The Purification of Hydrophilic Compounds by SepaFlash™ HILIC Cartridges

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Chromatography Application Note
AN001

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Abstract

Reverse Phase Chromatography (RPC) is the first choice in separation modes for regular samples. It is more universal compared with other separation modes. RPC has become the most widely employed purification method due to its satisfying resolution power. However, RPC cannot be used for separating hydrophilic compounds, which can hardly be retained by reversed-phase columns. The SepaFlash™ Hilic flash cartridge is a good choice for separating these strong polar and hydrophilic compounds due to its bonded phase with hydrophilic groups (as shown in Figure1).

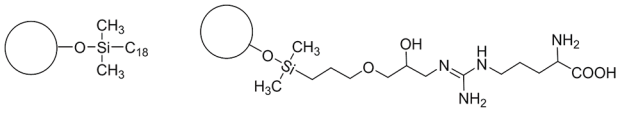

Figure 1. The stationary phases of the two SepaFlash™ flash cartridges.

The SepaFlash™ Hilic flash cartridges from Santai Technologies Inc. combined with a flash preparative chromatography system shows superior performance in separation. This application concerns the purification of a mixture of two strong polar compounds by the SepaFlash™ Hilic flash cartridge as well as the SepaFlash™ C18 flash cartridge in reversed-phase mode. The results indicate that the SepaFlash™ Hilic flash cartridge has better resolution power than the C18 ones, providing a set of efficient and practical solutions for the separation and purification of strong polar compounds.

Experiment

The cartridges employed in this application were a 25g SepaFlash™ Hilic flash cartridge as well as a 25g SepaFlash™ C18 flash cartridge. The sample was a mixture of cytosine and vitamin C (as shown in Figure 2). The experiments were performed on a flash preparative chromatography system under the same sample loading conditions. The results clearly showed the differences in selectivity for these two cartridges.

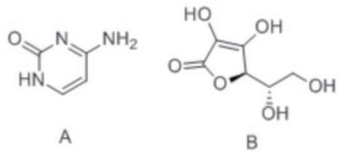

Figure 2. The structural formula of two strong polar compounds. (A: cytosine. B: vitamin C.)

The experimental parameters were summarized in Table 1.

Table 1. The experimental parameters.

Instrument	A flash preparative chromatography system	
Cartridges	25g SepaFlash™ Hilic flash cartridge 25g SepaFlash™ C18 flash cartridge (Order number: SW-5222-025-SP)	
Wavelength	220 nm, 254 nm	
Mobile phase*	A: 200 mM ammonium acetate (pH 9.0) in H2O B: acetonitrile	
Flow rate	15 ml/min	
Loading capacity	10 mg of the mixture	
Gradient	% B	Time / (min)
	95	0
	95	5
	80	10
	70	13
	70	30

*Note:The eluting condition for 25g SepaFlash™ C18 Cartridge is 0.1% trifluoroacetic acid in H2O/acetonitrile (19:1, V/V) with isocratic gradient.

Results and Discussion

According to the chromatogram (as shown in Figure 3 and Figure 4), we could accurately obtain the retention time and peak width of the two components. Next, the peak resolution could be calculated by the following formula:

$$R = 1.18\,(t_2 - t_1) / [(W_{1/2})_1 + (W_{1/2})_2]$$

where t_1 and t_2 were the retention time of the two spectrum peaks, respectively, and $(W_{1/2})_1$ and $(W_{1/2})_2$ were the corresponding peak width measured at the half peak height.

Based on the above mentioned calculating method, the peak resolution for the SepaFlash™ C18 flash cartridge was about 1.53. For the SepaFlash™ Hilic

flash cartridge the peak resolution was approximately 5.97. The results showed that the resolution power of the SepaFlash™ Hilic flash cartridge was much better than the SepaFlash™ C18 flash cartridge while handling with strong polar compounds.

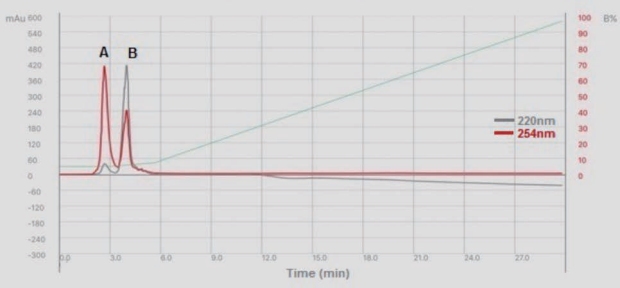

Figure 3. Separation of the sample using a SepaFlash™ C18 flash cartridge.

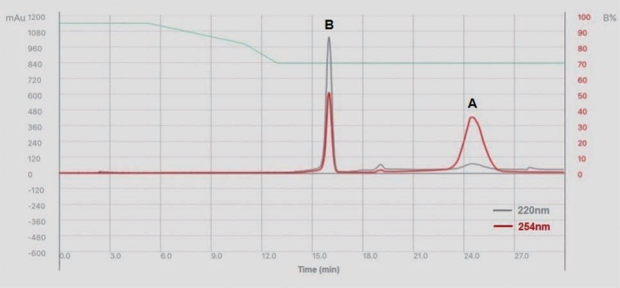

Figure 4. Separation of the sample using a SepaFlash™ Hilic flash cartridge.

The experimental results showed that the mixture of cytosine and vitamin C was successfully retained and separated on the SepaFlash™ Hilic flash cartridge, suggesting its excellent selectivity for these compounds. Furthermore, the two compounds of strong polarity were merely retained and not baseline separated on the SepaFlash™ C18 flash cartridge, even though eluting with the mobile phase of acetonitrile in 95% H2O. The reason for these results could be that the stationary phases bonded with C18 do not have enough polar interactions with the sample, while the hydrophilic groups of HILIC cartridge showing good interactions with the compounds of strong polarity. Therefore, this application note provided us a set of efficient and practical solutions for the separation and purification of strong polar compounds.

About SepaFlash™ Hydrophilic Chromatography Columns

SepaFlash™ hydrophilic cartridges include a series of cartridges pre-packed with different materials, such as Hilic cartridges, cyano cartridges (-CN), amino cartridges (-NH2), diol cartridges (-DiOH), and etc (as shown in Table 2 and Figure 5). These cartridges provide us multiple options for separating and purifying strong polar or hydrophilic compounds.

Table 2. Hydrophilic Cartridge Series*

Spherical Silica Matrix	• Spherical Hilic, Particle size: 20 – 45 μm, Pore size: 100 Å
	• Spherical CN, Particle size: 20 – 45 μm, Pore size: 100 Å
	• Spherical NH2, Particle size: 20 – 45 μm, Pore size: 100 Å
	• Spherical DiOH, Particle size: 20 – 45 μm, Pore size: 100 Å

*Notes:Please check the ordering information on the company' website for specific products.

Figure 5. SepaFlash™ hydrophilic cartridges.

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