The Purification of Highly Polar Impurities in Antibiotics by C18AQ Columns

SANTAI TECHNOLOGIES®

Chromatography Application Note
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Mingzu Yang, Bo Xu
Application R&D Center

Introduction

Antibiotics are a class of secondary metabolites produced by microorganisms (including bacteria, fungi, actinomycetes) or similar compounds which are chemically synthesized or semi-synthesized. Antibiotics could inhibit the growth and survival of other microorganisms. The first antibiotic discovered by human, penicillin, was discovered by British microbiologist Alexander Fleming in 1928. He observed that the bacteria in the vicinity of the mold could not grow in the staphylococcus culture dish which was contaminated with mold. He postulated that the mold must secrete an antibacterial substance, which he named penicillin in 1928. However, the active ingredients were not purified at that time. In 1939, Ernst Chain and Howard Florey of Oxford University decided to develop a drug that could treat bacterial infections. After contacting Fleming to obtain strains, they successfully extracted and purified penicillin from the strains. For their successful development of penicillin as a therapeutic drug, Fleming, Chain and Florey shared the 1945 Nobel Prize in Medicine.

Antibiotics are used as antibacterial agents to treat or prevent bacterial infections. There are several main categories of antibiotics used as antibacterial agents: β-lactam antibiotics (including penicillin, cephalosporin, etc.), aminoglycoside antibiotics, macrolide antibiotics, tetracycline antibiotics, chloramphenicol (total synthetic antibiotic), and etc. The sources of antibiotics include biological fermentation, semi-synthesis and total synthesis. The antibiotics produced by biological fermentation need to be structurally modified by chemical methods due to chemical stability, toxic side effects, antibacterial spectrum and other issues. After chemically modified, the antibiotics could achieve increased stability, reduced toxic side effects, expanded antibacterial spectrum, reduced drug resistance, improved bioavailability, and thereby improved effect of drug treatment. Therefore, semi-synthetic antibiotics are currently the most popular direction in the development of antibiotic drugs.

In the development of semi-synthetic antibiotics, antibiotics have the properties of low purity, lots of by-products and complex components since they are derived from microbial fermentation products. In this case, the analysis and control of impurities in semi-synthetic antibiotics is particularly important. In order to effectively identify and characterize impurities, it is necessary to obtain a sufficient amount of impurities from the synthetic product of semi-synthetic antibiotics. Among the commonly used impurity preparation techniques, flash chromatography is a cost-effective method with advantages such as large sample loading amount, low cost, time saving, etc. Flash chromatography has been more and more employed by synthetic researchers.

In this post, the main impurity of a semi-synthetic aminoglycoside antibiotic was utilized as the sample and purified by a SepaFlash® C18AQ cartridge combined with the flash chromatography system SepaBean® machine. The target product meeting the requirements was successfully obtained, suggesting a highly efficient solution for the purification of these compounds.

Experimental Section

The sample was kindly provided by a local pharmaceutical company. The sample was a kind of amino polycyclic carbohydrates and its molecular structure was similar with aminoglycoside antibiotics. The polarity of the sample was rather high, making it very soluble in water. The schematic diagram of the sample's molecular structure was shown in Figure 1. The purity of the raw sample was about 88% as analyzed by HPLC. For the purification of these compounds of high polarity, the sample would be barely retained on the regular C18 columns according to our previous experiences. Therefore, a C18AQ column was employed for the sample purification.

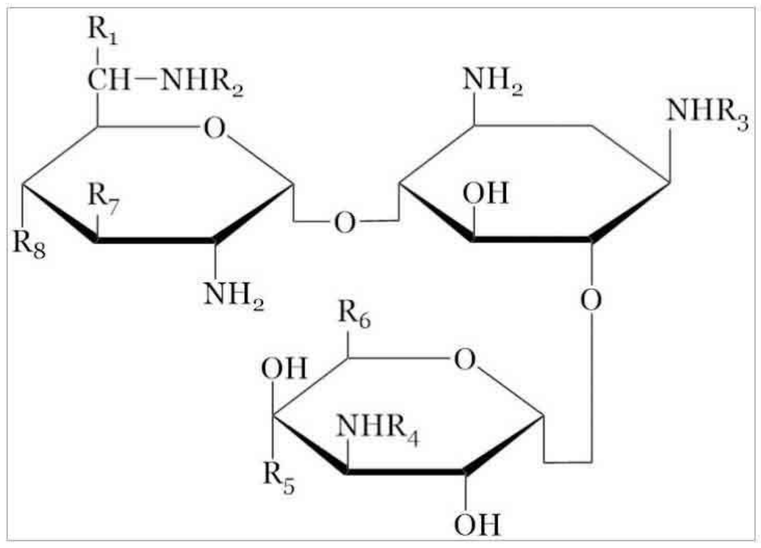

Figure 1. The schematic diagram of the sample's molecular structure.

To prepare the sample solution, 50 mg crude sample was dissolved in 5 mL pure water and then ultrasonicated in order to make it become a completely clear solution. The sample solution was then injected into the flash column by an injector. The experimental setup of the flash purification was listed in the Table 1.

Instrument	SepaBean® machine 2	
Cartridges	12 g SepaFlash® C18AQ RP flash cartridge (spherical silica, 20 - 45 µm, 100 Å, Order number: SW-5222-012-SP(AQ))	
Wavelength	204 nm, 220 nm	
Mobile phase	Solvent A: Water Solvent B: Acetonitrile	
Flow rate	15 mL/min	
Sample loading	50 mg	
Gradient	Time (min)	Solvent B (%)
	0	0
	19.0	8
	47.0	80
	52.0	80

Table 1. The experimental setup for flash purification.

Results and Discussion

The flash chromatogram of the sample on the C18AQ cartridge was shown in Figure 2. As shown in Figure 2, the highly polar sample was effectively retained on the C18AQ cartridge. After lyopholization for the collected fractions, the target product had a purity of 96.2% (as shown in Figure 3) by HPLC analysis. The results indicated that the purified product could be further utilized in the next step research and development.

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

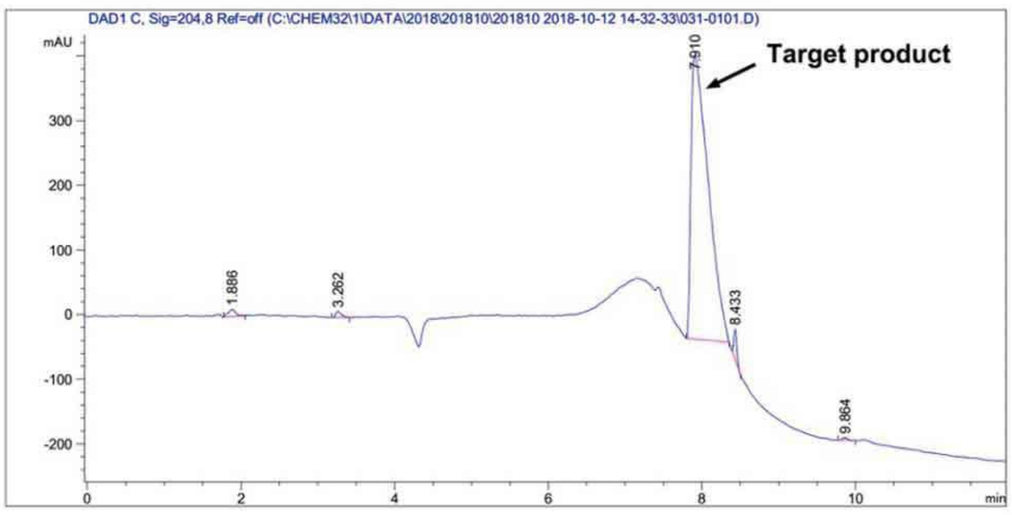

Figure 3. The HPLC chromatogram of the target product.

In conclusion, SepaFlash® C18AQ RP flash cartridge combined with the flash chromatography system SepaBean® machine could offer a fast and effective solution for the purification of highly polar samples.

About the SepaFlash® C18AQ RP flash cartridges

There are a series of the SepaFlash® C18AQ RP 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/en/.

Santai Technologies Inc.
Address: No. 78 Qingyang Road, Xinbei District, Changzhou, Jiangsu Province, China
Website: www.santaitech.com

CHINA
Tel.: +86 (519) 8515 0175
Fax: +86 (519) 8515 3561
Email: info@santaitech.com
Website: www.santaitech.com

CANADA
Tel: +1 418-580-0437
Order mail: ca_order@santaitech.com
Support mail: ca_support@santaitech.com

INDIA
Tel: +91 937-181-2696
Order mail: in_order@santaitech.com
Support mail: in_support@santaitech.com