SepaFlash HILIC ARG Cartridge and Its Application in the Purification of Amino Acid Derivatives

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Chromatography Application Note AN033

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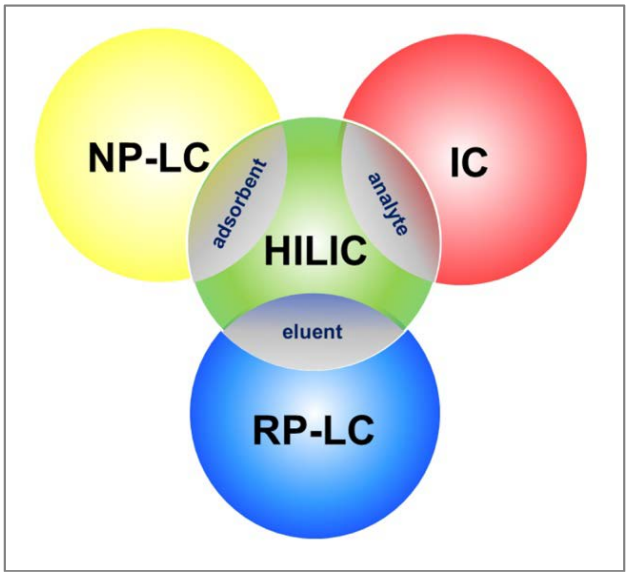

Introduction

Reversed phase liquid chromatography (RPLC) is by far the most widely used liquid chromatography separation technology. RPLC plays a leading role in the separation and purification of moderately polar or some highly polar compounds. Alkyl bonded phase LC columns, such as C18, C8, C4, etc. are usually the first choice for separating these compounds. With the rapid development of biomedicine, scientific researchers are presenting increasing demand on the separation of highly polar compounds, including small molecule organic acids, amino acids and their derivatives, nucleic acids, peptides and etc. However, conventional LC columns with alkyl bonded phase have poor retention for these highly polar samples. Considering this situation, numerous brands of manufacturers have introduced hydrophilic interaction chromatography (HILIC) columns that can improve the retention of highly polar compounds. For instance, there are plenty of LC columns used for analytical area, including InfinityLab Poroshell 120 HILIC series and Zorbax HILIC series from Agilent, Atlantis HILIC series and XBridge HILIC series from Waters, Accucor HILIC series and Syncronis HILIC series from Thermo Fisher, etc.

In Flash chromatography, SepaFlash HILIC ARG cartridge is specifically developed by Santai Technologies to improve the retention for compounds of high polarity. With the bonding of arginine molecules to the surface of silica, the interaction between the stationary phase surface and the molecules of high polarity is enhanced, thereby the retention of such molecules on the cartridge is improved. The retention mechanism in HILIC is a multi-mode one mixed with liquid-liquid distribution, ion exchange and hydrogen bonding. The schematic diagram of the separation mechanism in HILIC is shown in Figure 1. Same as conventional RPLC, the mobile phase used in HILIC is usually water/acetonitrile system or water/methanol system, except that the elution order in HILIC is exactly the opposite of RPLC. In RPLC, organic phases such as acetonitrile or methanol are solvents with higher elutropic strength. In contrast, organic phase becomes the weaker one in HILIC, meanwhile water becomes the solvent with the highest elutropic strength. For example, when setting the eluting gradient profile in HILIC mode, we usually start with acetonitrile/water (V : V = 95 : 5) and water ratio is increased during the separating procedure to elute out the highly polar samples gradually.

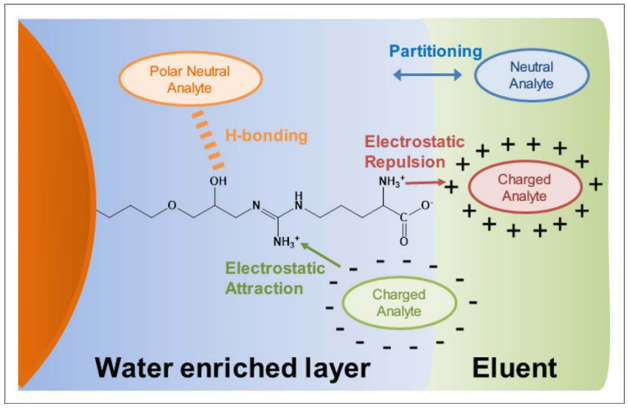

Figure 1. The schematic diagram of the separation mechanism in HILIC mode.

In this post, the sample is an amino acid derivative kindly provided by a synthetic lab from a pharmaceutical R&D company. The sample has high polarity and good solubility in water. However, the sample had poor retention on the hydrophilic C18AQ cartridge. For the analysis of raw sample, analytical amino column combined with buffer salt solution as the mobile phase was used. Considering the challenge of buffer salt removal in post separation sample process, HILIC ARG cartridge was used for the sample purification. The application engineers from Santai Technologies utilized SepaFlash HILIC ARG cartridge in combination with a Flash chromatography system SepaBean machine T to purify the sample. As a result, the target product meeting the purity requirement were successfully obtained, suggesting an efficient and cost effective solution for the purification of these highly polar compounds.

Experimental Section

1.Sample information

The sample used in this application was kindly provided by a pharmaceutical R&D company. The sample was an amino acid derivative that was highly polar and has a good solubility in water. The chemical structure cannot be obtained due to confidential reasons.

2.Sample purification by Flash chromatography

The sample was purified by a Flash chromatography system SepaBean machine T according to the parameters as shown in Table 1.

Instrument	SepaBean™ machine T			
Flash Cartridge	12 g SepaFlash C18AQ reversed phase cartridge (spherical silica, 20 - 45 μm, 100 Å, Order number:SW-5222-120-SP(AQ))		12 g SepaFlash HILIC ARG cartridge (spherical silica, 20 - 45 μm, 100 Å, Order number:SW-5622-012-SP)	
Wavelength	220 nm; 254 nm			
Mobile phase	Solvent A: Water; Solvent B: Acetonitrile			
Flow rate	15 mL/min			
Sample loading	0.5 mL (50 mg)			
Gradient	Time (min)	Solvent B (%)	Time (min)	Solvent B (%)
	0	0	0	95
	8.5	0	2.0	95
	12.5	17	14.0	78
	12.6	37	17.5	78
	17.3	80	20.0	62
	21.0	80	25.0	62

Table 1. The experimental setup for Flash purification.

Results and Discussion

According to previous experience of the purification of these highly polar samples, we used an aqueous C18AQ cartridge as a start to purify the sample. The Flash chromatogram of the sample was shown in Figure 2.

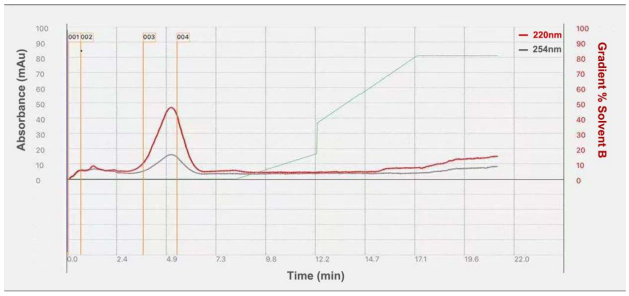

Figure 2. The Flash chromatogram of the sample on a C18AQ cartridge.

As shown in Figure 2, the sample could not be well retained on the C18AQ cartridge. The sample was eluted out from the cartridge by the mobile phase in about one column volume (CV), resulting poor separation between the target product and the impurities in raw sample. The reason behind this might be the existence of a large number of polar groups in the sample structure, such as Serine, Threonine, Asparagine, Glutamine, etc (Figure 3). On the other hand, there might be plenty of electrically charged groups in the sample structure, including Aspartate, Glutamate, Lysine, Arginine, Histidine, etc. These polar or electrically charged groups cannot have enough hydrophobic interactions with the hydrophobic C18 carbon chains, resulting poor retention of the sample on the C18AQ cartridge.

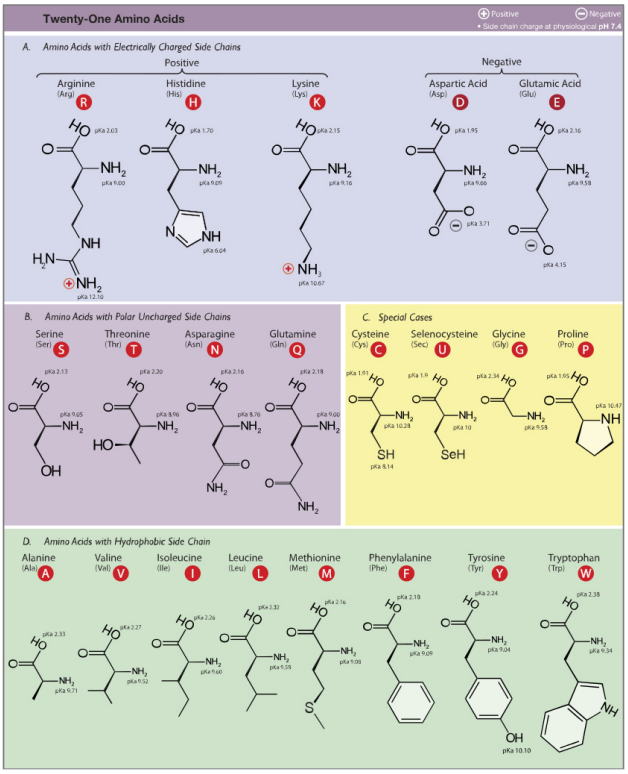

Figure 3. The chemical structure of twenty-one amino acids (reproduced from Wikipedia).

Based on the previous experimental results, we tried with a SepaFlash HILIC ARG cartridge to purify the sample. The Flash chromatogram was shown in Figure 4.

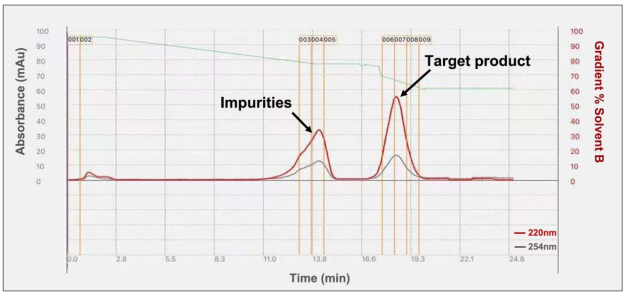

Figure 4. The Flash chromatogram of the sample on a HILIC ARG cartridge.

As shown in Figure 4, the sample could be well retained on the SepaFlash HILIC ARG cartridge. With the help of mixed separation mode in HILIC, the components in the sample have different retention time on the cartridge due to their different polarity or electric charge state. The resolution for the target product and the impurities in the raw sample were good. After post-separation process of the collected fractions, the target product meeting the purity requirement was obtained.

Conclusion

For the separation and purification of the highly polar amino acid derivative samples, HILIC ARG cartridge is a good choice due to its multi-mode separation mechanism, including liquid-liquid partitioning, ion exchange and hydrogen bonding.

User Guide for the SepaFlash HILIC ARG Cartridges

1.The Choice of Mobile Phase

As a start, water/acetonitrile is recommended as the mobile phase in HILIC mode. To keep the stationary phase in wet condition, at least 5% of the water phase should be maintained in the mobile phase. Furthermore, the water phase should not exceed 50% in the mobile phase when setting the elution gradient profile. As a replacement for acetonitrile in order to gain more selectivity for the analytes, other organic solvents including methanol, acetone, isopropanol, etc. could be considered.

2.The Choice of Buffer Salt

When buffer salt is needed as an additive to the mobile phase, it is suggested that avoid using phosphate buffer salts since these salts have poor solubility in organic solvents and might induce salt precipitation during separation, thus resulting over pressure in chromatography system. As a recommendation, the ammonium salts including ammonium acetate and ammonium formate have good performance in reproducibility and maintaining good peak shape. Moreover, ammonium salts are easy to remove when purified sample needs to be further processed. The suggested concentration for the ammonium salts as an additive to the mobile phase is 10 mM.

3.The Choice of Sample Dissolution Solvents

Avoid using pure water to dissolve the sample when using a HILIC cartridge. It is recommended to use 100% organic solvents as the sample dissolution solvent, including acetonitrile, methanol, isopropanol, etc. If the sample cannot be completely dissolved in the above mentioned organic solvents, add water or DMSO appropriately to dissolve the sample and then dilute with those organic solvents.

4.Cleaning and Storage of HILIC Cartridges

Cleaning: Use acetonitrile/water (V:V=50:50) as the cleaning solvent. If there are some components strongly retained on the cartridge, use acetonitrile/water (V:V=5:95) to thoroughly clean the cartridge and then replace with acetonitrile/water (V:V=95:5) for cartridge storage.

Storage: Use acetonitrile/water (V:V=95:5) as the cartridge storage solvent. Do not store the cartridge in solvents containing buffer salts. Store the cartridge well-capped and wet in proper storage solvent. Never dry out the HILIC cartridge since it will induce channeling due to expansion and contraction of the stationary phase.

About SepaFlash HILIC ARG Cartridges

There are a series of the SepaFlash HILIC ARG cartridges with different specifications from Santai Technologies (as shown in Table 2).

Item Number	Column Size	Sample Size	Max.Pressure (psi/bar)
SW-5622-004-SP	5.4 g	5.4 mg – 108 mg	400/27.5
SW-5622-012-SP	20 g	20 mg – 0.40 g	400/27.5
SW-5622-025-SP	33 g	33g – 0.66 g	400/27.5
SW-5622-040-SP	48 g	48 mg – 0.96 g	400/27.5
SW-5622-080-SP	105 g	105 mg – 2.1 g	350/24.0
SW-5622-120-SP	155 g	155 mg – 3.1 g	300/20.7
SW-5622-220-SP	300 g	300 mg – 6.0 g	300/20.7
SW-5622-330-SP	420 g	420 mg – 8.4 g	250/17.2

Table 2. SepaFlashTM HILIC ARG flash cartridges. Packing materials: High-efficiency spherical ARG-bonded silica, 20 - 45 μm, 100 Å.

For further information on detailed specifications of SepaBean machine, or the ordering information on SepaFlash series flash cartridges, please visit our website: www.santaitech.com/index/.

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