The Purification of Diastereomers by SepaFlash C18 Reversed Phase Cartridge

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Chromatography Application Note AN032

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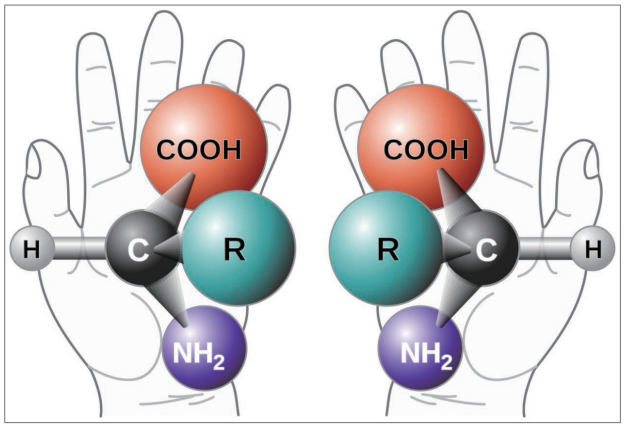

Introduction

The existence of chiral molecules is a common phenomenon in nature. The term chirality is derived from the Ancient Greek word for hand, χείρ (cheir). Just as people's left and right hands can only be mirror images of each other instead of completely overlapping, the chiral molecules cannot overlap completely either, no matter how they are rotated. As shown in Figure 1, the simplest amino acid, alanine, exists in two configurations, namely: *(S)*-alanine and *(R)*-alanine. These two configurations of alanine have different spatial structures since the four groups connected to the central carbon atom are arranged in different order in space.

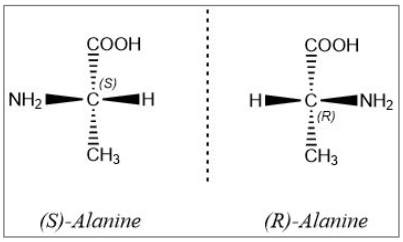

Figure 1. The chemical structure of the alanine enantiomers.

In 1848, French chemist and biologist Louis Pasteur discovered two different configurations of tartaric acid, marking the discovery of the chirality of organic molecules. It was not until more than one century that human realized that chiral phenomena play a key role not only in the life characteristics of plants and animals, but also in the pharmaceuticals, agriculture and chemical industry [1-3]. All proteins, peptides, amino acids, nucleotides, carbohydrates and some alkaloids, hormones, etc. are chiral compounds [4, 5]. At present, about 60% of the clinically used drugs are chiral compounds. It is estimated that about 88% of the chiral drugs used for treatment are racemic compounds. Chirality has now become a focal issue for academic research and pharmaceutical development [6-8].

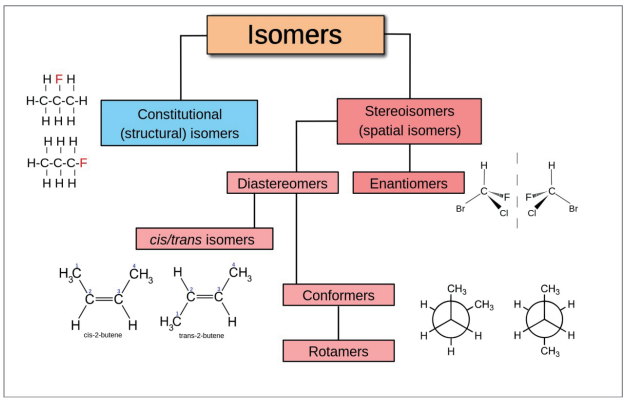

Figure 2. The classification of isomers.

In stereochemistry, chiral molecules belong to the category of isomers, that is, molecules which are chiral to each other have the same molecular formula and different spatial structures. As shown Figure 2, isomers can be divided into two categories: constitutional (structural) isomers and stereoisomers (spatial isomers). The stereoisomers can be further divided into two sub categories: enantiomers and diastereomers. Enantiomers are also called optical isomers. A pair of enantiomers are mirror images that cannot be overlapped with each other. Enantiomers have exactly the same physical properties but may have substantially different biological effects. For the separation of enantiomers, it is required to utilize chiral chromatography columns or supercritical fluid chromatography (SFC) [9-11]. Diastereomers are stereoisomers that have two or more chiral centers but are not mirror images of each other, including cis/trans isomers, conformers, rotamers and diastereomeric optical isomers. Diastereomers have different physical properties and often different chemical reactivity, making it is possible to separate these compounds by chromatography or recrystallization.

In this application note, the sample to be purified was a pair of diastereomers obtained by organic synthesis, which was kindly provided by a

synthetic lab from a pharmaceutical R&D company. Conventional normal phase chromatography cannot achieve the purification purpose, nor is preparative HPLC suitable due to its limited sample loading capacity and the cost issues. Therefore, the application engineers from Santai Technologies utilized SepaFlash C18 reversed phase cartridges in combination with a Flash chromatography system SepaBean machine T to purify the sample. The target products meeting the purity requirement were successfully obtained, suggesting an efficient and cost effective solution for the purification of these diastereomers.

Experimental Section

1. Sample information

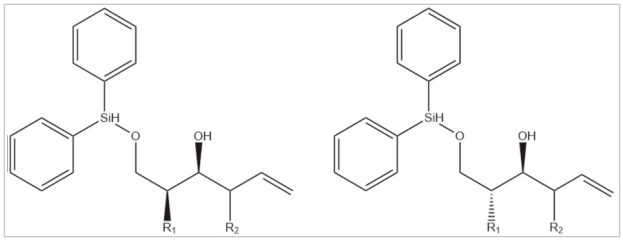

Figure 3. Two possible configurations of the sample molecule.

As shown in Figure 3, there are two possible configurations of the sample molecule since there are two chiral centers in its structure formula.

2. Analysis of the raw sample

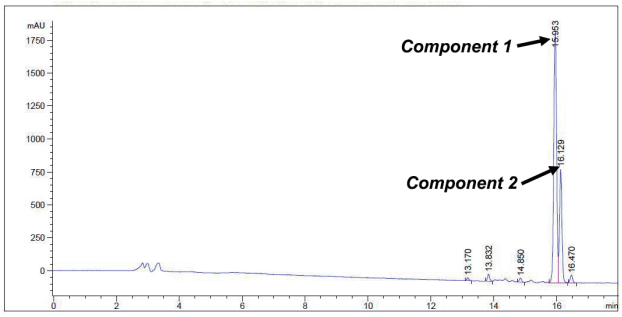

Figure 4. The HPLC chromatogram of the raw sample.

The raw sample was analyzed by HPLC for Flash chromatography method development. As shown in Figure 4, the two components in the sample, meaning the diastereomers of the sample molecule, have very similar retention time on the chromatogram, which poses challenges for the subsequent sample purification by Flash chromatography. To improve the resolution, column stacking is a simple and feasible way to achieve the goal in Flash chromatography (please refer to other application notes published by Santai Technologies, such as this one: *AN014 The Improvement of the Resolution by Column Stacking and Its Application*). In the subsequent Flash chromatography separation, two Flash cartridges were stacked and sample loading volume was also investigated.

3. 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	25g SepaFlash C18 reversed phase cartridges, two cartridges stacked (spherical silica, 20 - 45 μm, 100 Å, Order number: SW-5222-025-SP)	
Wavelength	220 nm, 254 nm	
Mobile phase	Solvent A: Water Solvent B: Acetonitrile	
Flow rate	15 mL/min	
Sample loading	0.75 mL (100 mg)	
Gradient	Time (min)	Solvent B (%)
	0	20
	56	83
	95	83
	100	87
	100.5	100
	105	100

Table 1. The experimental setup for Flash purification.

Results and Discussion

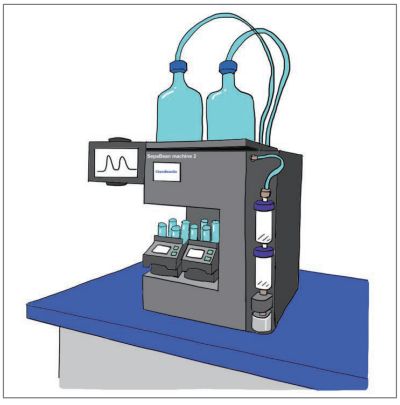

Figure 5. The schematic diagram of two stacking Flash cartridge installed on a SepaBean machine T.

As shown in Figure 5, two SepaFlash C18 reversed phase cartridge were stacked and installed on a SepaBean machine T in order to improve the resolution for the sample components. The Flash chromatogram of the sample was shown in Figure 6.

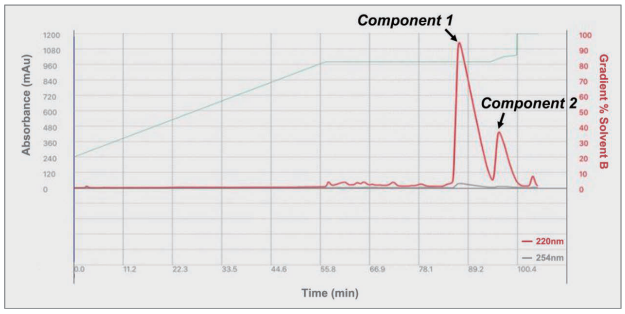

Figure 6. The Flash chromatogram of the sample.

As shown in Figure 6, two components in the sample were collected respectively and then further analyzed by HPLC. As shown in Figure 7, the purity of the two collected fractions exceeded 98%, indicating the purity requirement of the target product could be satisfied.

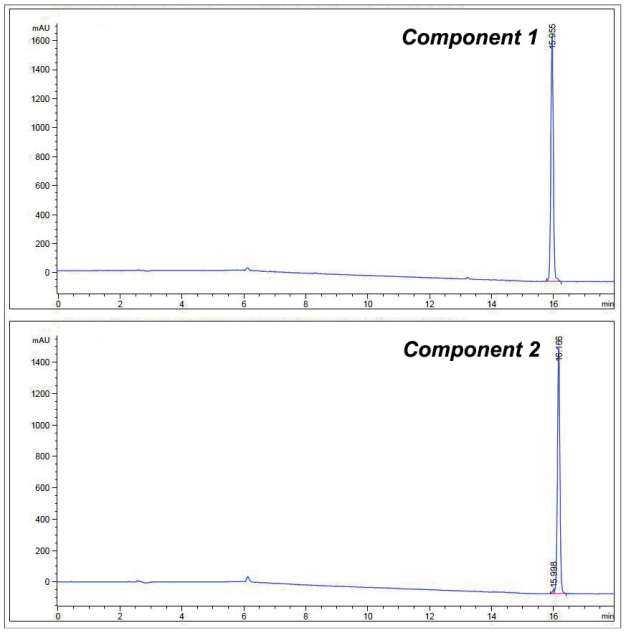

Figure 7. The HPLC chromatogram of the two collected fractions.

Conclusion

In this application note, two stacking 25 g SepaFlash C18 reversed phase cartridges were utilized for the purification of 100 mg diastereomer crude sample. In the following scale up of the Flash chromatography method, the sample loading amount could be easily scaled up to around 600 mg with the 330 g cartridge pre-packed with the same stationary phase. Compared with chiral chromatography column or preparative HPLC, Flash chromatography method has the advantages including higher throughput as well as lower cost.

For further information on detailed specifications of SepaBean machine, or the ordering information on SepaFlash series Flash cartridges, please visit our website: www.santaitech.com/index/.

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