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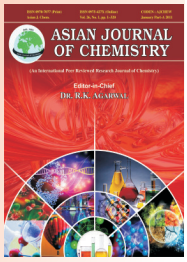

RP-HPLC Determination of Taxol and 10-Deacetylbaccatin III in Different Harvesting Time of *Taxus chinensis Var. Mairei* from China

LI-PING ZHU[1], HONG-TAO YANG[2], YUN-FANG GUAN[2] and KE YUAN[1,*]

[1]The Nurturing Station for the State Key Laboratory of Subtropical Silviculture, Zhejiang Agriculture and Forestry University, Lin' an 311300, P.R. China
[2]The Traditional Chinese Medicine Processed Samples of Co. Ltd. in East China of Hangzhou, Lin' an 311300, P.R. China

*Corresponding author: E-mail: yuan_ke001@163.com

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Taxus chinensis var. mairei is well known as anticancer plant. Its main anticancer compounds taxol and 10-deacetylbaccatin III and other diterpenoids have a strong anticancer effects, on clinical practice for the treatment of advanced breast cancer, ovarian cancer and other cancers, has a significant effect. This study established a method for the determination of taxol and 10-deacetylbaccatin III of *Taxus chinensis var. mairei* and to carry out an experiment for the quantitative determination of taxol and 10-deacetylbaccatin III of branches and leaves in different harvesting time of *Taxus chinensis var. mairei*. An HPLC method was adopted with Sunfire ODS C18 column (250 × 4.6 mm, 5 µm) with the mobile phase consisting of water-acetonitrile (A-B) by gradient program (0-20 min, 30 % A; 20-35 min, 30 % A → 55 % A, 35-43 min, 55 % A). The flow rate was 1.0 mL min^{-1} with the column temperature was 30 °C and UV detection wavelength at 232 nm. The linear range of taxol was 0.020-0.400 µg (r = 0.9993) with the average recovery of 100.49 %, the linear range of 10-DAB III was 0.010-0.200 µg (r = 0.9995) with the average recovery of 99.65 %. The content of the order of two active ingredients of different growth years of *Taxus chinensis var. mairei* is: 3 years > 4 years > 2 years > 1.5 years > 5 years. In different harvesting period, the highest content of taxol and 10-deacetylbaccatin III of *Taxus chinensis var. mairei* is harvested in May and June. The method is simple, accurate and reproducible, and it is applicable for the quality control of *Taxus chinensis var. mairei*.

Keywords: RP-HPLC, *Taxus chinensis var. mairei*, Taxol, 10-Deacetylbaccatin III.

INTRODUCTION

Taxus mairei belongs to *Taxus genus* of *Taxaceae* also known as yew or the red building has eleven species in the world[1]. There are six species in China, just like *Taxus madia, Taxus yunnanensis, Taxus cuspidate, Taxus chinensis var. mairei*., *Taxus wallichiana Zucc* and *Taxus chinensis*[2,3]. The *Taxus genus* is the taxol's raw material which is a new developed anticancer medicine in recent years. *Taxus chinensis* contains a variety of taxane analogues, including more than 10 kinds of composition with antitumor activity[4].

Taxus chinensis var. mairei[5] also named the beautiful-Taxus mairei belongs to the species of Coniferae and Taxaceae as well as Taxus genus[6]. It was called the national key protected wild plant which approved by the State Council on August 4,1999. The distribution of the *Taxus chinensis var. mairei* mainly in the South of the Yangtze river basin in China, in the forest which about 1000-2000 m above the sea level[7]. Since the 1970s American scientist get the taxol of anticancer drug from the *Taxus brervifolia*. Plants of the *genus taxus chinensis* have been paid much attention by people[8-9]. Nearly forty years *Taxus chinensis var. mairei* has become a research hotspot in the field of natural drugs in the world. Now *Taxus chinensis var. mairei* is planting on large areas of China, in order to replace the wild species.

Taxol is one of the three international achievements of antitumor medicine, which was detached from the tree bark of the *Taxus chinensis* in the pacific and was allowed to come into the market in America in 1992[10,11]. The ability of resisting the cancer is higher than that of *cis*-platinum, etoposide, adriamycin. It has obvious inhibition effect on the breast cancer, the lung cancer, ovarian cancer, mesothelioma. Therefore, it becomes the most effective antitumor medicine[12-14]. At present, the majority of the taxol are obtained from the plants like *Taxus chinensis*. However, because the taxol only take up a little part in the plants and the resource of natural *Taxus chinensisis* limited, people rely on other ways to get taxol for many years so that the problem of insufficient of taxol can be solved.

10-Deacetylbaccatin III belongs to taxanes that coexists with taxol in the *Taxus chinensis var. mairei*. They not only have certain anticancer activity, but can also be modifying the structure to synthesis the taxol and new taxol analogues and to get the taxol drug of efficient and low toxicity of the second

generation, all of those alleviate the pressure of taxol in short supply on the market in a certain extent[15].

The RP-HPLC method established in this paper measures simultaneously the contents of taxol and 10-deacetylbaccatin III of *Taxus chinensis var. mairei* in different harvesting time from China and exploring the change of taxol and 10-deacetylbaccatin III contents, in order to provide a reference for how to use this medicinal resources in much smarter ways.

EXPERIMENTAL

The HPLC system consisted of a waters 2695-2996 system (Waters Corp., MA, USA), equipped with a binary solvent manager, an auto-sampler and a waters 2996 diode array detector (DAD), was used for liquid chromatographic analysis, the Milli-Q super-purified water device; Shumei KQ2200DE ultrasonic cleaning instrument was used for extraction, AUTO SCIENCE solvent filtration device.

Taxol and 10-deacetylbaccatin III standards were provided by Xian Guanyu Bio-Tech Co., Ltd. (the purity is over 98 %). The HPLC grade acetonitrile were purchased from Tedia (Tedia Co., OH, USA), Deionized water was purified by a Milli-Q system from Millipore (Bedford, MA, USA). All the other chemicals and solvents used in sample preparation were of analytical grade.

This experiment used samples of *Taxus chinensis var. mairei* were collected from the planting base of *Taxus chinensis var. mairei* of Cixi in Zhejiang Province, China. Those samples were identified by Professor Lu-Huan Lou of Zhejiang Agricultural and Forestry University. The collected samples were dried at 50 °C and then smash into powder by 60 mesh sieves.

RESULTS AND DISCUSSION

HPLC chromatographic conditions: An HPLC method was adopted with Sunfire ODS C18 column (250 × 4.6 mm, 5 μm) with the mobile phase consisting of water- acetonitrile (A-B) by gradient program (0-20 min, 30 % A; 20-35 min, 30 % A → 55 % A, 35-43 min, 55 % A). The flow rate was 1 mL min^{-1} with the column temperature was 30 °C and UV detection wavelength at 232 nm. The injection volume was 1.0 μL. The separation between the chromatographic peak of adjacent degrees were greater than 1.5, number of theoretical pedal > 30000. Chromatograph chart as shown in Fig. 1

Preparation of standard solution: Precision weigh taxol reference substance 5 mg, dissolved it to volumetric flasks in 25 mL methanol, shake well, get the taxol standard solution (0.20 mg min^{-1}). Precision weigh 10-deacetylbaccatin III reference substance 5 mg, put it in the 25 mL volumetric flasks with added methanol for obtaining the 10-deacetylbaccatin III standard solution (0.10 mg min^{-1}). Accurately weigh 1 mL of the taxol standard solution (0.20 mg min^{-1}) and 1 mL of the 10-deacetylbaccatin III standard solution (0.10 mg min^{-1})

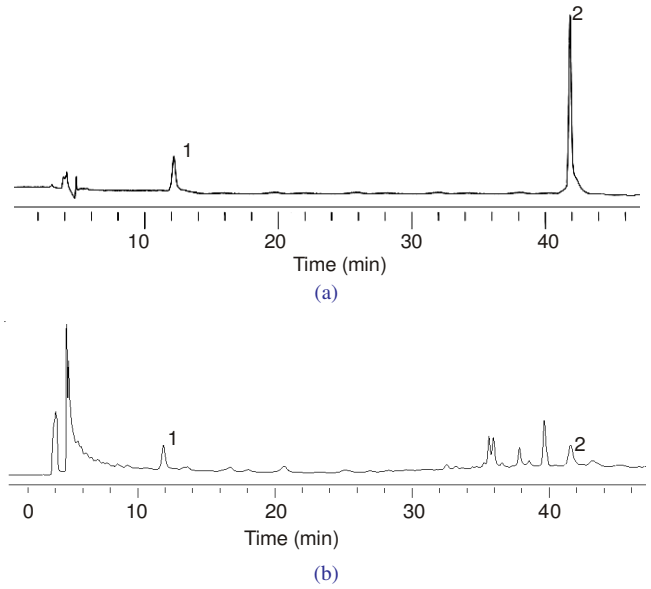

Fig. 1. Chromatogram of reference substances (a) and sample (b). 1 (10-deacetyl-bacratin III) 2 Taxol

respectively and put them in 10 mL volumetric flask and then resolve them by using methanol and regard them as the mixed standard solution. Avoid light kept at low temperature and to use it right after it was ready.

Preparation of sample solution: The collected samples of *Taxus chinensis var. mairei* were accurately weighed (approximately 1.0 g) and ultrasonic-extracted 0.5 h with 50 mL of methanol for three times, the extracts of the collected samples of *Taxus chinensis var. mairei* were concentrated into about 18 mL at 45 °C by the rotary evaporator, then added 2 mL distilled water, after mixed, added 30 mL of petroleum ether extracting for 3 to 4 times until the petroleum ether layer was nearly colourless. The methanol phase were evaporated to remove methanol, then added 30 mL methylene chloride and 15 mL distilled water extraction. The water layer extraction two times again by 20 mL of methylene chloride, the methylene chloride extraction that has been combined was evaporated to dryness under reduced pressure, next, dissolved them to a volumetric flasks use 25 mL methanol. then filtered it through 0.45 μm filtration membrane, get the sample solution.

Calibration curve drawing: The mixed reference solution injected for analysis purposes according to the chromatographic conditions, the sampling volume were 1, 3, 5, 7, 10, 15, 20 mL and record the peak area. As a result, the standard curves were obtained by plotting peak area (y) *vs*. the content of authentic standards (x (ug)) of each compound and were fitted to linear regression y = ax + b in Table-1.

Precision experiment: Taking the mixed reference fluid and continually injecting for 5 times, the result is the RSD of taxol and 10-deacetylbaccatin III are 1.58 % and 1.13 % respectively, which shows a high accuracy.

TABLE-1
CALIBRATION CURVES AND CORRELATION COEFFICIENTS OF TWO COMPONENTS IN THE MIXED REFERENCE FLUID (n = 6)

Components	Regression equation	r	Linear range (μg)
Taxol	$Y = 1.70 \times 10^6X - 3.78 \times 10^4$	0.999 3	0.020-0.400
10-DAB III	$Y = 6.40 \times 10^5X + 4.06 \times 10^2$	0.999 5	0.010-0.200

Stability of the samples: Taking the same sample solution and injecting at different time 0, 2, 4, 6, 8, 10, 12 h, we found that the RSD of the peak area of the taxol and 10-deacetylbaccatin III are 1.98 % and 1.26 %, respectively, which shows a good stability within 12 h.

Reproducibility: Precision weigh the same medicinal materials of *Taxus chinensis var. mairei*, the reproducibility was assessed by analyzing the same medicinal materials of *Taxus chinensis var. mairei*, using the above method. The RSD of the peak area of the taxol and 10-deacetylbaccatin III are 1.52 % and 1.23 %, respectively. Thus, the result indicated that the method shows a good reproducibility

Recoveries: Precision weigh the *Taxus chinensis var. mairei* that the content of the taxol and 10-deacetylbaccatin III is known 6 copies and each has 1 g with precision, then adding a certain amount of taxol and 10-deacetylbaccatin III reference solution, after that, according to the preparation method of sample solution and the above chromatography conditions we determine the samples content. The results showed that the average recoveries of taxol and 10-deacetylbaccatin III were 100.49 % and 99.65 %, respectively.

Determination of the content of the sample: The *Taxus chinensis var. mairei* of different growth years and different harvesting period were accurately weighed (1.0 g) respectively. Then use the preparation method of sample solution and the above chromatography conditions we determine the samples content, the result of the content of two components in *Taxus chinensis var. mairei* were shown in Tables 2 and 3.

Selection of detection wavelength: We found the wavelength of maximum absorption of taxol is 227 nm, while the wavelength of maximum absorption of 10-deacetylbaccatin III is 232 nm by UV spectroscopy scanning. In order to facilitate testing, finally we chose 232 nm as the monitoring wavelength.

Selection of moving phase: Taxol and 10-deacetylbaccatin III easily dissolved in acetonitrile-water system, so choose the system as mobile phase, by changing the proportion of acetonitrile, we could make the target peak and the impurity peak completely separated from each other.

Selection of extracting conditions: Through observing and studying the extraction effects of different extraction methods, different extraction solvent and extraction times on taxol and 10-deacetylbaccatin III, we found that under the extraction method of at 45 °C and ultrasonic extracted for three times, 0.5 h per time, moreover the dosage of methanol is in turn 50, 30 and 20 times of sample volume, the taxol and 10-deacetylbaccatin III of *Taxus chinensis var. mairei* could be fast and efficient extracted completely.

Inspection of extraction solution of methylene chloride: Observing and studying the extraction volume (10, 20, 30 mL) and extraction number of times (1, 2, 3, 4 times), we found that a small amount of extraction can save the solvent and make the target component extracted completely, so we finally decided to use 30 mL methylene chloride extraction 1 time first, then use 20 mL methylene chloride extraction 2 times.

The analysis results showed that in the different growth years of *Taxus chinensis var. mairei*, the highest contents of taxol and 10-deacetylbaccatin III of the medicinal materials is in the third year, which means the effective composition contents of 3-year-old *Taxus chinensis var. mairei* is higher than others. Compared with other harvesting period of 3-year-old *Taxus chinensis var. mairei*, the highest content of effective elements of the medicinal materials is in May and June. Thus we predicted the the best harvesting time of *Taxus chinensis var. mairei* is in May and June. Based on the results and our comprehensive consideration, we predicted that May and June may be the best collection time of 3-year-old *Taxus chinensis var. mairei*.

This experiment adopts the method of ultrasonic extraction and the extraction process was optimized. RP-HPLC method is simple, economic and reliable. The separation degree is far more than the demand of quantitative analysis. The method is simple, accurate and reproducible and the experimental results can provide certain reference basis for the quality control of *Taxus chinensis var. mairei.*

TABLE-2
CONTENT OF TWO COMPONENTS IN *Taxus chinensis va. mairei collected* IN DIFFERENT GROWTH YEAR (n = 3)

No	Time (year)	Taxol		10-DAB III	
		Content (mg g^{-1})	RSD (%)	Content (mg g^{-1})	RSD (%)
1	1.5	0.2892	1.45	0.5159	1.25
2	2	0.3276	2.06	0.6673	2.04
3	3	0.3930	1.33	0.9470	1.64
4	4	0.3375	1.92	0.6213	2.15
5	5	0.1539	1.67	0.1788	2.23

TABLE-3
CONTENT OF TWO COMPONENTS IN *Taxus chinensis va. mairei* COLLECTED IN VARIOUS PERIODS (n = 3)

No	Time (month)	Taxol		10-DAB III	
		Content (mg g^{-1})	RSD (%)	Content (mg g^{-1})	RSD (%)
1	2012-03-10	0.2533	1.44	0.4214	1.57
2	2012-04-16	0.2940	1.92	0.4668	1.91
3	2012-05-14	0.3510	2.31	0.7229	2.28
4	2010-06-03	0.3601	1.34	0.9323	1.53
5	2010-07-22	0.3365	1.57	0.6295	1.26
6	2010-08-13	0.2990	1.89	0.6028	1.06

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