

RediSep Rf Gold™ High Performance Amine Columns

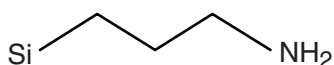
Use, Cleaning, and Storage Instructions

Overview

Proper cleaning and storage of Teledyne Isco reusable RediSep Rf Gold amine columns will help extend column life and improve column consistency and reliability.

Column Use

The RediSep Rf Gold amine functionalized silica is a short carbon tether end-capped with a polar primary amine functionality.



You can use RediSep Rf Gold amine columns under normal- or reversed-phase conditions. The sample load on the amine column should be 0.1 to 2 percent of the column media weight.

Initial Wash

Prior to initial use of RediSep Rf Gold amine columns, wash the dry column with 2–3 column volumes (CV) of methanol. This methanol wash will remove residual underivatized silica.

Post-separation Care

For normal phase separation methods, add a column wash step after the separation (Figure 1). Write the method so the system washes the column with one to two CV of a high percentage (100%) of the polar organic solvent. This is typically Solvent B, ethyl acetate or methanol. After 1–2 CV of wash, return the percent organic to the starting condition for 2–3 CV. This prepares the instrument for the next separation run's equilibration conditions. Refer to Table 1 for column volume data.

For reversed-phase methods, also write a column wash step after the separation. The system should wash the column with 1–2 CV of a high percentage (100%) of the organic (B) solvent. After 1–2 CV of wash, return the percent organic to the starting condition for 2–3 CV to prepare for the next separation run's equilibration conditions.

CAUTION

Air purging a RediSep Rf Gold amine column after separation will cause the stationary phase to expand and contract. This might induce channeling, which degrades column performance. Turn off the air purge feature on chromatography systems that include a post-run air purge.

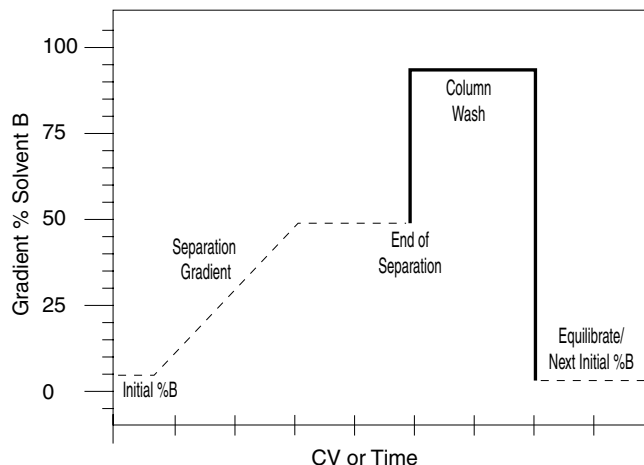


Figure 1: Post-separation column flushing

Storage

With proper care and storage, you can use RediSep Rf Gold amine columns thirty times or more, and store the columns for two months or longer with no loss in performance. Some general rules for storage of RediSep Rf Gold amine columns are:

- Flush the column with 3–5 column volumes of storage solvent.
- Store columns wet and well capped in 100% isopropanol.
- The storage solvent should be miscible with solvent remaining in the column (see *Post-separation Care*). If not, first pump an intermediate solvent through the column to displace the wash solvent.

Table 1: Column Data

Column Weight (grams)	CV (mL)	Flow Rate (mL/min)	Sample Mass
5.5	5.7	18	5.5 mg to 110 mg
15.5	16.8	30	15.5 mg to 310 mg
30	32.9	35	30 mg to 600 mg
50	52.9	40	50 mg to 1 g
100	109	60	100 mg to 2 g
150	162	85	150 mg to 3 g
275	302	150	275 mg to 5.5 g
415	453	150	415 mg to 8.3 g
950	1027	250	0.9 g to 19 g
1900	2053	300	1.9 g to 38 g

Sample Run

The sample run purifies a 1:1 mixture of nicotine and 1-methylbenzimidazole (Figure 2) using the parameters in Table 2. The chromatogram is illustrated in Figure 3.

Table 2: Sample Run Parameters	
Chromatography mode:	Normal phase
Wavelength:	254 nm
Sample loading type:	Pre-loaded on silica gel solid sample cartridge
Mobile phase:	Solvent A: Hexane Solvent B: Ethyl Acetate
Flow Rate:	Per Table 1.

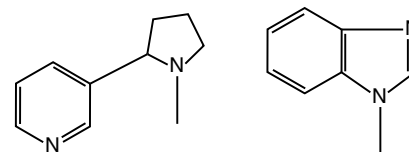


Figure 2: Compound Mixture Nicotine (left) and 1-methylbenzimidazole (right)

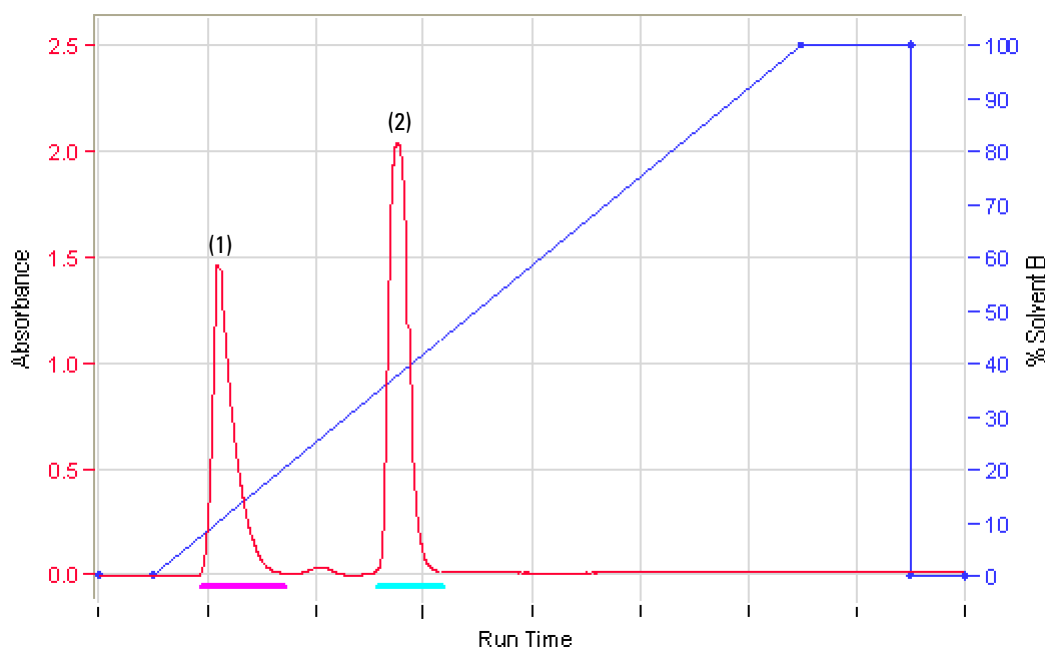


Figure 3: Sample run on RediSep Rf Gold amine
Columns Nicotine (1) and 1-methylbenzimidazole (2)

Summary

RediSep Rf Gold amine stationary phase is favorable for performing separations in a normal phase mode capable of employing a wide variety of solvents—including those typically used in reversed phase separations. Clean columns with high (100%) polar organic solvent. Never dry the column and store columns wet and capped with 100% isopropanol.

Assistance Available

Should you need assistance with these instructions, please contact the Teledyne Isco Chromatography Laboratory.

Teledyne Isco, Inc.

P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 775-2965 • Phone: (402) 464-0231 • Fax: (402) 465-3001
E-mail: IscoService@teledyne.com