

# The Application of SepaFlash HILIC Cartridges in Synthetic Intermediates Purification

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## Abstract

The compounds of strong polarity or hydrophilicity could barely be retained in reversed-phase chromatography. In the meantime they could hardly be dissolved in the mobile phase of normal phase chromatography. Therefore HILIC cartridges were employed for the separation and purification of these samples with specific characteristics. In the application note, a 40g SepaFlash HILIC flash cartridge (as shown in Figure 1) was utilized to purify strong hydrophilic samples. As a comparison, a 40g SepaFlash C18 flash cartridge was also used in a parallel experiment. The results showed that SepaFlash HILIC flash cartridge is a good choice for the separation and purification of samples with strong polarity or hydrophilicity.

## Experiment

The cartridges employed in this application were a 40g SepaFlash™ HILIC flash cartridge as well as a 40g SepaFlash™ C18 flash cartridge. The chemical structure of the stationary phases in these two flash cartridges was shown in Figure 1.

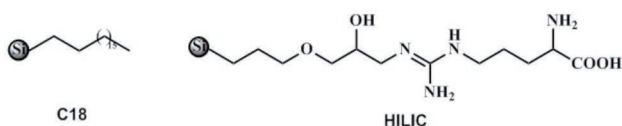


Figure 1. The stationary phases of the two SepaFlash™ flash cartridges.

The sample used in the experiment was a mixture of product and impurities from a synthetic procedure (as shown in Figure 2). The experiments were performed on a flash preparative chromatography system under the same sample loading conditions. The experimental parameters were summarized in Table 1.

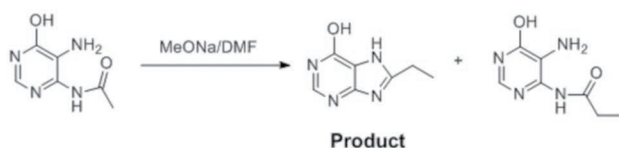


Figure 2. The chemical structure of the sample mixture.

Instrumentation	A flash preparative chromatography system			
Cartridges	40g SepaFlash™ HILIC flash cartridge (Order number: ) 40g SepaFlash™ C18 flash cartridge (Order number: SW-5222-040-SP)			
Wavelength	254 nm (detection), 280 nm (monitoring)			
Mobile phase	Solvent A: water Solvent B: acetonitrile			
Flow rate	30 ml/min			
Loading capacity	100 mg of the mixture			
Gradient	Settings for C18 cartridge		Settings for HILIC cartridge	
	Solvent B (%)	Time (min)	Solvent B (%)	Time (min)
	5	0	95	0
	5	3	95	15
	22	9	50	17
22	12	/	/	

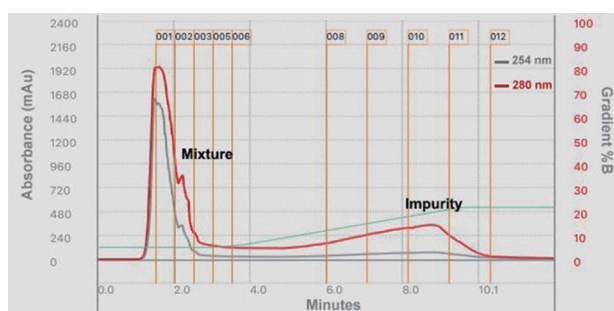


Figure 3. The chromatogram of the sample mixture in C18 flash cartridge.

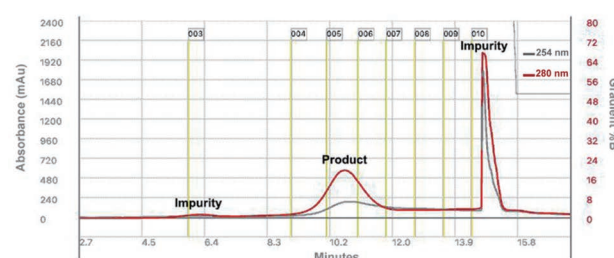


Figure 4. The chromatogram of the sample mixture in HILIC flash cartridge.

## Results and Discussion

As shown in Figure 3, the sample of strong hydrophilicity has poor retention time and bad peak shape when separated by the C18 flash cartridge. The peaks of the product and one of the impurities were merged as a broad peak. In contrast, the chromatogram of HILIC flash cartridge (as shown in Figure 4) clearly showed that the product was successfully separated from two impurities while the peak shape was greatly improved comparing with

the results from C18 flash cartridge. By comparing the separation results of C18 and HILIC cartridges, it was found that for the compounds of strong polarity, the HILIC cartridges could offer adequate retention time and higher peak resolution. On the contrary, these compounds have poor retention as well as possible strong irreversible adsorption on the traditional C18 reversed-phase cartridges, making SepaFlash HILIC cartridges a good choice for the separation and purification of polar or hydrophilic samples.

### Suggestions for Using HILIC Cartridges

HILIC is the abbreviation for hydrophilic interaction liquid chromatography. To some extent, the elution sequence in HILIC separation mode is the same with normal phase chromatography. It means that the compounds of weak polarity are eluted preferentially from the column, while strong polar compounds have stronger retention on the column and are eluted later than weak polar ones.

#### The Choice of the Mobile Phases

In the HILIC separation mode, the order of eluent strength is as follows: tetrahydrofuran (THF) < acetone < acetonitrile (ACN) < propanol < methanol (MeOH) < water. The eluent strength increases with increasing solvent polarity. The most popular solvent system is the ACN-water system, which always has good peak resolution and decent peak shape when being used in HILIC cartridges. At least keep 5% polar solvent in the mobile phase, e.g. 5% water or 3% MeOH + 2% water, to ensure the packing materials in HILIC cartridge are always wetted by water.

Always keep the percentage of organic solvent in mobile phase not lower than 40% when running gradient elution. For example, for ACN-water system, keep the percentage of ACN in mobile phase not lower than 40%.

#### Recommendation for General Eluting Gradient Profile

When handling with a new sample, the recommended eluting gradient profile is shown in Table 2. If the sample could be properly retained and then eluted from the column, further optimization on other parameters (e.g. pH, ionic strength, buffer concentration, etc.) could be performed.

**Table 2. General eluting gradient profile.**

	% ACN in water	Time (min)
Gradient	90	0
	90	2.5
	50	10
	50	13

#### Cleaning and regeneration of the HILIC cartridge

When separating samples by HILIC cartridges, if chromatographic resolution is deteriorated, or peak shape broadens, or column pressure goes higher or other abnormal situation occurs, it is probably due to some dirty stuff of strong retention to the stationary phase of the cartridge which changes the column capacity so that the interaction between the sample and the stationary phase is compromised. In this circumstance, the cartridge should be thoroughly cleaned and maintained.

##### •Cleaning

To remove polar contaminant, flush the HILIC cartridge with 50% ACN in water at low flow rate for 30 column volumes (CV). In case of no effect, a solvent consisting of 5% ACN in water should be used for washing the cartridge. Then the percentage of ACN in water should be smoothly adjusted to 95% and further test should be conducted.

##### •Preservation

The HILIC cartridge should be preserved in the solvent consisting of 95% ACN in water. NEVER preserve the cartridge in the mobile phase with buffer salts. If mobile phase with buffer salts was used, deionized water should be employed to wash the cartridge for 10 CVs and then replaced by 95% ACN in water for cartridge preservation.

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