmed to vary the wavelength throughout a run.)

In this analysis, the buffer concentration has been increased in order to give the buffer greater strength. This will help to compensate for the miscellaneous compounds in a “real” sample which could affect the pH of the mobile phase.

The final step is to validate the method. In doing this it is important to understand the robustness of the separation by investigating the effects of buffer strength, slight differences in organic percentage and slight differences in pH. Note that peaks should not move or change drastically. Most importantly, there should be no interference from impurities of actual samples.

