

Desalting Samples with RediSep Rf Gold[®] C18Aq Columns

Abstract

Compounds are often purified with solvents that contain salts or buffers that need to be removed prior to subsequent studies with the compound. The RediSep Rf Gold[®] C18Aq columns have the ability to adsorb compounds under highly aqueous conditions and are ideal for removing the salt from a sample. The method described also removes a large portion of water from samples dissolved in aqueous solvents facilitating evaporation. The procedure is similar to solid phase extraction, but on a larger prep scale.

Background

Compounds run on ion exchange media are often eluted with buffers that are difficult to remove from the sample after purification. Buffers, such as phosphate salts, are often used to improve resolution during purification on C18 by maintaining the pH of the solvent at a value such that the eluted compounds remain in a consistent ionization state.

The RediSep Rf Gold[®] C18Aq columns are well suited to desalting applications because they resist phase collapse. Samples requiring desalting are generally dilute and the large volume of water eventually causes phase collapse with other types of C18 (Figure 1).

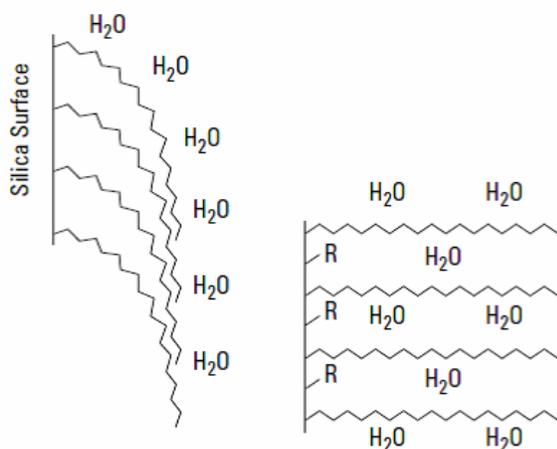


Figure 1: C18 chains are fully extended when organic solvent is present but undergo “phase collapse” (left) under highly aqueous conditions. Hydrophilic groups (right) reduce phase collapse (right).

The general procedure for desalting samples is as follows:

1. Remove any organic solvents by rotary evaporation. Organic solvents tend to reduce the binding of compounds to C18. Stop evaporation if the sample appears to be precipitating.
2. Condition the RediSep Rf Gold[®] C18Aq column by washing with an organic solvent such as methanol or acetonitrile for 5 column volumes (CV) followed by water for 5 CV.
3. Load the sample. For desalting purposes, load up to 5% by weight on the column because the desired compound is captured from the salt solution and released. For example, a 5.5 g RediSep Rf Gold[®] C18Aq column (PN 69-2203-558) can be used to capture up to ~250 mg compound. If further purification is performed after washing off the salt, use a 1% load. Larger volumes can be loaded with a CombiFlash[®] sample load pump (PN 60-524-7007). Since the sample is being loaded in a weak, polar solvent the total volume injected may exceed the column volume without sample loss.
 - a. Place the Rf+ in manual control, and select **Liquid Injection (Pumps Disabled)** from the **Valve Position** control. Place the column on the instrument and load the sample.
 - b. For an Rf-150, place the column on the system and load the sample.
 - c. Verify the column has been conditioned according to the manufacturer’s instructions.
4. Wash the salt from the column using manual control. On the Rf+, select **Through column and flow cell** from the **Valve Position** control. On an Rf-150, connect the solvent line to the column shuttle. Set the flow rate to the value listed on the column label and press the **Prime A** button. Wash the column for at least one, and preferably five, column volumes.
5. Elute the compound. Change the method to a step gradient after 1 CV. The step should go to 100% B. If the compound requires further purification, use a gradient suitable for the purification. From the **Run Requirement** screen, select **None-On Column** from the **Sample Loading Technique** control because the column was equilibrated and the sample is on the column.

Always run a small sample to verify the compound will adsorb on the column before running the entire sample mixture.

Experiment and Results

The example in Figure 2 is brilliant blue purified from a WAX column eluted with 1M NaH₂PO₄ in water. As there was no organic solvent involved, the mixture was not evaporated prior to loading on the column.

The procedure listed above was altered slightly for this run; the column was not washed prior to the run as described in step 4, but was washed with water for 10 CV during the run to demonstrate that the compound remained on the column during the wash. This compound elutes with 30% methanol in water on a RediSep Rf Gold[®] C18Aq column¹ under gradient conditions and demonstrates that compounds that normally elute with high water concentrations can be desalted with this method. The solvent used to elute the sample from the C18Aq column is methanol.

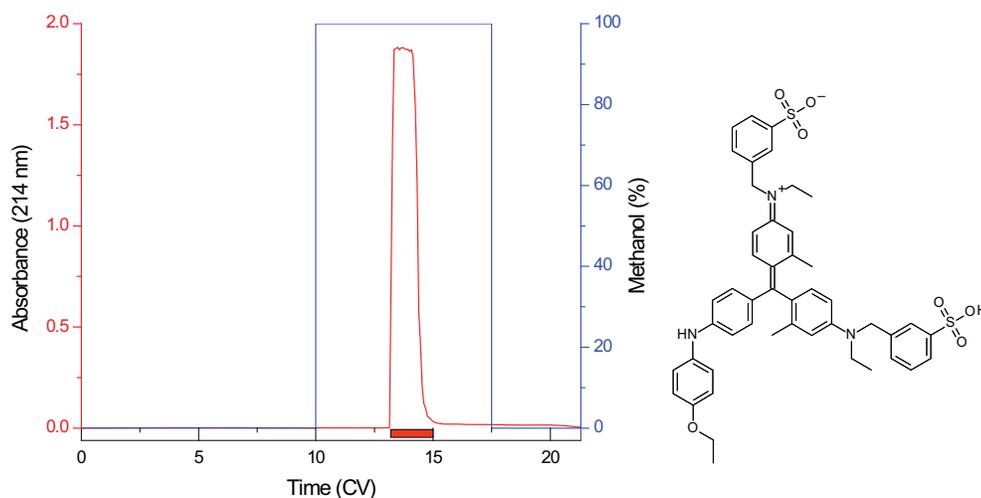


Figure 2: Desalting of brilliant blue with a RediSep Rf Gold[®] C18Aq column

The step gradient provides the following features:

- Run time and solvent usage is reduced.
- The compound elutes in a sharper band reducing the volume of solvent to evaporate, saving time.
- There is relatively little water in the sample, so drying time is reduced because methanol is easier to evaporate.

Conclusion

RediSep Rf Gold[®] C18Aq columns provide a convenient way to desalt samples. Other benefits include removing most of the water and reducing the volume of the fractions to facilitate evaporation. If desired, a regular gradient can also be run if the compound requires further purification instead of the step gradient.

1. http://www.isco.com/WebProductFiles/Applications/101/Application_Notes/AN76_RediSep_Rf_Gold_C18A_for_Highly_Aqueous_Mobile_Phases.pdf retrieved 15 Aug 2012

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