The Application of SepaBean machine Equipped with ELSD for the Purification of PEG Derivatives

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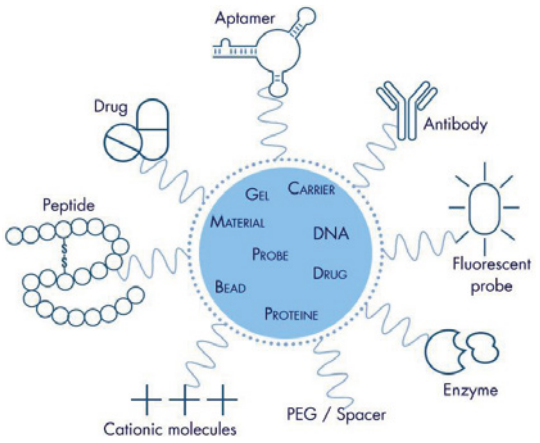
Chromatography Application Note



Introduction

Polyethylene glycol (PEG) is a polymer of ethylene glycol with a relative molecular weight of 200 to 8000 or above. It is composed of repeated oxyethyl groups, which not only has good water solubility, but is also soluble in organic solvents such as benzene, acetonitrile and ethanol. The characteristics of PEG molecules are as follows:

1. Low dispersion: The dispersion with a relative molecular mass (Mr) of less than 5000 is 1.01, and the dispersion with a molecular weight (Mr) of more than 5000 is 1.1, with a wide distribution and greater selectivity;

2. Unique amphiphilic: unique structure makes it soluble in both organic solvents and water;

3. Non-toxic: Studies show that polyethylene glycols greater than 1000 are non-toxic and have been used in various foods, cosmetics and drugs;

4. Biodegradable: Polyethylene glycol is directly eliminated in the body without any structural changes. Metabolites with a molecular weight of less than 20,000 can be metabolized by the kidneys, and larger molecules can be metabolized by the digestive system.

IDue to the above mentioned characteristics, PEG has been widely used in the pharmaceutical industry [1-4]. The covalent attachment of PEG to a drug or therapeutic protein can "mask" the agent from the host's immune system (reducing immunogenicity and antigenicity), and increase its hydrodynamic size (size in solution), which prolongs its circulatory time by reducing renal clearance [5]. PEGylation can also provide water solubility to hydrophobic drugs and proteins. Many small molecule drugs, especially the anti-tumor drugs, use PEGylation technology to improve its biocompatibility [6, 7], the most representative of which are paclitaxel [8] and camptothecin [9]. The polyethylene glycol (PEG) supported small molecules can transfer many of its excellent properties to the conjugate which make the polymer has excellent biocompatibility. Thus, the polymer can be dissolved in the tissue fluid, and can be quickly excreted by the body without any toxic side effects. Many anti-tumor drugs are modified by high molecular weight PEG to achieve passive targeted administration of tumor tissue [10, 11]. Having proven its pharmacological advantages and acceptability, PEGylation technology is the foundation of a growing multibillion-dollar industry [12].

In this post, the sample used was an oligomeric PEG derivative which was kindly provided by a pharmaceutical R&D company. UV detector is not a proper one for the detection of this sample in which no sufficient active chromophores are contained for UV characterization. The application engineers from Santai Technologies utilized a Flash chromatography system SepaBean machine T which was equipped with SepaFlash FP LT-ELSD for the sample purification. As a result, the target product meeting the purity requirement was successfully obtained, suggesting an efficient and cost-effective solution for the purification of these PEG derivative compounds.

Experimental Section

1.Sample information

The sample used in this application was a small molecule drug modified by oligomeric PEG, which was kindly provided by a pharmaceutical R&D company. The chemical structure of the sample molecule was shown in Figure 1. No specific structure can be given due to confidentiality requirements.

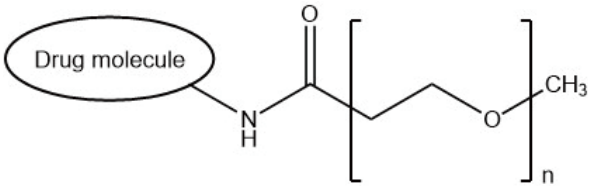


Figure 1. The schematic diagram of the sample chemical structure.

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	25 g SepaFlash silica cartridge (irregular silica, 40 - 63 μm, 60 Å, Order number: S-5101-0025)	
Detection channels	254 nm; 280 nm; ELSD	
Detection parameters for ELSD channel	Evaporator temperature: 40 °C Gas pressure: 3.2 bar Signal gain: 8 Filter constant: 4 s	
Mobile phase	Solvent A: dichloromethane; Solvent B: methane	
Flow rate	35 mL/min (Split flow for ELSD: 0.5 mL/min)	
Sample load	0.5 mL	
Gradient	Time (min)	Solvent B (%)
	0	0
	5.0	10
	15.0	20
	20.0	30

Table 1. The experimental setup for Flash purification.

Results and Discussion

The Flash chromatogram of the sample on a silica cartridge was shown in Figure 2.

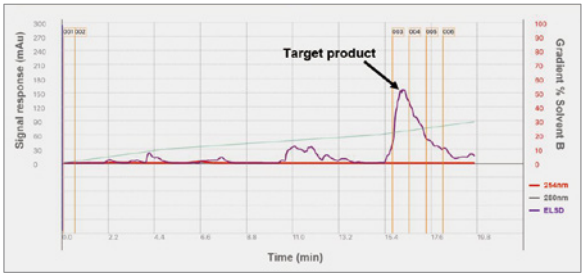


Figure 2. The Flash chromatogram of the sample on a silica cartridge.

According to the chromatogram as shown in Figure 2, due to the lack of chromophore groups which can be detected by UV detector, the sample molecule has no signal in UV channels. In contrast, ELSD based on the principle of sample particle scattering detection can well detect the signal of these sample molecules. As a complement to UV–Vis detector, ELSD can expand the application range of Flash chromatography and provide signal detection tools for many samples that cannot be detected by UV–Vis detector.

The fractions collected in Flash chromatography was further identified by TLC. Dichloromethane/methane (V:V=9:1) was used as the development solvent for the sample spots on TLC plate. When the TLC plate development was completed, iodine vapor was utilized to visualize the spots on TLC plate (as shown in Figure 3). Confirmed by TLC identification, the collected fraction could meet the purity requirement and be further utilized in next step research and development.

Figure 3. The TLC identification results for raw sample and collected fractions.

In conclusion, for the purification of PEG derivatives without UV absorption, the Flash chromatography system SepaBean machine T equipped with SepaFlash FP LT–ELSD provides the customer a feasible solution to efficiently and economically purify such samples.

About SepaFlash FP LT–ELSD

The detailed parameters of SepaFlash FP LT–ELSD presented by Santai Technologies is listed in Table 2.

Detection	Photodiode
Light source	Blue LED, built-in elapsed time counter
Temperature range	Ambient to 100 ℃
Eluent flow rate	100 μL/min to 5 mL/min
Typical sensitivity	100 ng
Analog output	0 - 1 Volt
Gas supply	Nitrogen or air, 2.0 bar (less than 3L/min)
Selection & display	OLED display and keypad
System control	Remote control by SepaBean App
Dimension (W x H x D)	250 x 330 x 530 mm
Weight	15 kg (33 lb)

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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