RediSep Gold® Silica and Highly Polar Solvents

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Chromatography Application Note AN84

Abstract

RediSep Gold silica columns are suitable for highly polar solvent systems such as those used for Hydrophilic Interaction Liquid Chromatography (HILIC), including methanol and water. The use of these solvents leads to improved purifications, reduced requirements to use chlorinated solvents, and faster solvent evaporation due to reduced volumes of water compared to reverse phase. Solvent modifiers can be used for HILIC to improve peak shape, but the pH should remain below pH 7. This application note evaluates silica leaching from RediSep Gold silica in both 100% methanol and water. Purification of highly polar compounds is demonstrated, as is the use of trifluoroacetic acid (TFA) as a modifier.

Background

Compounds including nucleotides, dyes, and many basic compounds are very polar and are difficult to purify without resorting to correspondingly polar solvents on silica gel. HILIC is increasingly used to analyze these compounds with HPLC. Common HILIC media include diol, amine, and bare silica. Many of these compounds could be purified on silica with high concentrations of methanol.

It is commonly held that silica gel dissolves in methanol or water. However Alexander et al. demonstrated the solubility of silica in methanol to be 0.0014%[1]; the same study demonstrated aqueous silica solubility of 0.01%. Both solubility measurements were obtained after equilibrating for several months, much longer than the residence time in a flash column. This same study also demonstrated a large increase in silica solubility above pH 8.

HILIC is also called aqueous normal phase. *Table 1* lists solvents commonly used for this phase.

Table 1: List of solvents suitable for Aqueous Normal Phase Chromatography

acetone	ethanol
acetonitrile	methanol
isopropanol	tetrahydrofuran

Experimental and Results

The experiments utilized a mixture of caffeine and adenine adsorbed on silica gel and placed in a solid load cartridge. A CombiFlash® system was run for all experiments. Compounds were detected at 210 or 270 nm. RediSep Gold silica columns (24 g, PN 69-2203-346) were used for all experiments except where indicated.

High Concentrations of Methanol with RediSep Gold Silica

A gradient of 1 to 100% methanol in dichloromethane was used for this experiment. Detection was at 270 nm. The gradient started at 1% methanol to better equilibrate the column and improve the peak shape of caffeine which elutes first.

Figure 1 shows the purification of adenine from dichloromethane. Adenine elutes at ~46% methanol. Both peaks have good peak shape with slight tailing of the adenine peak. This compares favorably to the reversed phase elution using a water/methanol gradient (Figure 2). This run used a 30 g RediSep Gold C18 column and both solvents contained 0.1% TFA.

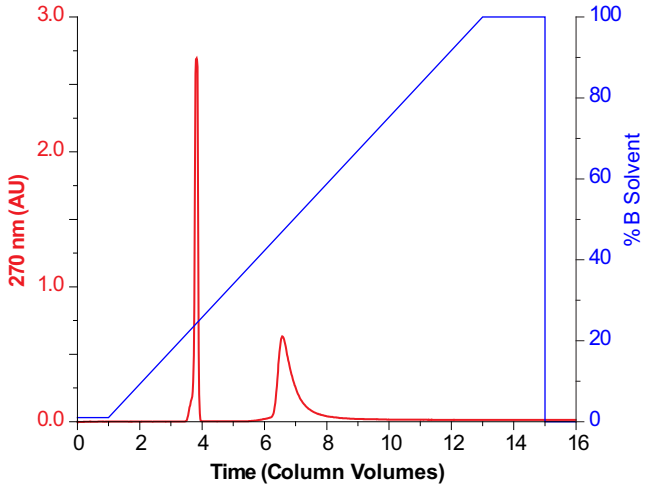

Figure 1: Purification of adenine from caffeine using a dichloromethane/methanol gradient

An 85 g RediSep Gold silica column (PN 69-2203-348) was flushed with 10 column volumes (1250 mL) of methanol. The column was air-purged at the end of the wash; the purge and column washings were combined. The mixture as well as a methanol blank (1250 mL) were dried on a rotary evaporator in a tared flask. The column eluent weighed 24 mg while the solvent blank was 8 mg, demonstrating minimal bleeding of silica from the column using 100% methanol.

Aqueous Normal Phase with RediSep Gold Silica

A gradient of 1 to 30% water in acetonitrile was used for this experiment. Detection was at 210 nm. The gradient started at 1% water to hydrate the column prior to eluting the compounds.

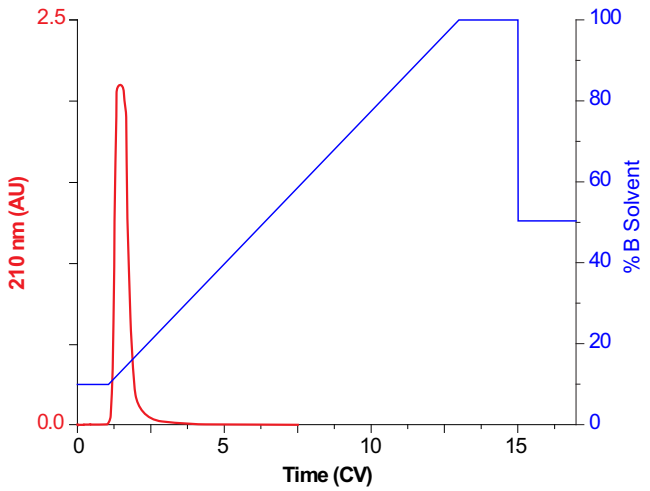

Figure 2: Caffeine and adenine elute together on C18

The elution of adenine and caffeine from silica using aqueous normal phase is shown in *Figure* 3. Both compounds exhibited good peak shape. The adenine peak was improved with the substitution of 0.1% TFA in water for plain water (*Figure 4*).

TFA was used instead of ammonium hydroxide to ensure the column was run at a low pH. Although silica has minimal solubility in water below pH 8, it dissolves readily above this pH.

A gram of Redi*Sep* Gold silica was mixed with 100 mL 18 MΩ water in a plastic bottle and allowed to sit with occasional stirring for 48 hours. The water was decanted from the silica which settled to the bottom of the bottle. The concentration of silica was measured to be 25 PPM, compared to 0 PPM for the water blank.

Conclusion

Redi*Sep* Gold silica columns are suitable for purifying compounds using high concentrations of methanol or even water without leaching silica. The ability to run in very polar solvents allows the purification of very polar compounds where other purification techniques fail to resolve the desired compounds. Modifiers can be used to improve the peak shape, but the pH of the solvent system should remain below pH 7 to avoid dissolving silica.

Polar compounds are eluted mostly in organic solvents from silica compared to reverse phase, where polar compounds elute in highly aqueous solutions. The high organic solvent content of the eluent speeds drying compared to reverse phase, where lyophilization may be required.

The ability to use water on Redi*Sep* Gold silica columns allows users to avoid chlorinated solvents. The elution of caffeine and adenine were similar using dichloromethane or water.

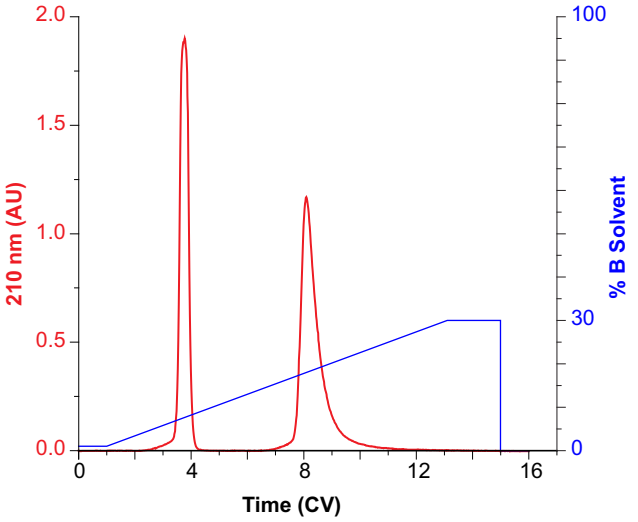

Figure 3: Elution of caffeine and adenine using silica HILIC on a Redi*Sep* Gold Silica column

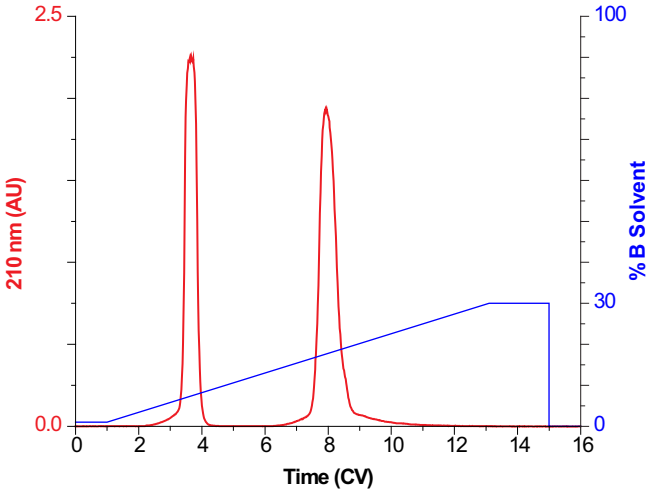

Figure 4: Improved adenine peak symmetry with 0.1% TFA in water

References

1. Alexander, G.B.; Heston, W.M.; Iler, R.K. The Solubility of Amorphous Silica In Water *J. Phys. Chem.* **1954**, *58(6)*, 453-455

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