ELSEVIER

Journal of Chromatography A, 750 (1996) 253–256

JOURNAL OF CHROMATOGRAPHY A

Determination of taxol in *Taxus* species grown in Hungary by high-performance liquid chromatography–diode array detection Effect of vegetative period

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Abstract

The influence of a vegetative period on the taxol content in the needles of *Taxus brevifolia* grown in Hungary was determined using porous graphitized carbon column and a HPLC–diode array detection system. The relative standard deviation of the retention time of taxol peak was 1.24%, the peak symmetry 1.06–1.07 indicating the reliability of the HPLC systems. It was found that the accuracy of the peak purity test can be enhanced by carrying out the test at various wavelengths. It was established that the taxol content is considerably higher in the winter months and taxol is purer in the same period. Taxol content also depends on the production site and taxol extracted from the foliage contains more taxoteres than taxol extracted from bark.

Keywords: Taxol; *Taxus baccata*

1. Introduction

Taxol, a highly functionalized taxane diterpene amide ([2a*R*-[2aα,4β,4aβ,6β,9α(a*R**,β*S**),11α,-12α, 12aα, 12bα]]-β-(benzoylamino)-α-hydroxybenzenepropanoic acid 6,12b-bis(acetyoxy)-12-(benzoyl-oxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a, 8,13,13-tetramethyl-5-oxo-7,11-methano-14-cyclodeca[3,4]benz[1,2-b]oxet-9-yl ester was firstly isolated from the bark of *Taxus brevifolia* [1]. Taxol has been successfully used for the treatment of various malignant tumors such as ovarian carcinomas [2–4], metastatic breast cancer [5,6], non small-cell-lung cancer [7,8] and adenocarcinoma and squamous cell carcinoma of the esophagus [9]. Taxol showed other biological effects too, i.e. it inhibited progression of congenital polycistic kidney disease in mice [10].

The growing demand for taxol stimulated research for other natural sources of taxol [11]. Thus, it has been established that not only *T. brevifolia* but other *Taxus* species such as *T. baccata* [12], *T. cuspidata* [13], *T. wallichiana* [14], and *T. xmedia* cv. Hicksii [15,16] also contain taxol. The chemistry and biology of taxol have been reviewed many times [17–19].

Many efforts have been devoted for the development of HPLC methods for the separation and quantitative determination of taxol in various *Taxus* species. A wide variety of supports (cyano [20], silica [21], pentafluorophenyl- [22], phenyl- [23,24], octadecyl- [25,26] and octyl-coated silica [27], porous graphitized carbon [28] and diphenylmethylsilyl [29]) have been tested for their capacity to separate taxol from the other impurities in the plant extracts.

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0021-9673/96/$15.00
PII S0021-9673(96)00541-9

Not only isocratic but also gradient elution has been used for the separation [30].

As it has been previously indicated that the age of foliage can influence the taxol content in *T. brevifolia* [31], the objective of our investigation was the elucidation of the effect of vegetative period on the taxol content in *Taxus* species grown in Hungary using high-performance liquid chromatography (HPLC) combined with diode array detection system (DAD).

2. Experimental

The influence of the vegetative period on the taxol content in the needles of *T. baccata* was studied in samples collected monthly in the arboretum in Budapest from December 1993 to December 1994. As our preliminary investigations indicated that the needles of *T. baccata* from the arboretum in Kámon (West Hungary) contain the highest quantity of taxol, the taxol content of four clones of *T. baccata* grown in Kámon was also determined (sampling time February 1995). To compare the taxol content of needles and bark, samples of bark were used as controls. Extraction was carried out as described in ref. [32]. Measurements were carried out on a porous graphitized carbon (PGC) column (Shandon Hypercarb 100×4.6 mm I.D., particle size 7 μm; Shandon Scientific, Cheshire, UK). The HPLC equipment consisted of a ISCO model 2350 HPLC pump and a ISCO model 2360 gradient programmer (Isco, Lincoln, NE, USA), a Rheodyne injector with a 20 μl sample loop (Rheodyne, Cotati, CA, USA), a Waters 991 photodiode array detector (Millipore, Waters, Milford, MA, USA), a NEC Power Mate SX/16 computer (NEC Technologies, Boxborough, USA) and a Waters 5200 printer plotter (Millipore, Waters). The detection wavelength range was 225–399 nm, the calibration curve and the qualitative evaluation was carried out at 228 nm at the absorption maximum of taxol. The flow-rate was 0.8 ml/min. The eluent was a mixture of 1,4-dioxane–water (46:54, v/v). The experiments were carried out at room temperature (22–24°C). Standard of taxol (from *Taxus brevifolia*, purity min.95%) was purchased from Sigma (Deisenhofen, Germany) and was used without further purification. Each determination was carried out in quadruplicate and the relative standard deviation of the peak position, asymmetry factor and the quantity of taxol was calculated. Peak purity test at various detection wavelengths (228, 238 and 248 nm) was carried out for the taxol peak of each sample. The use of this procedure was motivated by the finding that the results of the peak purity test may depend on the wavelength used [33].

3. Results and discussion

It was established that the anticancer drug taxol was present in measurable amounts in each sample. The mean, the relative standard deviation of the taxol content in the needles of *T. baccata* collected in the arboretum in Budapest and the results of peak purity test carried out at the maximum absorbance of taxol (match %) are compiled in Table 1 and visualized on Fig. 1. The data in Table 1 clearly show that the taxol content of needles markedly depends on the vegetative period of the plant, it is considerably higher in the winter months. This finding indicates that the needles harvested between October and February represent a better source of taxol than the needles harvested in any other period. It is interest-

Table 1
Effect of vegetative period on taxol content (mg/g) in the needles of *Taxus baccata*

Date of sampling		Mean	R.S.D.	Match %
Month	Year			
December	1993	0.039	3.14	65.37
February	1994	0.037	2.87	66.12
March	1994	0.032	3.51	66.03
April	1994	0.029	2.92	65.92
May	1994	0.028	3.36	63.97
June	1994	0.026	3.65	58.15
July	1994	0.029	3.43	59.65
August	1994	0.030	2.89	59.02
September	1994	0.032	3.28	60.05
October	1994	0.037	3.40	60.12
November	1994	0.036	3.16	60.09
December	1994	0.039	2.98	62.39

R.S.D.= relative standard deviation. Match %=results of peak purity test carried out at the maximum absorbance of taxol.

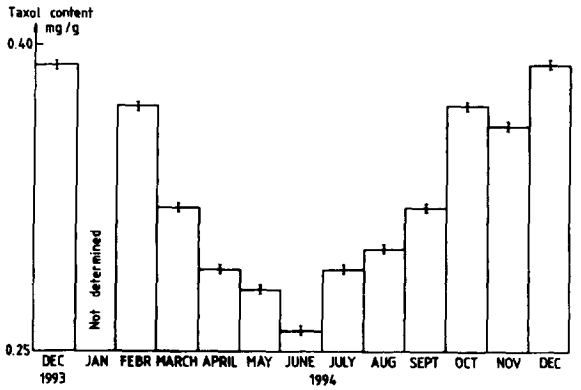

Fig. 1. Effect of vegetative period on the taxol content in the needles of *Taxus baccata*.

ing to note that peak purity tests indicate that the taxol peak is less homogeneous in the extracts from needles than in the extracts from bark (match %= 92.43). This result may be due to the fact that other taxoters can also be present in the extracts of the foliage of *T. baccata* [34]. Their quantity is markedly lower in the extract of bark. However, it can also be established that the match values of the peak purity test are higher in the period between December and May. This finding also indicates that the most appropriate period for harvesting the needles of *T. baccata*, for the production of taxol, is the winter months. These needles not only contain a higher quantity of taxol but the purity is also higher.

The taxol content of the various *T. baccata* clones grown in Kámon, Hungary and the relative standard deviation of the measurements are compiled in Table 2. The data in Table 2 indicate that the taxol content of *T. baccata* needles grown in Kámon is higher than that of the plants grown elsewhere in Hungary. This finding draws the attention to the fact that the climatic conditions, soil composition, etc., may have a considerable influence on the taxol content in the needles of *T. baccata*, and the taxol content may considerably depend on the site of production.

It was interesting to note that the results of peak purity tests slightly differ according to the wavelength used (Table 3). This effect is higher in the case of needles than in the case of bark. This discrepancy can be tentatively explained by the supposition that the extract of needles contains various other taxoteres with absorption spectra slightly different from that of taxol. The spectral differences results in the marked decrease of matching values of the peak purity test. This finding suggests that the validity of the peak purity test can be considerably enhanced when the test is carried out at various wavelengths.

The relative standard deviation of the retention time of the peak of taxol was 1.24%, indicating the good reproducibility and stability of the HPLC–DAD sytem used for the investigations. The values of peak symmetry were between 1.06–1.07, that means that the peak distorsion was minimal on a PGC column using dioxane–water eluent mixture.

It can be concluded from the data that the needles of *T. baccata* contain more taxol in the winter period and the taxol is purer. Not only the vegetative period but also the climatic conditions may have a considerable effect on the taxol content of *T. baccata* needles. The reliability of the peak purity test can be enhanced by carrying out the test at various wavelengths. HPLC–DAD using a PGC proved to be a suitable method to study the dependence of taxol content on the vegetative period and cultivating site of *T. baccata*.

Table 2
Taxol content (mg/g) in the needles of the various clones of *Taxus baccata* grown in the arboretum in Kámon (West Hungary)

No. of clone	Mean	R.S.D.
1	0.034	3.21
2	0.037	2.96
3	0.041	3.38
4	0.045	3.37

R.S.D.=relative standard deviation

Table 3
Effect of the wavelength selection on the results of peak purity test (match %) in the extracts of needles and bark of *Taxus baccata*

Wavelength (nm)	Bark	Needles
228	92.43	63.67
238	92.49	60.86
248	92.30	60.39

Acknowledgments

This work was supported by the grant OTKA T7340.

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