

RediSep[®] Rf Reversed Phase C18 Columns

Chromatography Application Note AN2
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Use, Cleaning, and Storage

Application Overview

Proper preconditioning, cleaning, and storage of reusable Teledyne Isco RediSep Rf reversed phase C18 and RediSep Rf Gold[®] high performance C18 columns will help to extend column life and improve column consistency and reliability.

General Method

Initial Preconditioning

Flush the column with six column volumes (CV) of 1:1 water:acetonitrile prior to immediate use. Alternatively, pump 2CV of 100% acetonitrile through the column, then cap and store it for one hour before use. Refer to Table 1 for column volume data.

Table 1: Column Data

Column Media Weight (g)	Column Volume (mL)	Optimal Flow Rate (mL/min)
RediSep Rf C18 Columns		
4.3	4.1	18
13	12.9	30
26	26.4	35
43	42.4	40
86	87.7	60
130	132.6	85
240	242.8	150
360	361.1	150
RediSep Rf Gold High Performance C18 Columns		
5.5	4.6	18
15.5	13.5	30
30	26.4	35
50	42.6	40
100	87.7	60
150	129.7	85
275	243.4	150
415	363.5	150
950	825	250
1900	1650	300

Sample Loading Capacity

The sample load on the column should be 0.1 to 2 percent of the column media weight.

Post-run Care

With proper care, RediSep Rf C18 columns may be used thirty times or more. Reversed phase methods should be written so that at the end of the separation, the column is washed with 2–4CV of a high percentage (100%) of the organic (B) solvent. After 2–4CV of wash, the percent organic can be returned to the starting condition for 2–3CV to anticipate the next run column equilibration conditions (see Figure 1). Alternatively, the percent organic can be changed to 80% in anticipation of column storage.

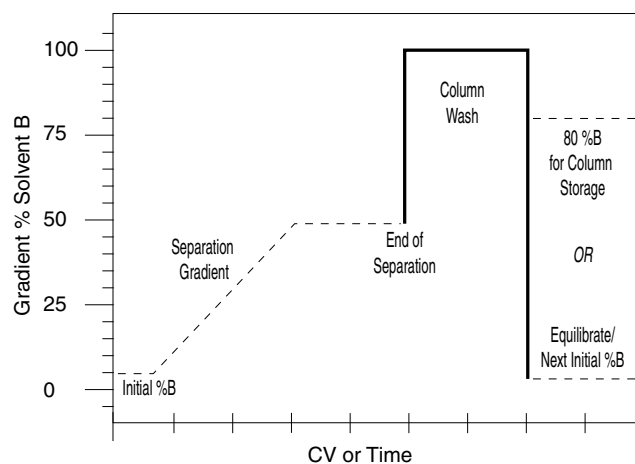


Figure 1: Post Run Column Flushing

Storage

It is possible to store C18 columns for long periods with no loss in performance. Following are some general rules for storage for reversed phase C18 columns:

- *Once wetted, never dry out a reversed phase column.* This will induce channeling due to expansion and contraction of the stationary phase. The air purge feature (if available on the Flash chromatography system) should be turned off.
- *Store columns wet and well-capped* in 10–30% water and 70–90% organic solvent such as acetonitrile, methanol, or ethanol.

Summary

Precondition columns before initial use. Clean columns with high (100%) organic solvent. Never dry the column and store wet and capped with 70–90% organic solvent.

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