

RediSep Rf Gold[®] C18Aq

for Highly Aqueous Mobile Phases

Chromatography Application Note
AN76

Abstract

RediSep Rf Gold C18Aq media consists of C18 bonded to silica with modifications to show improved performance under conditions where the solvent contains a high concentration (>50%) of water. If compounds elute with more than 50% organic solvent, the RediSep Rf Gold C18 media shows improved performance. Purifications of water-soluble organic compounds are demonstrated.

Background

Why RediSep Rf Gold C18Aq?

Standard C18 columns are designed to reduce the interactions of unreacted surface silanols by end-capping with a methyl group or another non-polar entity. This limits their selectivity towards highly polar compounds due to “phase collapse” (see Figure 1).

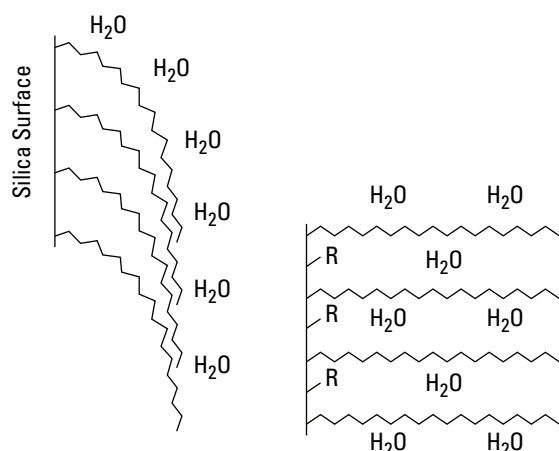


Figure 1: C18 chains are fully extended when organic solvent is present but undergo “phase collapse” (left) under highly aqueous conditions. Hydrophilic groups (R) reduce phase collapse (right).

Phase collapse causes reduced retention due to decreased interaction of the sample with the C18 chains. Irreproducible runs are also another symptom of phase collapse.

RediSep Rf Gold C18Aq media, with its hydrophilic groups, reduces tendencies toward phase collapse and allows better retention of highly polar samples.

What types of compounds run best on RediSep Rf Gold C18Aq?

Compounds that require more than 50% water in the mobile phase for elution are candidates for purification with RediSep Rf Gold C18Aq. Examples include water soluble dyes, glycosylated compounds, and other materials containing multiple polar groups.

RediSep Rf Gold C18Aq columns are a preparative alternative to analytical separations currently performed with hydrophilic C18 columns.

When should I instead choose RediSep C18 Gold?

Although RediSep Rf Gold C18Aq columns work well under all percentages of organic solvent, improvements in resolution are seen using RediSep Rf Gold C18 for compounds that elute using more than 50% organic solvent.

Use and Care of RediSep Rf Gold C18Aq

RediSep Rf Gold C18Aq columns are dry packed and may be stored indefinitely under these conditions. Prior to use, the column should be activated with the organic solvent used in the purification. Activate the column by washing with ~6 column volumes of solvent containing ~80% of the organic solvent used in the mobile phase. After the initial wash, the column should not be allowed to dry out.

RediSep Rf Gold C18Aq can be run with acetic acid, trifluoroacetic acid, or other modifiers to improve peak shape. After use, these modifiers should be washed from the column to improve column life.

RediSep Rf Gold C18Aq columns should be stored in at least 50% organic solvent with the end caps in place.

Experimental and Results

Water soluble dyes

50 mg each of tetrazine and brilliant blue were dissolved in 2.0 mL water. Of this solution, a 1.0 mL sample was injected onto a 30 g RediSep Rf Gold C18 and a RediSep Rf Gold C18Aq column. The columns were run according to the conditions listed in Figure 2.

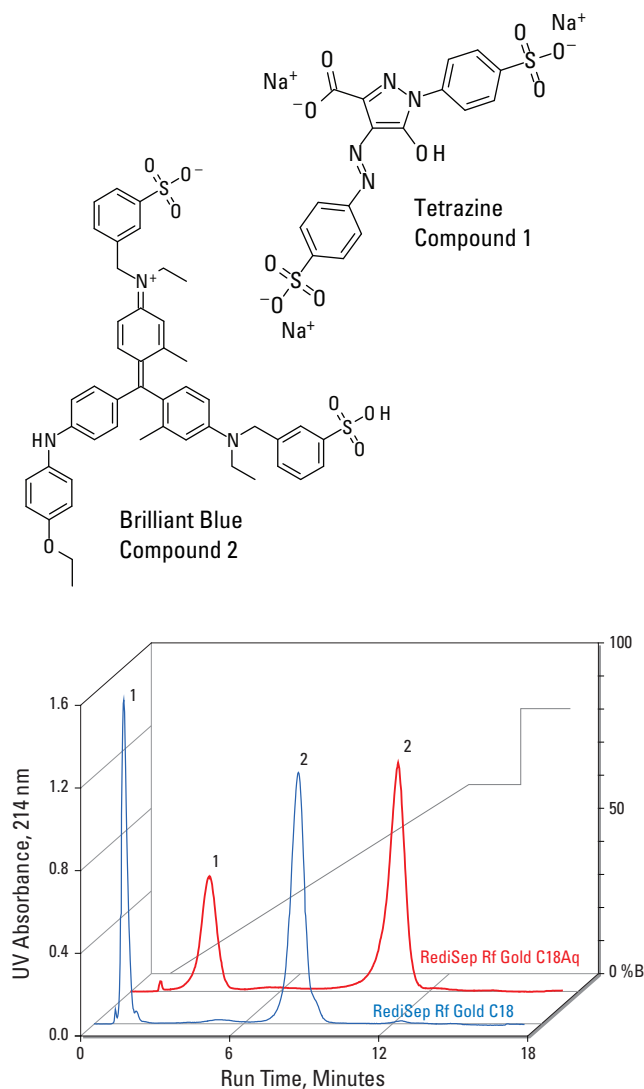


Figure 2: Comparison of RediSep Rf Gold C18Aq and standard RediSep Rf Gold C18 with sample requiring highly aqueous mobile phase. The mobile phase for both runs was water (A) and methanol (B) at 35 mL/min. Compound 1 is not retained on the RediSep Rf Gold C18 column under low organic solvent conditions.

The RediSep Rf Gold C18Aq column displayed increased retention of both compounds with better resolution between compounds.

Customer Sample

A customer sample was run on both the RediSep Rf Gold C18 and RediSep Rf Gold C18Aq columns. The sample load was three times greater on the RediSep Rf Gold C18Aq. The method for the RediSep Rf Gold C18Aq was extended during the run because of the better retention exhibited by this column. A maximum of 30% methanol was used for both runs with 0.1% TFA modifier.

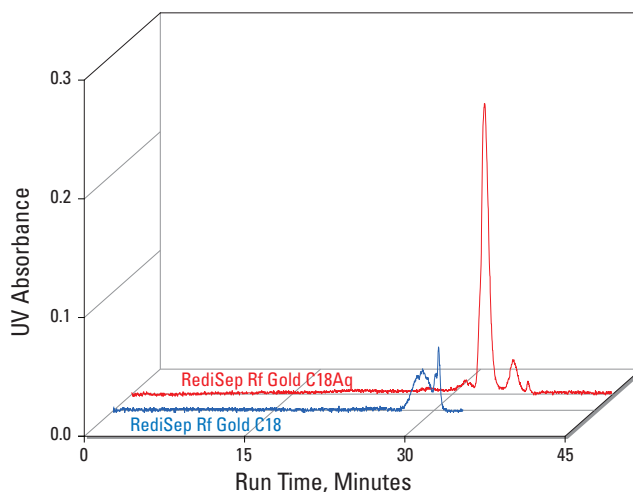


Figure 3: RediSep Rf Gold C18Aq purification of a customer's water soluble sample compared to RediSep Rf Gold C18. The sample load on the C18Aq column is three times that of the C18 column.

Conclusion

The compound was able to interact with the RediSep Rf Gold C18Aq under highly aqueous conditions because the chains remained rigid. The bands are tighter, resulting in sharper peaks and better resolution. The impurities are completely removed as compared to the RediSep Rf Gold column (Figure 3).

Because the chains remain extended with C18Aq media, it is able to load a greater amount of material as opposed to standard C18 when the mobile phase has a high water content.

February 16, 2011

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