Chimeric Diol Column Behavior using Aqueous Solvents

with Redi*Sep* Rf Gold® Diol Columns

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Chromatography Application Note AN86

Abstract

Diol columns are a versatile column. Used with organic solvents, the diol column acts as a normal phase column with reduced retention compared to silica. Under aqueous conditions, diol columns run as normal phase, but may exhibit reverse phase behavior at times. This application note explores those compounds and solvent conditions that cause reverse phase behavior in diol columns. Polar compounds run under aqueous conditions cause the column to act as either normal or reverse phase while non-polar compounds under the same conditions run purely as reverse phase.

Background

Diol columns have been marketed as being "useful in normal and reverse phase"[1] with little indication about how to determine the conditions for "normal" and "reverse" phase. For organic solvents, diol columns act as "normal phase"[2].

A column acts as "normal phase" if a compound exhibits reduced retention as the solvent polarity is increased. Conversely, a column showing reduced compound retention with reduced solvent polarity is acting as "reversed phase". It is possible for a column to exhibit both normal and reverse phase behavior at the same time with a mixture of compounds.

Experimental and Results

FD&C Blue #1 (1.0 g) was dissolved in water and mixed with Celite 541 (18.0 g); butyl paraben (1.0 g) was dissolved in methanol and added to the dye/Celite mixture. The sample mixture was dried under vacuum and was used for all experiments; 200 mg of this mixture was used for each run.

A 15.5 g diol column (Teledyne Isco, PN 69-220-3515) was used for all experiments. The column was run isocratically for 20 column volumes (see details of *figures 1 and 2* for solvent concentrations) using water and acetonitrile as solvents. The column was washed after 20 column volumes. A Combi*Flash*® Rf 200 with UV-vis detection (PN 68-5230-008) was used for all runs; butyl paraben was detected at 254 nm while the dye was monitored at 628 nm. The chromatograms are plotted separately for each compound.

Figure 1 shows that FD&C Blue #1 exhibits minimum retention at 50% acetonitrile in water. As the concentration of water increases from 0 to 50%, the retention time of the dye decreases consistent with normal phase behavior. As the solvent polarity is decreased (0 to 50% acetonitrile) the retention time again decreases, consistent with reverse phase.

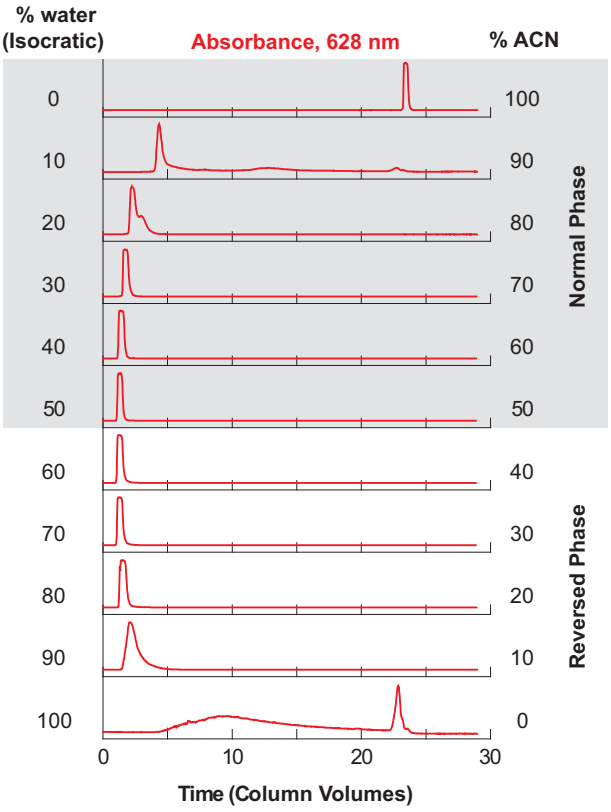

Figure 1: FD&C Blue #1 exhibits both normal and reverse phase behavior on a diol column. Shaded area denotes "normal phase" behavior for this column and compound.

Butyl paraben (*figure 2*) is a non-polar compound that exhibits purely reverse behavior on a diol column under aqueous conditions. Note that using solely organic solvents, the diol column will act as a normal phase column.

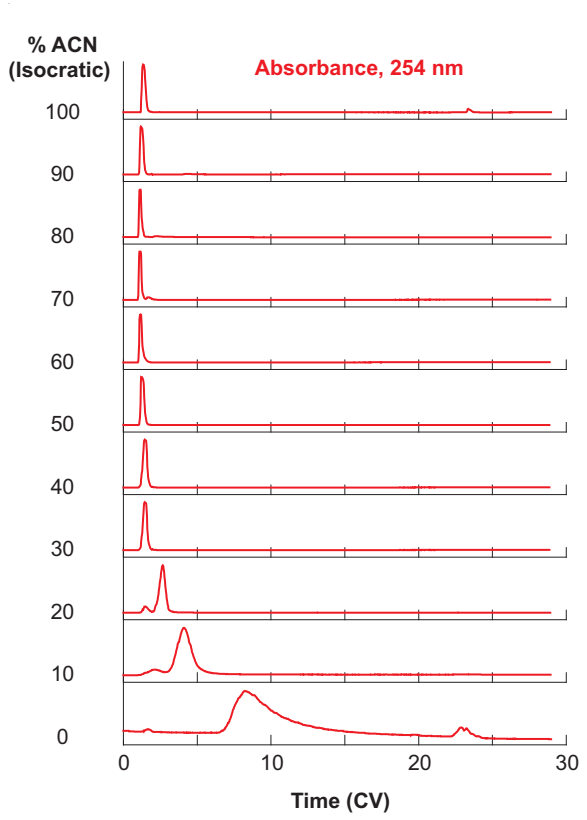

Figure 2: Butyl paraben exhibits reverse phase behavior on a diol column under aqueous conditions

Conclusion

Diol columns can exhibit both normal and reverse phase behavior under aqueous conditions. Diol behavior can be summarized as:

- Diol columns run as normal phase when using only organic solvents.
- Non-polar compounds will run as reverse phase under aqueous conditions and will not work well in HILIC purifications.
- Water soluble compounds can show both normal phase and reverse phase behavior. They will generally show normal phase behavior from 0 to 40 or 50% water, which corresponds to the commonly used range for aqueous normal phase.
- More polar organic solvents (such as methanol) are likely to show a reduced range of normal phase behavior with water soluble compounds.

References

1. Diol Phase. http://www.justchromatography.com/wiki/diol-phase, 7 October 2011
2. Diol Columns – pretend they're normal phase. http://www.isco.com/WebProductFiles/Applications/101/Poster_and_Paper_Reprints/Diol_Columns-Pretend_They're_Normal_Phase.pdf (accessed Feb 2012).

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