The Purification of Small Molecule Peptide by SepaFlash HP Bio Series Flash Cartridge

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Chromatography Application Note AN006

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Introduction

With the development of biotechnology as well as peptide synthesis technology, more and more peptide drugs have been developed and applied clinically. Due to its wide indications, high safety and significant effect, peptide drugs are widely used in the prevention, diagnosis and treatment of many diseases such as tumor, hepatitis, diabetes, AIDS, etc [1-3]. Among the production methods of peptide drugs, solid phase synthesis method has the advantages of high yield as well as the ability to realize automation. However, its shortcomings are also obvious that the intermediate products cannot be purified per step, and the final product must be purified by reliable method. The commonly used purification methods for peptides include ion exchange chromatography (IEC) and reversed-phase high performance liquid chromatography (RP-HPLC), which have the disadvantages of low sample loading capacity, high cost of separation media, complicated and costly separation equipment, etc. In this application note, thymopentin (hereinafter referred to as TP-5) was chosen as the sample. Peptide products of high purity (>94%) was successfully obtained by a single sample injection on a SepaFlash HP Bio series flash cartridge combined with a preparative flash chromatography system, suggesting a fast, highly efficient and low cost solution for the purification of such small molecule peptide samples.

Experimental

Sample information

The sample used in this application note is TP-5, which is the amino acid residue fragment of Thymosin II at position 32-36. The molecular formula of TP-5 is C30H49N9O9 (as shown in Figure 1). Its molecular weight is 679.41 Da. The raw material of TP-5 used in this application note is purchased from Wuxi Asiapeptide Biotechnology Co., Ltd.

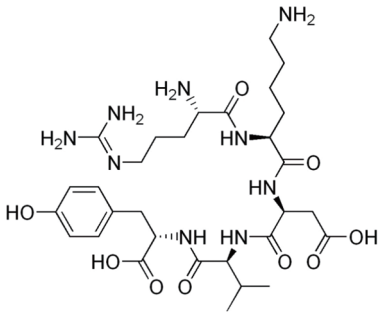

Figure 1. The chemical structure of TP-5.

TP-5 can induce the T cell differentiation and promote the development and activation of T lymphocyte subsets. In clinical practices, TP-5 is mainly used in the treatment of tumors, herpes simplex, rheumatoid arthritis, AIDS, severe immunodeficiency syndrome, chronic hepatitis B and other diseases [4-7].

Instruments and Consumables

A 12g SepaFlash HP Bio Series C18 reversed-phase cartridge (order number: Bio-SW-5223-012-SP) combined with a preparative flash chromatography system was utilized for peptide sample purification.

The raw material and purified product of TP-5 was analyzed by a HPLC system (Agilent 1100) with an analytical C18 reversed-phase column (Diamonsil, 5μm, 4.6 x 150 mm, Dikma). The parameters of the mobile phases and eluting gradient were shown as Table 1.

Table 1. The parameters of the mobile phases and eluting gradient for HPLC analysis.

Mobile phase	Solvent A: water (5% ACN,0.05% TFA) Solvent B:ACN	
Flow rate	1.0 ml/min	
Gradient	% Solvent B	Time / (min)
	0	0
	0	3.0
	30	6.0
	100	12.5
	0	13.0
	0	15.0

The molecular weight of the purified TP-5 product was identified by a LC-MS system, in which a HPLC (LC-10A, Shimadzu) was hyphenated with a MS detector (API 2000, AB Sciex). The HPLC analytical column used in LC-MS system was ZORBAX 3.5μm XDB-C18 (2.1 x 50 mm, Agilent). The parameters of the mobile phases and eluting gradient were shown as Table 2.

Table 1. The parameters of the mobile phases and eluting gradient for LC-MS analysis.

Mobile phase	Solvent A: water (5% ACN,0.05% Formic acid) Solvent B: ACN	
Flow rate	0.5 ml/min	
Gradient	% Solvent B	Time / (min)
	5	0
	60	2.0
	60	4.0
	5	4.2
	0	6.0

Flash Purification Procedure

45 mg of TP-5 raw material were dissolved in 1mL MeOH-water (V:V = 3:7) and then injected into flash cartridge by a sample injector. Afterwards the flash purification for the raw sample was performed. The related experimental parameters were summarized in Table 3.

Table 3. The experimental parameters of flash purification.

Instrument	A preparative flash chromatography system	
Flash cartridge	12g SepaFlash HP Bio C18 reversed-phase cartridge (Particle size: 15μm,Pore diameter: 100Å,Order number: Bio-SW-5223-012-SP)	
Sample loading	45 mg raw material, dissolved and loaded by a sample injector	
Wavelength	220 nm, 254 nm	
Mobile phase	Solvent A: water Solvent B: MeOH	
Flow rate	20 ml/min	
Gradient	% Solvent B	Time / (min)
	10	0
	90	10.0
	90	12.0

Results and Discussion

In this application note, 45 mg raw material of TP-5 (purity: 80.5%, as shown in Figure 2) were dissolved and loaded into a 12g SepaFlash HP Bio C18 reversed-phase cartridge. All the sample was successfully purified in a single run. The purification chromatogram was shown in Figure 3. The fractions from collection tube No. 002 and No. 003 were merged as the purified product. After lyophilization, the final product was weighed as 27.5 mg and the purity was 94.1% by HPLC analysis (as shown in Figure 4). The yield of purified TP-5 was calculated as 75.3%. The molecular weight of the product was further identified by LC-MS (as shown in Figure 5). In conventional preparative RP-HPLC method, multiple injections are usually required to purify the sample of the amount similar to this application note due to the limitation of loading capacity. In contrast, flash purification adopted in this application note could significantly shorten the whole experimental procedure, as well as saving human and material resources. Furthermore, the flash preparative method is easy to be scaled-up, suggesting a fast, highly efficient and low cost solution for the large scale purification of small molecule peptides.

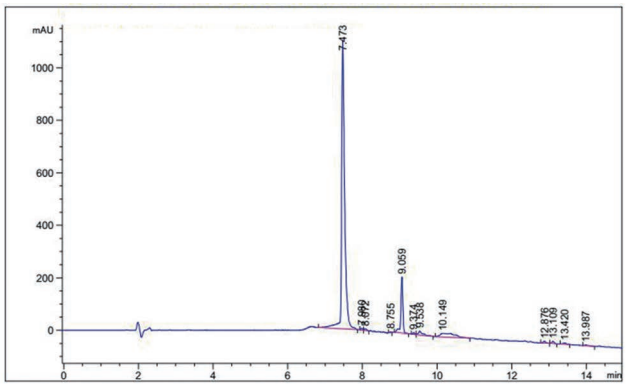

Figure 2. The chromatogram of TP-5 raw material by HPLC analysis.

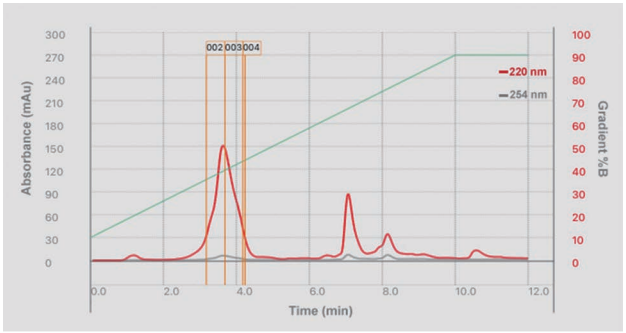

Figure 3. The chromatogram of TP-5 raw material by flash purification.

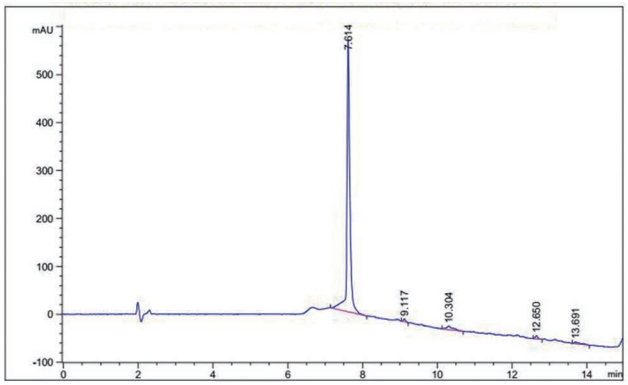

Figure 4. The chromatogram of purified TP-5 by HPLC analysis.

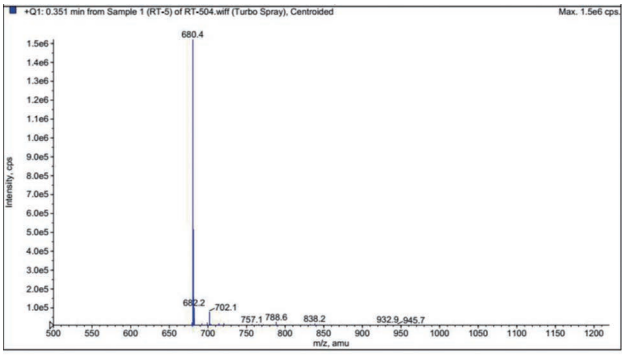

Figure 5. The MS results of purified TP-5.

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