The Purification of Taxus Extract by SepaBean™ machine

a santai science company
SANTAI TECHNOLOGIES quality since 2004

Chromatography Application Note
AN017

Meiyuan Qian, Yuefeng Tan, Bo Xu
Application R&D Center

Introduction

Taxus (Taxus chinensis or Chinese yew) is a wild plant protected by the country. It is a rare and endangered plant left behind by the Quaternary glaciers. It is also the only natural medicinal plant in the world. Taxus is distributed in the temperate zone of the northern hemisphere to the mid-subtropical region, with about 11 species in the world. There are 4 species and 1 variety in China, namely Northeast Taxus, Yunnan Taxus, Taxus, Tibetan Taxus and Southern Taxus. These five species are distributed in Southwest China, South China, Central China, East China, Northwest China, Northeast China and Taiwan. Taxus plants contain a wide variety of chemical components, including taxanes, flavonoids, lignans, steroids, phenolic acids, sesquiterpenes and glycosides. The famous anti-tumor drug Taxol (or Paclitaxel) is a kind of taxanes. Taxol has unique anticancer mechanisms. Taxol can “freeze” microtubules by combing with them and prevent microtubules from separating chromosomes at the time of cell division, thus leading to the death of dividing cells, especially rapidly proliferating cancer cells [1]. Furthermore, by activating macrophages, Taxol causes a decrease in TNF-α (tumor necrosis factor) receptors and release of TNF-α, thereby killing or inhibiting tumor cells [2]. Moreover, Taxol can induce apoptosis by acting on the apoptotic receptor pathway mediated by Fas/FasL or activating the cysteine protease system [3]. Due to its multiple target anticancer effect, Taxol is widely used in the treatment of ovarian cancer, breast cancer, non-small cell lung cancer (NSCLC), gastric cancer, esophageal cancer, bladder cancer, prostate cancer, malignant melanoma, head and neck cancer, etc [4]. Especially for advanced breast cancer and advanced ovarian cancer, Taxol has an outstanding curative effect, therefore it is known as “the last line of defense for cancer treatment”.

Taxol is the most popular anticancer drug in the international market in recent years and is considered to be one of the most effective anticancer drugs for humans in the next 20 years. In recent years, with the explosive growth of population and cancer incidence, the demand for Taxol has also increased significantly. Currently, Taxol required for clinical or scientific research is mainly extracted directly from Taxus. Unfortunately, the content of Taxol in plants is quite low. For example, the Taxol content is only 0.069% in the bark of Taxus brevifolia, which is generally considered to have the highest content. For the extraction of 1 g of Taxol, it requires about 13.6 kg of Taxus bark. Based on this estimate, it takes 3 – 12 Taxus trees which are more than 100 years old to treat an ovarian cancer patient. As a result, a large number of Taxus trees have been cut down, resulting in near extinction for this precious species. In addition, Taxus is very poor in resources and slow in growth, which makes it difficult for further development and utilization of Taxol.

At present, the total synthesis of Taxol has been successfully completed. However, its synthetic route is very complex and high-cost, making it have no industrial importance. The semi-synthetic method of Taxol is now relatively mature and is considered to be an effective way to expand the source of Taxol in addition to artificial planting. Briefly, in the semi-synthesis of Taxol, the Taxol precursor compound which is relatively abundant in Taxus plants is extracted and then converted into Taxol by chemical synthesis. The content of 10-deacetylbaccatin Ⅲ in the needles of Taxus baccata can be up to 0.1%. And the needles are easy to regenerate comparing with the barks. Therefore, the semi-synthesis of Taxol based on 10-deacetylbaccatin Ⅲ is attracting more and more attention from researchers [5] (as shown in Figure 1).

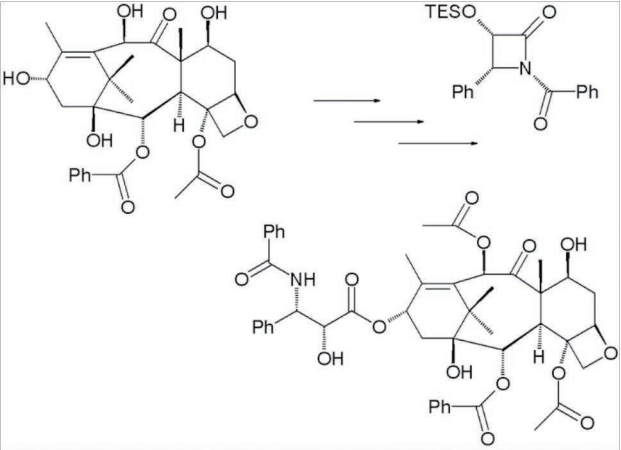

Figure 1. The semi-synthetic route of Taxol based on 10-deacetylbaccatin Ⅲ.

In this post, the Taxus plant extract was purified by a flash preparative liquid chromatography system SepaBean™ machine in combination with SepaFlash™ C18 reversed-phase (RP) flash cartridges produced by Santai Technologies. The target product meeting the purity requirements was obtained and can be used in subsequent scientific research, offering a cost-effective solution for the rapid purification of this kind of natural products.

Experimental Section

In this post, the Taxus extracts was used as the sample. The raw sample was obtained by extracting the Taxus bark with ethanol. Then the raw sample was dissolved in DMSO and loaded on the flash cartridge. The experimental setup of the flash purification is listed in the Table 1.

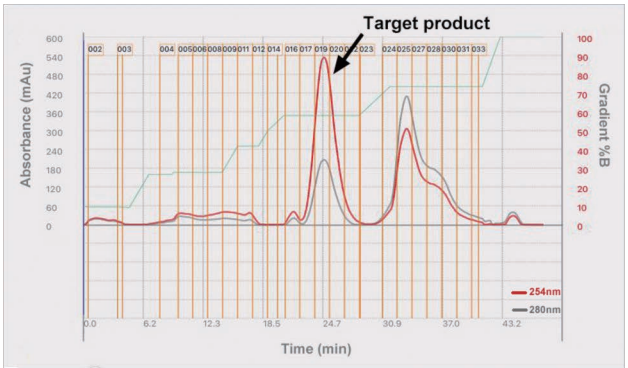

Figure 2. The flash chromatogram of crude extract from Taxus.

Instrument	SepaBean™ machine	
Cartridges	12 g SepaFlash™ C18 RP flash cartridge (spherical silica, 20 - 45 μm, 100 Å, Order number:SW-5222-012-SP)	
Wavelength	254 nm (detection), 280 nm (monitoring)	
Mobile phase	Solvent A: Water Solvent B: Methanol	
Flow rate	15 mL/min	
Sample loading	20 mg raw sample dissolved in 1 mL DMSO	
Eluting gradient	Time (min)	Solvent B (%)
	0	10
	5	10
	7	28
	14	28
	16	40
	20	60
	27	60
	30	72
	40	72
	43	100
	45	100

Table 1. The experimental setup for flash purification.

Results and Discussion

The flash chromatogram for the crude extract from Taxus was shown in Figure 2. By analyzing the chromatogram, the target product and the impurities achieved baseline separation. Furthermore, good reproducibility was also realized by multiple sample injections (data not shown). It will take about 4 hours to complete the separation in manual chromatography method with glass columns.

Comparing with traditional manual chromatography method, the automatic purification method in this post only requires 44 minutes to complete the whole purification task (as shown in Figure 3). More than 80% of the time and a large amount of solvent can be saved by taking automatic method, which can effectively reduce the cost as well as greatly improve the work efficiency.

Figure 3. The comparison of manual chromatography method with automatic purification method.

In conclusion, combing SepaFlash™ C18 RP flash cartridges with SepaBean™ machine can offer a fast and efficient solution for the rapid purification of natural products such as Taxus extract.

References

1. Alushin GM, Lander GC, Kellogg EH, Zhang R, Baker D and Nogales E. High-resolution microtubule structures reveal the structural transitions in αβ-tubulin upon GTP hydrolysis. Cell, 2014, 157 (5), 1117-1129.

2. Burkhart CA, Berman JW, Swindell CS and Horwitz SB. Relationship between the Structure of Taxol and Other Taxanes on Induction of Tumor Necrosis Factor-α Gene Expression and Cytotoxicity. Cancer Research, 1994, 54 (22), 5779-5782.

3. Park SJ, Wu CH, Gordon JD, Zhong X, Emami A and Safa AR. Taxol Induces Caspase-10-dependent Apoptosis, J. Biol. Chem., 2004, 279, 51057-51067.

4. Paclitaxel. The American Society of Health-System Pharmacists. [January 2, 2015]
5.Bruce Ganem and Roland R. Franke. Paclitaxel from Primary Taxanes: A Perspective on Creative Invention in Organozirconium Chemistry. J. Org. Chem., 2007, 72 (11), 3981-3987.

About the SepaFlash™ C18 RP flash cartridges

There are a series of the SepaFlash™ C18 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	5.4 g	5-15	400/27.5
SW-5222-012-SP	20 g	10-25	400/27.5
SW-5222-025-SP	33 g	10-25	400/27.5
SW-5222-040-SP	48 g	15-30	400/27.5
SW-5222-080-SP	105 g	25-50	350/24.0
SW-5222-120-SP	155 g	30-60	300/20.7
SW-5222-220-SP	300 g	40-80	300/20.7
SW-5222-330-SP	420 g	40-80	250/17.2

Table 2. SepaFlash™ C18 RP flash cartridges. Packing materials: High-efficiency spherical C18-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/index.php .

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