User Guide for Using, Cleaning and Storage of SepaFlash™ Bonded Series Flash Columns

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Chromatography Application Note

Application R&D Center

Introduction

With proper preconditioning, cleaning and storage, SepaFlash™ Bonded Series flash columns can be re-used and the column life will be extended. Moreover, the column consistency as well as reliability will also be improved.

General Method

Initial Preconditioning

Flush the column with 4 – 6 column volumes (CV) of methanol or acetonitrile to activate the SepaFlash™ Bonded Series flash columns. Then flush the column with another 2 CVs of 1:1 methanol:water or 1:1 acetonitrile:water. Finally equilibrate the column with 3 – 6 CVs of mobile phase which is intended to be used in flash chromatography. Refer to Table 1 for column volume data.

Post-run Cleaning

With proper cleaning after each run, SepaFlash™ Bonded Series flash columns may be used twenty times or more. After each run, flash column should be flushed with mobile phase which has higher elutropic strength. For example, if there are no acidic, basic or salty additives in the mobile phase used in separation, then 90% methanol in water is suggested as the cleaning solvent to flush the column for 5 CVs. In case there are salty additives included in the mobile phase as a buffer solution, then the aqueous part of the mobile phase should be replaced by pure water as the cleaning solvent to flush the column for 5 CVs. Afterwards the column should be flushed with 100% organic phase for another 5 CVs. The reason behind this cleaning manner is that the flash column might be damaged due to precipitation of salty additives resulting from direct flushing with pure organic phase.

Table 1: Column Data

Column Media Weight (g)	Column Volume (mL)	Optimal Flow Rate (mL/min)
SepaFlash™ Bonded Series, Irregular Silica Family,40-63 µm, 60 Å		
5.9	3.6	15
23	14	20
38	23	20
55	33	25
122	70	30
180	103	40
340	195	60
475	272	60
SepaFlash™ Bonded Series, Spherical Silica Family,20-45 µm, 100 Å		
5.4	4.3	15
20	16	20
33	26	20
48	38	25
105	83	30
155	122	40
300	236	60
420	331	60

Storage

To maintain the performance of SepaFlash™ Bonded Series flash columns, these columns should be kept in proper storage condition. Please follow these general rules as listed below:

- *Once wetted, never dry out a reversed phase column.* Since blow dry the column will induce channeling due to expansion and contraction of the stationary phase. Please make sure the air purge feature (if available on the flash chromatography system) is turned off.
- *Store the column well-capped and wet* in the solvent with high ratio of organic phase. The solvent used for column storage could be 80 – 95% acetonitrile, methanol or ethanol in water*.

*Note: For the user guide of SepaFlash™ amino columns, please refer to another application note published by Santai Technologies:
The Use and Maintenance Guide for SepaFlash™ Amino Columns.

Column Efficiency Testing Method

To evaluate the column performance, a standard testing method could be followed. The testing method is described in details as follows.

Dissolve 0.1 g caffeine, 0.4 g acetophenone and 7.5 g toluene in 100 mL methanol and sonicate the solution for 1 min to get a clear and transparent solution. Inject 0.5 mL of the sample solution with an injector onto a 12 g SepaFlash™ C18 cartridge (part number: SW-5201-012-IR). Then employ a water/methanol system as the mobile phase and set a gradient elution profile where methanol ratio is increased from 10% to 90% in 10 min and maintained for 2 min. A flash chromatogram is then obtained as shown in Figure 1.

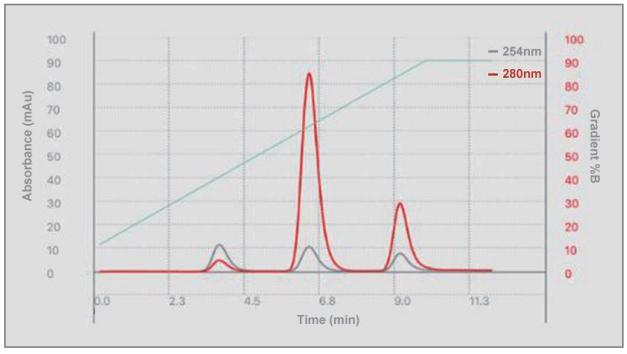

Figure 1. The flash chromatogram of standard sample mixture on a 12 g SepaFlash™ C18 cartridge.

According to the chromatogram as shown in Figure 1, the equation for calculating the theoretical plate number of the column is as follows:

$$n = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2$$

or:

$$n = 16\left(\frac{t_R}{W}\right)^2$$

Where n is the theoretical plate number, t_R is the retention time of a specific component, $W_{1/2}$ is the half width of the eluted peak corresponding to the specific component, W is the bottom width of the eluted peak corresponding to the specific component.
According to the above equation, calculate the theoretical plate number of a specific component from current column and compare the result with a newly unused column to determine whether the performance of current column is good or not.

More Tips for SepaFlash™ Bonded Series Columns

Please find more information of SepaFlash™ Bonded Series columns in Table 2, including chemical structure of stationary phase, particle size, typical applications, storage conditions and other characteristics.

Table 2. More Information of SepaFlash™ Bonded Series Columns

Bonded Phase	Structure	Characteristics	Typical Applications	Storage Conditions
Amine		Irregular, 40-63 μm Endcapping: Yes Amino content: 1.3 mmol/g	Good alternative for normal phase purification of compounds with high polarity, especially useful for polysaccharides.	Flush the cartridge with 3 CVs of 80% acetonitrile in water or 100% isopropanol. Store in 100% isopropanol with well-capped ends.
Diol		Spherical, 20-45 μm Endcapping: Yes Carbon content: 5.0%	Good alternative for difficult separation of samples with low to medium polarity. Offers a better retention time compared to normal phase.	Flush the cartridge with 3 CVs of 80% acetonitrile in water. Store in flush solvent with well-capped ends.
Cyano		Spherical, 20-45 μm Endcapping: Yes Carbon content: 5.5%	Versatile sorbent that can be used either as normal or reversed phase. Indicated for products with intermediate to extreme polarity. The slightly hydrophobic nature of cyano group offers alternative selectivities.	Flush the cartridge with 3 column volumes of 80% acetonitrile in water or 80% methanol in water. Store in flush solvent with well-capped ends.
C4		Spherical, 20-45 μm Endcapping: Yes Carbon content: 5.8%	Reversed-phase matrix can provide less retention of non-polar compounds than C18 and C8 and is useful in ion-pairing chromatography. Used to separate large biomolecules.	Flush the cartridge with 3 column volumes of 80% acetonitrile in water or 80% methanol in water. Store in flush solvent with well-capped ends.
C8		Spherical, 20-45 μm Endcapping: Yes Carbon content: 7.0%	Reversed-phase matrix with a moderate degree of hydrophobility that works well for separating a wide range of compounds. May be used as replacement of C18 when shorter retention times are desired or required.	Flush the cartridge with 3 column volumes of 80% acetonitrile in water or 80% methanol in water. Store in flush solvent with well-capped ends.
C18		Spherical or Irregular Endcapping: Yes Carbon content: 17%	Indicated for the purification of medium to high polarity compounds, they provide reproducible purification without the complexity and cost of preparative HPLC.	Flush the cartridge with 3 column volumes of 80% acetonitrile in water or 80% methanol in water. Store in flush solvent with well-capped ends.
C18AQ		Spherical, 20-45 μm Endcapping: Yes Carbon content: 10%	C18AQ silica is specially designed for the purification of highly polar or hydrophilic compounds, including peptides, antibiotics, glutamine derivatives, etc.	Flush the cartridge with 3 column volumes of 80% acetonitrile in water or 80% methanol in water. Store in flush solvent with well-capped ends.
SAX		Irregular, 40-63 μm Endcapping: No Carbon content: 8.0%	It is mainly used as a strong anion exchanger in ion chromatography and ion exchange SPE. It is especially used for the "Catch and Release" purification of weak acids.	Flush the cartridge with 10 column volumes of 80% methanol in water or 100% isopropanol. Store in flush solvent with well-capped ends.
SCX		Irregular, 40-63 μm Endcapping: No Carbon content: 10%	It is widely used for the scavenging of amines and other basic functionalities, including weakly basic anilines, borohydrides, and metals such as Ni and Ag.	Flush the cartridge with 10 column volumes of 80% methanol in water or 100% isopropanol. Store in flush solvent with well-capped ends.
ARG		Spherical, 20-45 μm Endcapping: Yes Carbon content: 8.0%	ARG Silica can separate hydrophilic compounds such as amino acid, peptide, vitamin and nucleic acid. ARG Silica is dedicated to the separation of various hydrophilic compounds.	Flush the cartridge with 3 column volumes of 80% acetonitrile in water or 80% methanol in water. Store in flush solvent with well-capped ends.
Phenyl		Spherical, 20-45 μm Endcapping: Yes Carbon content: 10%	Phenyl silica has unique retention for aromatic compounds. Therefore Phenyl silica is dedicated to the separation of the compounds with aromatic rings, including peptides and proteins.	Flush the cartridge with 3 column volumes of 80% acetonitrile in water or 80% methanol in water. Store in flush solvent with well-capped ends.
Phenyl-Hexyl		Spherical, 20-45 μm Endcapping: Yes Carbon content: 10%	Compared with Phenyl silica, Phenyl-Hexyl silica is more hydrophobic. Phenyl-Hexyl silica could be used for the separation of aromatic compounds while C4 or C18 silica shows poor resolution.	Flush the cartridge with 3 column volumes of 80% acetonitrile in water or 80% methanol in water. Store in flush solvent with well-capped ends.

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