Use of Redi*Sep* Gold® Amine Columns in the Weak Ion Exchange Mode

TELEDYNE ISCO
Everywhereyoulook™

Chromatography Application Note
AN99, August 2020

Abstract

The Redi*Sep* Rf Gold® Amine columns are useful for normal phase purifications, but can also be converted for use as a weak anion exchange (WAX) column. This application note describes the conversion of the amine column to a WAX column and an example run with the converted column. Washing and conditioning the column for another run is also described.

Background

Why Use Ion Exchange Resins?

Ion exchange resins are useful to purify ionic compounds from non-ionic materials. Ionic compounds may be resolved from each other based on their ionization at a given pH or buffer concentration.

Compounds that contain strongly ionizable groups such as sulfonates or phosphates can be captured and released on a WAX column. These compounds are generally very soluble in water and often difficult to purify with other techniques.

Redi*Sep* Rf Gold Amine columns contain an amino-propyl bonded phase which becomes a weak ion exchange (WAX) phase which can be used to isolate and purify compounds with strongly anionic groups after treatment with an acid or salt. Compounds with phenolic, carboxylic, and other weakly anionic groups can be purified on Redi*Sep* Rf SAX (strong ion exchange) columns.

Once the Redi*Sep* Rf Gold Amine column is converted for use as an ion exchange column, it is difficult to convert it back to an un-ionized amine column. The column should be labeled as a WAX column to avoid confusion with an unmodified column.

CAUTION

This procedure uses non-volatile salts. Use of non-volatile salts and buffers may reduce pump seal life. The chromatography system must be flushed with water after the runs are complete to prevent salts from precipitating in the pumps and lines. See Application Note #28 on the Teledyne ISCO web site[1] for a method to efficiently clean the entire system.

The compounds will be eluted with a large quantity of salt that generally needs to be removed. Many compounds can be desalted with a Redi*Sep* Rf Gold C18Aq column. Test the procedure on a small scale before purifying your entire compound mixture.

Experiment and Results

Column Capacity

Part #	Column size (grams)	Maximum sample Load (mMol)
69-2203-504	5.5	6.4
69-2203-505	15.5	18.1
69-2203-506	30	35.1
69-2203-507	50	58.5
69-2203-508	100	117
69-2203-509	150	175.5
69-2203-510	275	321.7

Table 1: RediSep Rf Gold Amine loading capacities when used as WAX columns

The maximum capacity of the Redi*Sep* Rf Gold Amine columns, when used as WAX columns, is shown in Table 1.

Column Preparation and Use

A new column should be conditioned with methanol or 2-propanol, followed by a wash with at least 10 column volumes (CV) of 5% acetic acid. The acetic acid converts the column from the free base to the ionic form with acetate as the counter-ion. Compound retention is improved with the use of buffers with lower selectivity than the functional groups on your compound. The selectivity order is:

- OH^- < acetate < formate < HCO_3^-< Cl^- < HSO_3^-< citrate (ions on right displace those on left)

The compound mixture should be dissolved in water and liquid-loaded on the column. If the compound is dissolved in a buffer, the buffer concentration should be kept below 0.05M to reduce competition on the column between the buffer and the desired compound. The column can be washed with water to elute neutral and un-retained compounds.

Elute the compound with the buffer. The example uses gradients that increases the ionic strength (salt

1. http://www.isco.com/WebProductFiles/Applications/101/Application_Notes/AN28_Solvent_Changing_on_CombiFlash_Systems.pdf retrieved 16 Aug 2012

concentration) until the compounds elute. The gradient can be a continuous or step-wise gradient.

Column Washing/Storage

After use, wash the column with 10 CV of 5% acetic acid in water. The acetate regenerates the column by displacing the counter-ion that was used last on the column. Follow the acetic acid wash with a 5 CV water wash. Store the column in 100% 2-propanol.

Purification of Brilliant Blue Dye

Brilliant blue (Figure 1) is a good example of both capture/release and purification of a compound. This compound possesses two sulfonate groups. A number of minor impurities were resolved from the main compound peak during the gradient elution.

Brilliant blue (50 g) was dissolved in 5 mL water and injected on a 15.5 g Red*Sep* Rf Gold Amine column (PN 69-2203-505) previously conditioned with 5% acetic acid. The mixture was eluted with a gradient from 0 to 100% B solvent consisting of 1.0M NaH2PO4 in water; the pH was not adjusted but measured to be pH 4.6.

Fractions from the main peak were combined and desalted using a 5.5 g Red*Sep* Rf Gold C18Aq column (PN 69-2203-558).

Conclusion

The Red*Sep* Rf Gold Amine column is versatile. It can be run as a normal phase column or as an ion exchanger. Once converted to ion exchange, it can be reused for other ion exchange purifications. The compounds are eluted using increasing buffer concentration.

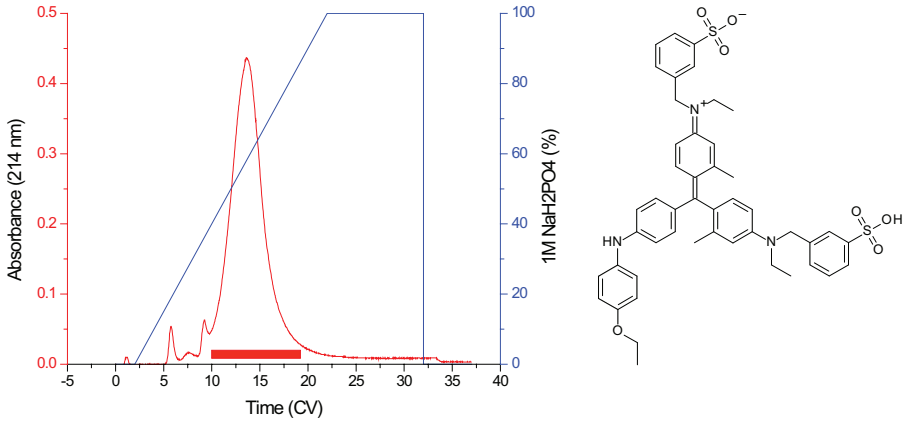

Figure 1: Purification of brilliant blue dye with a RediSep Rf Gold Column in WAX mode

Teledyne ISCO
P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3091