SepaFlash HILIC ARG cartridge and Its Application in the Purification of Oligosaccharides

Chromatography Application Note
AN027

Wenjun Qiu, Bo Xu
Application R&D Center

Introduction

Oligosaccharides is a group of sugar polymers composed of a few (usually 3 to 10 [1-3]) monosaccharide molecules which are polymerized by glycosidic bonds. Oligosaccharides are ubiquitous in the cell membrane of animal cells and have many functions including cell recognition and cell binding [4]. For example, glycolipid molecules play an important role in the immune response process [5]. In nature, oligosaccharides usually exist as part of glycoproteins and glycolipids. Oligosaccharides bind to lipids or appropriate amino acid side chains of protein molecules via N-linked or O-linked glycosidic bonds. N-glycosidically bonded oligosaccharides are always a class of pentasaccharides which bond to the asparagine of the amino acid side chains via a β-bond [6]. O-linked oligosaccharides are usually linked to threonine or serine in the amino acids side chain. According to nutrition experts and medical research results, oligosaccharides have functions similar to water-soluble dietary fiber, including promoting intestinal peristalsis, improving constipation, diarrhea and other issues. Since the small intestine of the human body can not completely consume oligosaccharides, part of the oligosaccharides that are not digested will be utilized by the intestinal microflora [7, 8]. As a result, the intestinal ecology is changed. The ecology of human digestive microflora is normalized and the number of beneficial microflora is increased, which helps to improve the digestion and movement of the intestine, reduce the absorption of toxins, prevent the incidence of intestinal cancer or enteritis, etc., as well as improve the blood lipid level. Therefore, oligosaccharides have been attracting more and more attentions from researchers for their application in medicine, nutrition, immunology, etc.

In this application note, a synthetic oligosaccharide molecule was used as the sample to be purified. The sample molecule has poor retention on the regular C18 flash cartridge due to its strong polarity. Furthermore, the sample molecule has very week UV absorbance, making it difficult to be detected by UV detector. Considering these factors, application engineers from Santai Technologies utilized a SepaFlashTM HILIC ARG cartridge with a flash system SepaBeanTM machine which is combined with an external ELSD to purify the sample. As a result, the target product meeting the purity requirement was successfully prepared, suggesting a feasible method for the purification of these highly polar oligosaccharide samples.

Experimental Section

The sample used in this application note was a synthetic oligosaccharide which was kindly provided by a university laboratory focusing on the application research of oligosaccharides. The sample contained other by-products with different degrees of polymerization, which were very polar and soluble in water. The chemical structure of the sample molecule is shown in Figure 1.

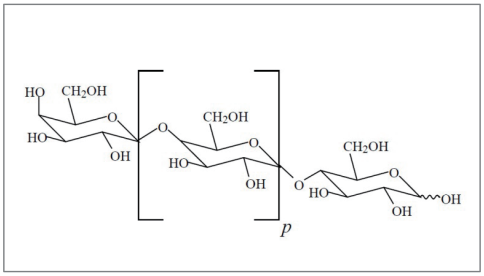

Figure 1. The chemical structure of oligosaccharide sample.

Due to the high polarity of the sample and its low solubility in organic solvents, the normal phase separation mode was excluded from consideration first. In reversed phase separation mode, C18 reversed phase cartridge was the most commonly used flash cartridge for the separation of various polar or non-polar samples. Therefore, we utilized a regular C18 reversed phase flash cartridge to purify the sample as a beginning step. A small amount of the sample was dissolved in water and then injected into the flash cartridge by an 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)		12 g SepaFlash™ HILIC ARG cartridge (spherical silica, 20 - 45 μm, 100 Å, Order number: SW-5622-012-SP)	
Wavelength	254 nm, 280 nm, ELSD			
Mobile phase	Solvent A: water Solvent B: acetonitrile			
Flow rate	30 mL/min			
Sample loading	30 mg			
Gradient	Time (min)	Solvent B (%)	Time (min)	Solvent B (%)
	0	10	0	95
	15.0	90	8.0	80
	/	/	15.0	80
			23.0	53
			25.0	53
			32.0	25
			37.0	25

Table 1. The experimental setup for flash chromatography.

Results and Discussion

The flash chromatogram of the sample by the regular C18 reversed phase cartridge was shown in Figure 2. As shown in Figure 2, the sample had poor retention on the regular C18 cartridge and was directly eluted out from the cartridge with the mobile phase due to its high polarity. Therefore the sample was not effectively purified from the impurities. Furthermore, it could also be seen from the chromatogram that the sample has no UV absorbance, which makes ELSD the inevitable choice for the detection of the sample.

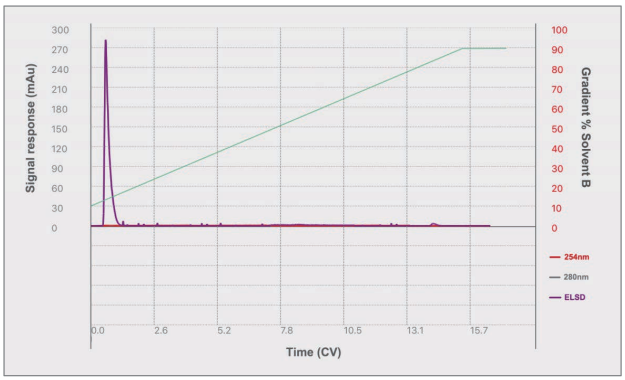

Figure 2. The flash chromatogram of the sample by a regular C18 reversed phase cartridge.

In the following step, we considered utilizing HILIC (hydrophilic interaction chromatography) mode for the purification of the sample. It is known to all that highly hydrophilic polar stationary phase was used in HILIC mode. For example, SepaFlashTM HILIC ARG cartridges from Santai Technologies are pre-packed with spherical silica bonded with highly polar Arginine groups (as shown in Figure 3), which have sufficient retention for hydrophilic samples. In HILIC mode, the water ratio in the mobile phase is gradually increased during the elution process, making it suitable for the separation and purification of the samples with higher polarity.

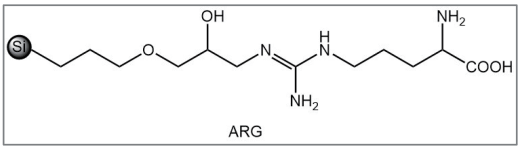

Figure 3. The schematic diagram of the stationary phase bonded to the surface of ARG separation media.

A SepaFlash™ HILIC ARG cartridge was used for the purification of the sample. The flash chromatogram was shown in Figure 4. As shown in Figure 4, the sample was well retained on the stationary phase of HILIC ARG cartridge in the HILIC mode. The target product was effectively separated from the impurities such as by-products. The collected fractions were further identified by TLC. Sulfuric acid/methanol (V:V=1:5) was used as the color developer for TLC analysis. After baked by a heat gun for 15 min, the TLC identification results was shown in Figure 5. It can be concluded that the target product was effectively isolated from impurities in the crude sample and can be used in next step research and development.

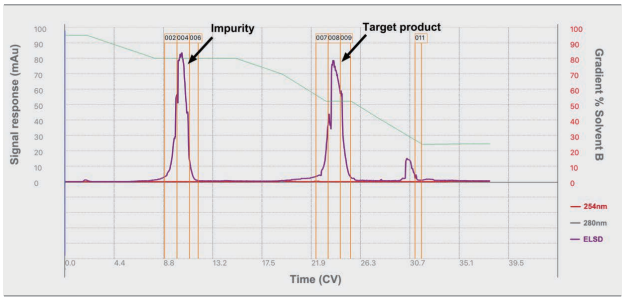

Figure 4. The flash chromatogram of the sample by a HILIC ARG cartridge.

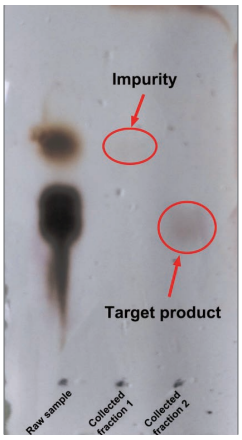

Figure 5. The TLC identification results of raw sample and collected fractions.

In conclusion, for the purification of oligosaccharide samples which have strong polarity as well as no UV response signal, combining SepaFlash™ HILIC ARG cartridge with a flash system SepaBean™ machine which is linked with an external ELSD is an effective and feasible solution.

About SepaFlash™ HILIC ARG flash cartridges

There are a series of the SepaFlash™ HILIC ARG flash 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
SW-5622-330-SP	420 g	420 mg – 8.4 g	250/17.2

Table 2. SepaFlash™ 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: http://www.santaitech.com/index/.

References

1.Oligosaccharides at the US National Library of Medicine Medical Subject Headings (MeSH)

2.Walstra P, Wouters JT, Geurts TJ (2008). Dairy Science and Technology (Second ed.). CRC, Taylor & Francis.

3.Whitney E, Rolfes SR (2008). Understanding Nutrition (Eleventh ed.). Thomson Wadsworth.

4.Varki A, (1993). Biological roles of oligosaccharides: all of the theories are correct. Glycobiology. 3 (2): 97-130.

5.Mattner J, DeBord KL, Ismail N, et al (2005). Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature. 434: 525-529.

6.Voet D, Pratt C (2013). Fundamentals of Biochemistry: Life at the Molecular Level (4th ed.). Hoboken, NJ: John Wiley & Sons, Inc. ISBN 978-0470-54784-7.

7.Bode L (2009). Human milk oligosaccharides: prebiotics and beyond. Nutrition Reviews. 67 (2): S183–91.

8.De Filippo C, Cavalieri D, Di Paola M, et al (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences. 107 (33): 14691–14696.

Santai Science Inc.

Santai Science Inc.
Website: www.santaisci.com

Office: 214 Brunswick, Pointe-Clarie, Montréal, H9R 1A6, Québec, Canada

CPS analitica | Calibre SCIENTIFIC
via neera 8/a 20141 Milano
Tel. +39 02.8954201 - Fax +39 02.89542022
www.cps.it - cps@cps.it