SepaFlashTM C18AQ Cartridge and Its Application in the Purification of Glutamine Derivatives

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Chromatography Application Note
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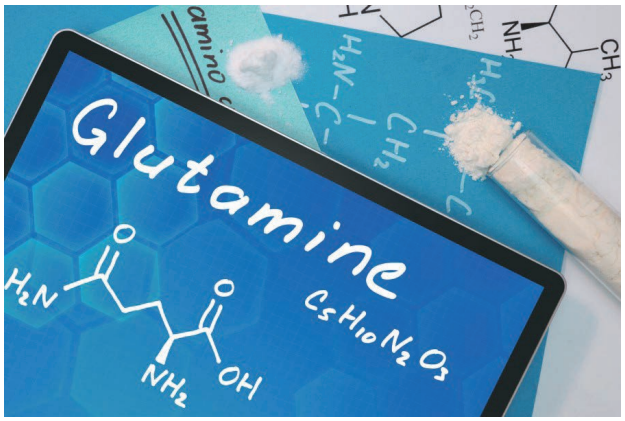

Introduction

Glutamine, or 2-amino-4-carbamoylbutanoic acid, has a molecular formula of C5H10N2O3 and a molecular mass of 146.16 g/mol. It is a polar and uncharged derivative of acidic amino acid glutamic acid or glutamate. It has a carboxamide group, which is neutral at physiological pH and can be changed to carboxylic acid by hydrolysis to form glutamate amino acid. The carboxamide group of the amino acid can form hydrogen bonds. Glutamine is a non-essential amino acid which can be synthesized by the human body itself and does not need to be obtained from external source [1]. Glutamine is one of the most abundant amino acid in the human body [2], and it can directly cross the blood-brain barrier through blood circulation in the human body. Glutamine has multiple functions in biochemistry. In addition to participating in protein synthesis, it can also help maintain the neutral pH of the liver by balancing acid-base levels [3]. Similar to glucose, glutamine can provide energy to the cell body [4]. It provides nitrogen to cells through anabolic reactions [5] and provides carbon in the citric acid cycle [6]. In the gastrointestinal system, glutamine is an important source of energy for small bowel movements [7].

In the research of anticancer drugs, cancer cells will sometimes exhibit what is called “glutamine addiction”, which has become a potential target for new anticancer therapies [8]. However, since glutamine is essential for many physiological processes in the human body, such as synaptic communication in the brain, it is not a viable treatment and very dangerous to simply remove glutamine from the human body. Therefore, researchers are now focusing their efforts on the protein targets associated with the glutamine metabolic pathway [9, 10]. In clinical nutrition studies, glutamine and its derivatives have demonstrated multiple efficacies in the treatment of various diseases including trauma, infection, critically ill patients, bone marrow transplantation, small bowel transplantation, etc. Its role includes maintaining glutamine concentration in skeletal muscle, improving nitrogen balance, promoting protein synthesis, avoiding intestinal mucosal atrophy caused by trauma, reducing intestinal mucositis caused by chemotherapy, improving human immunity, etc. [11] In conclusion, glutamine derivatives are now attracting more and more attentions from researchers in clinical nutrition research.

In this post, the sample used was a highly polar glutamine derivative which cannot be dissolved in regular organic solvents such as n-hexane, ethyl acetate, etc. The sample can barely retain on regular reversed phase C18 cartridge. Considering the specific sample properties, the application engineers from Santai Technologies utilized a hydrophilic SepaFlash™ C18AQ cartridge combining with a flash chromatography system SepaBean™ machine for the sample purification. As a result, the target product meeting the purity requirement was obtained, suggesting a feasible solution for the fast purification of highly polar glutamine derivative samples.

Experimental Section

The sample used in this application was a glutamine derivative which was kindly provided by a pharmaceutical company. The chemical structure of the sample molecule is shown in Figure 1. The purity of the raw sample is about 73% by HPLC as shown in Figure 2.

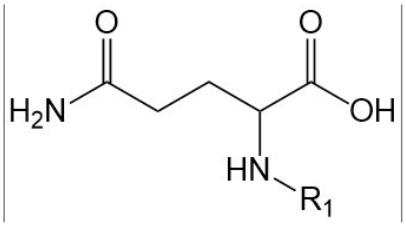

Figure 1. The chemical structure of glutamine derivative sample.

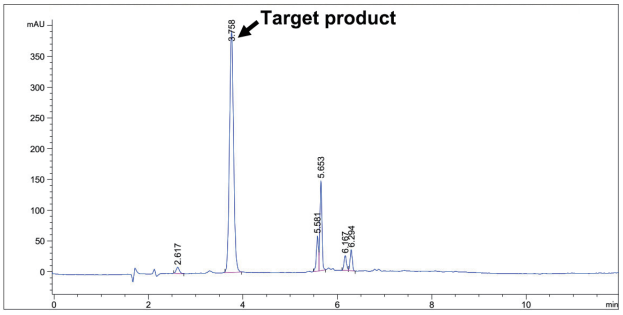

Figure 2. The HPLC chromatogram of the raw sample.

To prepare the sample solution, 1.5 g of the raw sample was dissolved in 1 mL DMSO and sonicated to get a clear and transparent solution. The sample solution was then loaded onto a flash cartridge by a liquid injector. The experimental setup of flash chromatography for the sample was listed in Table 1.

Instrument	SepaBean™ machine T			
Cartridges	12 g SepaFlash™ Bonded Series C18 cartridge (spherical silica, 20 - 45 μm, 100 Å, Order number:SW-5222-012-SP)		120 g SepaFlash™ Bonded Series C18AQ cartridge (spherical silica, 20 - 45 μm, 100 Å, Order number:SW-5222-120-SP(AQ))	
Wavelength	220 nm, 254 nm			
Mobile phase	Solvent A: Water Solvent B: Acetonitrile			
Flow rate	25 mL/min		40 mL/min	
Sample loading	300 mg		1.2 g	
Gradient	Time (min)	Solvent B (%)	Time (min)	Solvent B (%)
	0	0	0	0
	15	20	10.0	0
	/	/	12.0	2.0
			16.0	2.0
			17.5	95
			30.0	95

Table 1. The experimental setup for flash purification.

Results and Discussion

As a start, a regular C18 cartridge was used for the purification of small amount of the sample. The flash chromatogram of the sample by a regular C18 cartridge was shown in Figure 3.

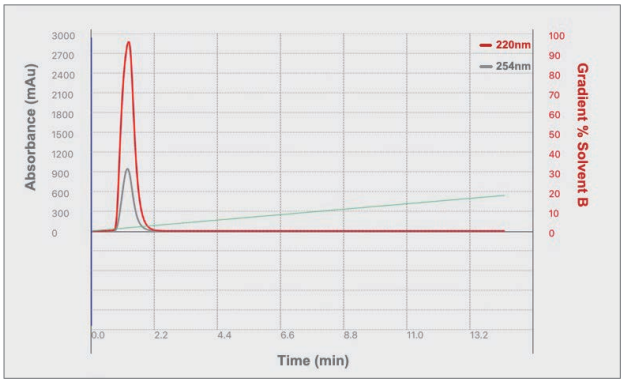

Figure 3. The flash chromatogram of the sample by a regular C18 cartridge.

As shown Figure 3, the sample had almost no retention on the regular C18 Cartridge. The sample was directly eluted out from the cartridge by the mobile phase with DMSO in the sample solution. So the target product was not effectively separated with the impurities in the raw sample. For this result, we believe that the reason can be attributed to the hydrophobic collapse of the stationary phase. As well known to all, the commonly used elution solvents in reversed phase chromatography can be ordered according to their elutropic strength: water < methanol < acetonitrile < ethanol < tetrahydrofuran < isopropanol. To assure good retention on the column for those samples which are highly polar or hydrophilic, high proportion of aqueous system is necessary to be used as the mobile phase. However, when using pure water system (including pure water or pure salt solution) as the mobile phase, the long carbon chain bonded on the stationary phase of C18 column tends to avoid the water and mix with each other, resulting in an instantaneous decrease in the retention capacity of the column or even no retention. This phenomenon is called “hydrophobic phase collapse” (as shown in the left part of Figure 4). Though this situation is reversible when the column is washed with organic solvents such as methanol or acetonitrile, it still can cause damage to the column. Therefore, it is necessary to prevent this situation from happening.

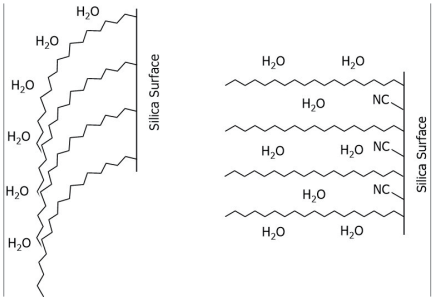

Figure 4. The schematic diagram of the bonded phases on the surface of silica gel in regular C18 column (left) and C18AQ column (right).

To address the above mentioned problems, the chromatographic packing materials manufacturers have made technical improvements. One of these improvements is making some modifications on the surface of the silica matrix, such as the introduction of hydrophilic cyano groups (as shown in the right part of Figure 4), to make the surface of the silica gel more hydrophilic. Thus the C18 chains on the silica surface could be fully extended under highly aqueous conditions and the hydrophobic phase collapse could be avoided. These modified C18 columns are called aqueous C18 columns, namely C18AQ columns, which are designed for highly aqueous elution conditions and can tolerate 100% aqueous system. C18AQ columns have been widely applied in the separation and purification of strong polar compounds, including organic acids, peptides, nucleosides and water-soluble vitamins.

In the following part, a C18AQ cartridge was used for the purification of the raw sample. The flash chromatogram of the sample by a C18AQ cartridge was shown in Figure 5. As shown in Figure 5 we can see that the glutamine derivative sample was well retained on the C18AQ cartridge and effectively purified from other impurities in the sample. After lyophilization of the collected fractions, the purity of the target product exceeded 98% as analyzed by HPLC (as shown in Figure 6). The target product could be utilized for next step research and development.

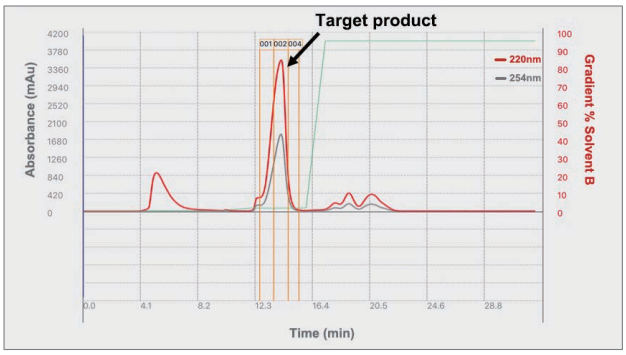

Figure 5. The flash chromatogram of the sample by a C18AQ cartridge.

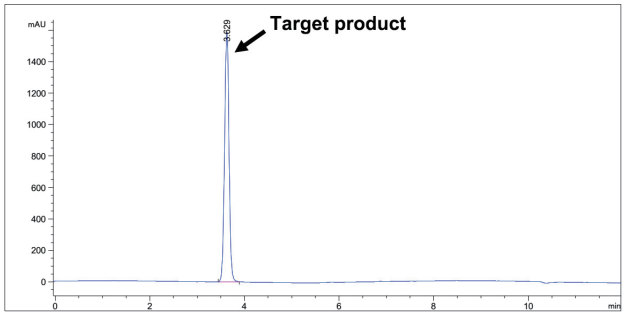

Figure 6. The HPLC chromatogram of the purified target product.

Conclusion

Due to the hydrophobic phase collapse, the application of regular C18 cartridge for the purification of highly polar glutamine derivative samples was strictly limited. In contrast, the improved C18AQ cartridge could overcome the above mentioned effect and was successfully applied for the purification of such kind of samples in this post. In conclusion, for the purification of glutamine derivative samples which have strong polarity, combining SepaFlash™ C18AQ cartridge with a flash chromatography system SepaBean™ machine is an effective and feasible solution.

About the SepaFlash® Bonded Series C18AQ flash cartridges

There are a series of the SepaFlash® C18AQ flash cartridges with different specifications from Santai Technology (as shown in Table 2).

Item Number	Column Size	Flow Rate (mL/min)	Max.Pressure (psi/bar)
SW-5222-004-SP(AQ)	5.4 g	5-15	400/27.5
SW-5222-012-SP(AQ)	20 g	10-25	400/27.5
SW-5222-025-SP(AQ)	33 g	10-25	400/27.5
SW-5222-040-SP(AQ)	48 g	15-30	400/27.5
SW-5222-080-SP(AQ)	105 g	25-50	350/24.0
SW-5222-120-SP(AQ)	155 g	30-60	300/20.7
SW-5222-220-SP(AQ)	300 g	40-80	300/20.7
SW-5222-330-SP(AQ)	420 g	40-80	250/17.2

Table 2. SepaFlash® C18AQ RP flash cartridges. Packing materials: High-efficiency spherical C18(AQ)-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: http://www.santaitech.com/index/.

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