The Separation and Purification of Glutamine Derivatives by C18AQ Reversed Phase

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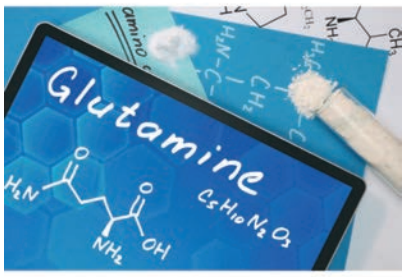
The separation and purification of a polar, uncharged amino acid derivatives that are insoluble in organic solvents seems an impossible mission for purification by traditional normal phase flash chromatography. Due to hydrophobic phase collapse, regular C18 reversed phase might not suitable for the purification of these samples either. This article investigates an alternative aqueous C18 reversed phase and found it could offer good retention for these samples, suggesting a satisfactory choice for the separation and purification of such samples.



Glutamine, or 2-amino-4-carbamoylbutanoic acid, has a molecular formula of $C_5H_{10}N_2O_3$ and a molecular mass of 146.16 g/mol. It is a polar and uncharged derivative of acidic amino acid glutamic acid or glutamate with a carboxamide group, which is neutral at physiological pH and can be changed to a 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 synthesised by the human body itself and does not need to be obtained from external sources [1]. Glutamine is one of the most abundant amino acids in the human body [2], and it can cross the blood-brain barrier directly through blood circulation. 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 work, 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 be retained on regular reversed phase C18 cartridges. Considering the specific sample properties, the application engineers from Santai Technologies utilised a hydrophilic SepaFlash™ C18AQ cartridge combined with a SepaBean™ flash chromatography system for the sample purification. As a result, the target product which met the purity requirement was obtained, suggesting a feasible solution for the fast purification of highly polar glutamine derivative samples.

Experimental Section

The glutamine derivative sample used in this application 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 was about 73% by HPLC as shown in *Figure 2*.

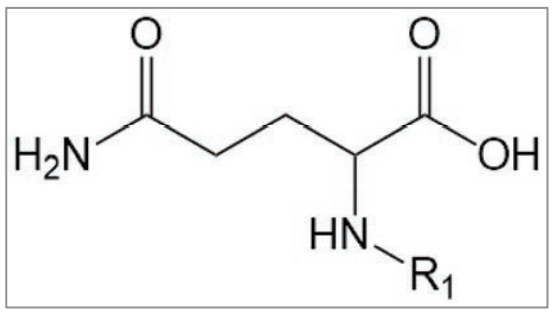


Figure 1. The chemical structure of glutamine derivative sample.

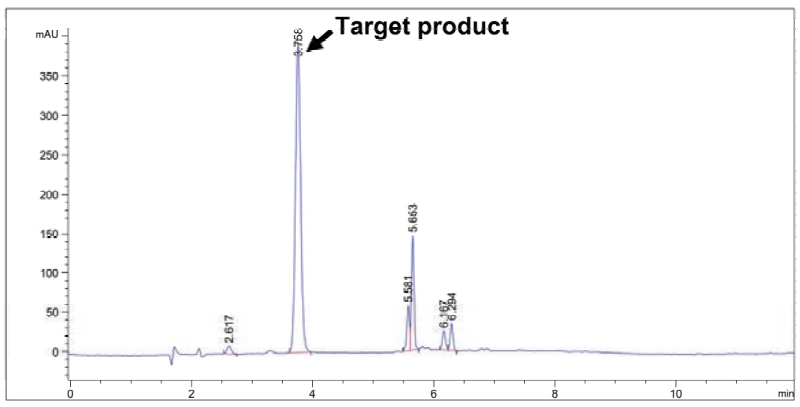


Figure 2. The HPLC chromatogram of the raw sample.

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 for flash chromatography of the sample is listed in *Table 1*.

Table 1. The experimental setup for flash purification.

Instrument	SepaBean™ machine T	
Flash cartridge	12 g SepaFlash™ Bonded Series C18 cartridge (spherical silica, 20 - 45 μm, 100 Å)	120 g SepaFlash™ Bonded Series C18AQ cartridge (spherical silica, 20 - 45 μm, 100 Å)
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

	Time (min)	Solvent B (%)	Time (min)	Solvent B (%)
Gradient	0	0	0	0
	15	20	10.0	0
	/	/	12.0	2.0
	/	/	16.0	2.0
	/	/	17.5	95
	/	/	30.0	95

Results and Discussion

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

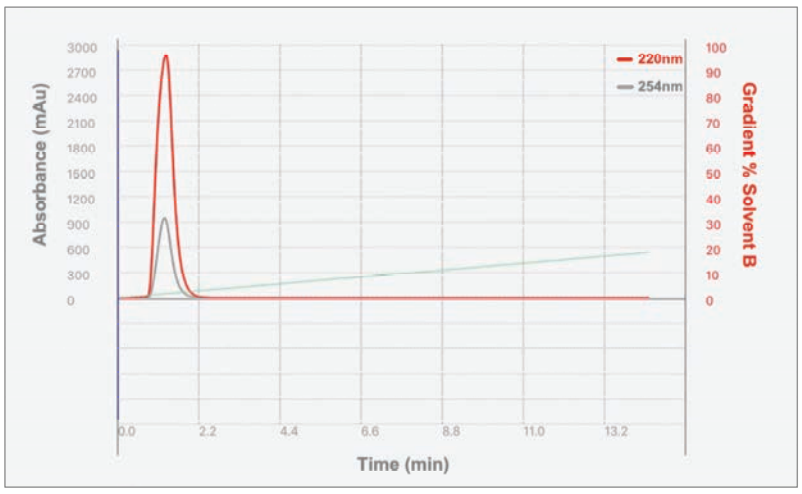


Figure 3. The flash chromatogram of the sample by a regular C18 cartridge.

As shown in *Figure 3*, the sample had almost no retention on the regular C18 Cartridge. The sample was eluted immediately from the cartridge by the mobile phase employing DMSO as the sample solution. So the target product was not effectively separated from the impurities in the raw sample. It was believed from this result that the reason for no retention was due to hydrophobic phase collapse of the C18 stationary phase. 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, and subsequent separation, for those samples which are highly polar or hydrophilic, high proportions of aqueous mobile phase are required in the. However, when using a 100% aqueous 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 can still cause damage to the column. Therefore, it is necessary to prevent this situation from happening.

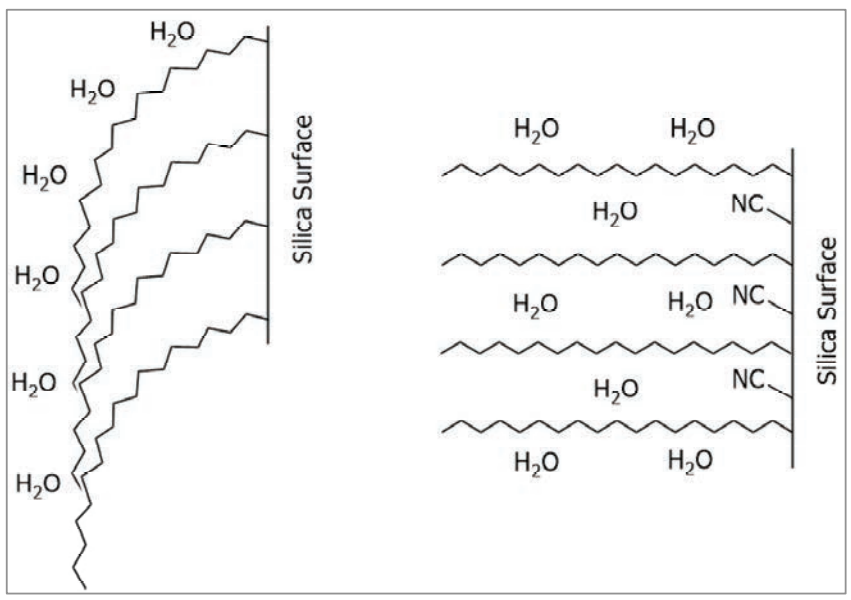


Figure 4. The schematic diagram of the bonded phases on the surface of silica gel in regular C18 column (left) and C18AQ column (right).

To address the above mentioned problem, the chromatographic packing materials manufacturers have made technical improvements. One of these improvements is making modifications to the surface of the silica matrix, such as the introduction of hydrophilic cyano groups (as shown in the right part of *Figure 4*), making the surface of the silica gel more hydrophilic. Therefore the C18 chains when secondarily bonded onto the silica surface remain fully extended under highly aqueous conditions and hydrophobic phase collapse can 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.

A C18AQ cartridge was then used for the purification of the raw sample. The flash chromatogram of the sample by a C18AQ cartridge is shown in *Figure 5* where it can be seen that the glutamine derivative sample was well retained on the C18AQ cartridge and effectively purified from other impurities in the sample. After lyophilisation of the collected fractions, the purity of the target product exceeded 98% as analysed by HPLC (as shown in *Figure 6*). The target product could be utilised for next step research and development.

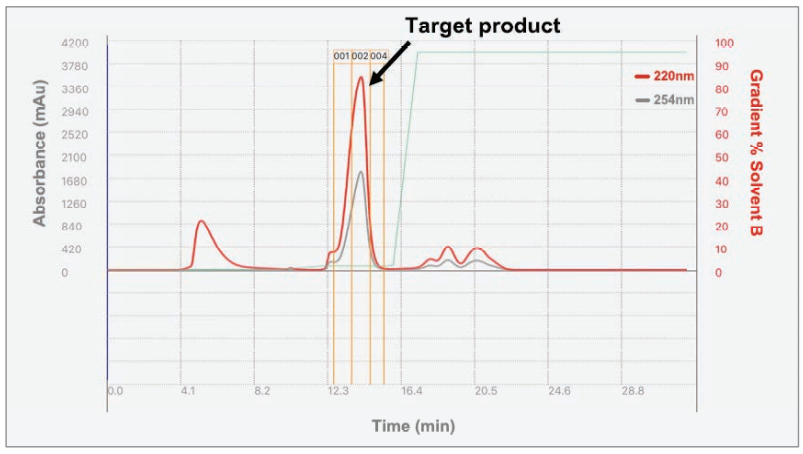


Figure 5. The flash chromatogram of the sample by a C18AQ cartridge.

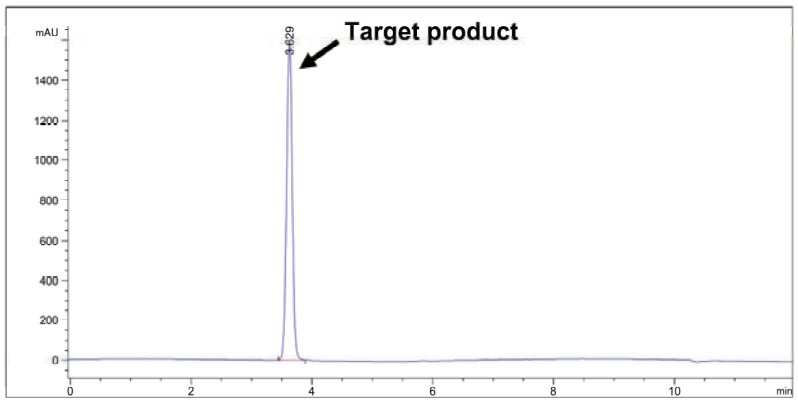


Figure 6. The HPLC chromatogram of the purified target product.

Conclusion

Due to hydrophobic phase collapse, the application of regular C18 flash cartridges for the purification of highly polar glutamine derivative samples was strictly limited. In contrast, the improved C18AQ cartridge overcame the above mentioned effect and was successfully applied for the purification of the samples in this article. 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.

References

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