

# The Use of RediSep Rf Gold® C18 Columns at High pH

## Abstract

There is a need to purify acid labile compounds using reverse phase at high pH. RediSep Rf Gold C18 columns are able to purify compounds with mobile phases up to pH 10 with minimal degradation. A RediSep Rf Gold C18 column was run for 8 hours with a pH 10 mobile phase. There was only a minimal change in resolution over this time period.

Instructions are provided for cleaning the column after running in high pH mobile phases.

## Overview

Many compounds generate multiple ionic species during the course of C18 purification which cause broadened peaks that reduce resolution. If the interconversion of the compounds is slow, each ionic compound may appear as an individual peak. Trifluoroacetic acid (TFA) is generally used for ion pairing (with basic compounds) or ion suppression (acidic compounds) since C18 columns are compatible with low pH.

There is a need to purify compounds on reverse phase column at higher pH. Many compounds are acid sensitive yet form peak-broadening ionic species at neutral pH. Purifying the compounds at basic pH would improve their purity and yield. C18 columns are not gen-

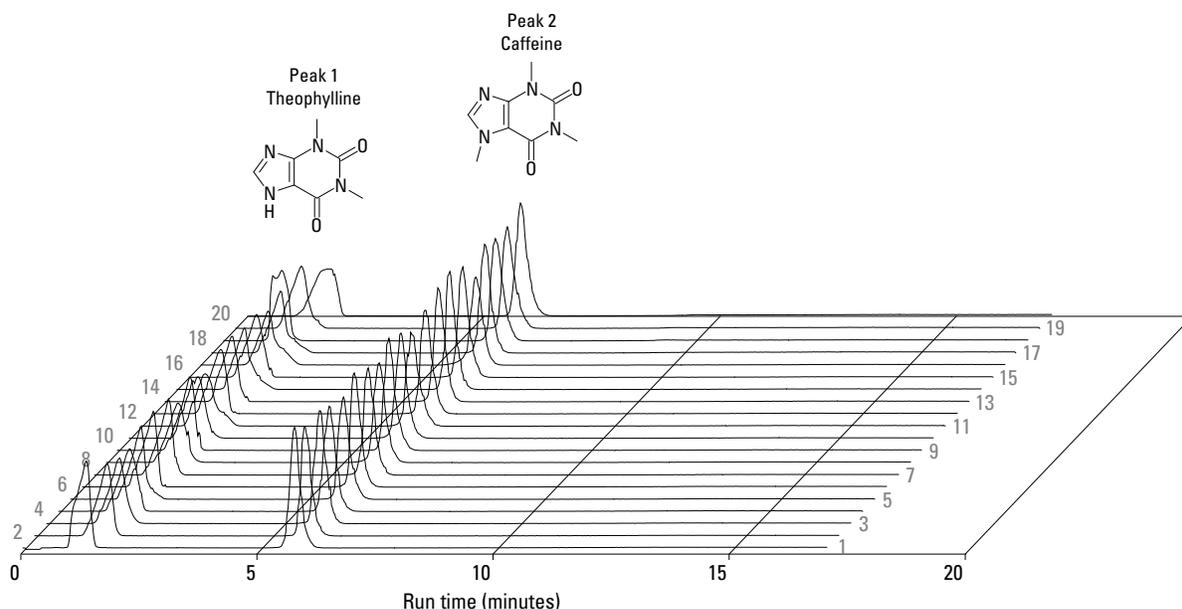
erally stable at pH greater than 7.5 because the basic solution dissolves the silica underlying the C18 chains, eventually washing the C18 away and causing a decrease in column performance. These changes take the form of reduced plate counts or changes in retention time. RediSep Rf Gold C18 columns are end-capped to resist attack of the silica by the mobile phase.

RediSep Rf Gold C18 columns can be used for purifying compounds up to pH 10 with only minimal change over time. Compared to steel C18 prep HPLC columns, RediSep Rf Gold C18 columns are inexpensive and useful for milligram to gram scale purifications.

## Results and Discussion

### Purification of xanthine alkaloids at pH 10

Caffeine and theophylline were dissolved in water and repeatedly injected onto a RediSep Rf Gold C18 15.5 g column. A standard C18 method was run on a CombiFlash Rf system with a gradient hold at 100% B at the end of the run. Solvent A was dionized water adjusted to a pH of 10.0 with ammonium hydroxide. Solvent B was methanol. The compound mixture was injected twenty times. The column was equilibrated with 5 column volumes of 5% methanol/95% pH 10 water prior to each run.



**Figure 1: Twenty repeated injections** of caffeine and theophylline in a pH 10 mobile phase on a 15.5 g RediSep Rf Gold column

Each of the twenty runs in Figure 1 required a total time of ~24 minutes, including equilibration, to give a total of 8 hours total exposure time at pH 10. The RediSep Rf Gold C18 column still showed good purifications after this time.

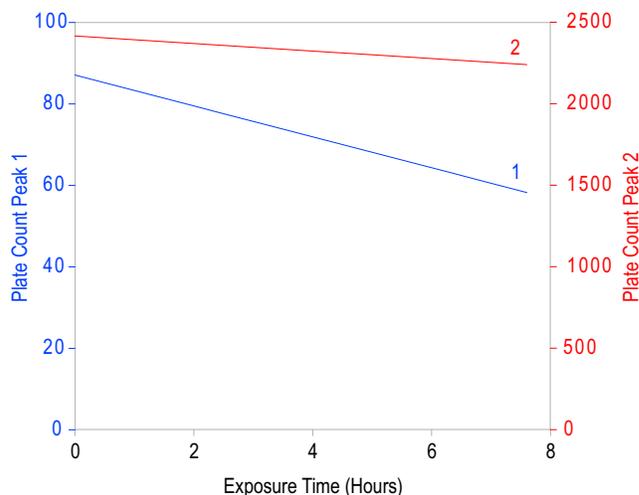
Figure 2 shows that RediSep Rf Gold columns can run for several hours exposed to pH 10 mobile phases. Like other silica-based C18 columns, the RediSep Rf Gold columns will degrade over time. However, the degradation is slow enough that the column is still usable for many runs. The degradation is reduced as the pH is lowered or exposure time is decreased.

### Removal of the basic solvent from the column

After use, the column was washed with 5 column volumes of methanol. The column was then run with a standard gradient method from 5 to 95% methanol in unbuffered water. The extended wash is intended to remove as much of the ammonium hydroxide from the column as possible. After washing, store the column in 85% methanol or acetonitrile. The column can be stored indefinitely without further degradation as long as it does not dry out.

### Conclusion

Although RediSep Rf Gold columns are not immune from damage from high pH mobile phases, they are able to provide purifications of compounds for many runs. This allows reverse phase purification of acid-sensitive compounds with mobile phases with a pH greater than 7. This study was done at pH 10; mobile phases at lower pH should show longer column life.



**Figure 2: Change in plate count** from multiple runs at pH 10

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