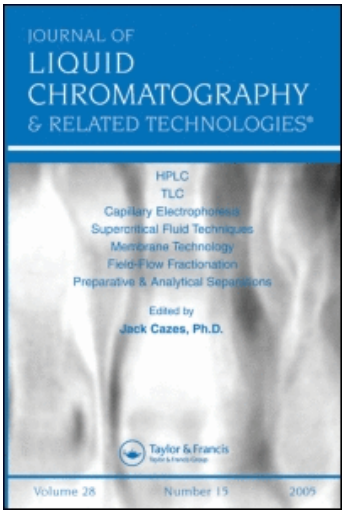

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HPLC DETERMINATION OF TAXOL AND RELATED COMPOUNDS IN *TAXUS* PLANT EXTRACTS

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ABSTRACT

A sensitive and reproducible HPLC method, using a Curosil G column and gradient elution, was developed for the routine analysis of taxol (I) and six related taxanes, 10-desacetyl-7-epi-taxol (II), cephalomannine (III), 10-desacetyl taxol (IV), baccatin III (V), 10-desacetyl-7-epi-baccatin III (VI), and 10-desacetyl baccatin III (VII) in *Taxus* plant extracts. The method was linear for taxanes I-VII within the concentration range tested of 0.1μg - 2.4 μg injected. Purging and regeneration procedures were used which allowed more than 600 injections to be made onto the same column without the development of backpressure problems.

The day-to-day variation in the peak area of taxanes I-VII was minimum (C.V. = 9.8%, 9.1%, 5.8%, 7.7%, 7.4% and 7.8% respectively, n = 12). Variation within a day in the peak area of taxanes I-VII was even less (C.V. = 1.5%, 1.3%, 1.4%, 1.4%, 1.4%, 1.5% and 1.5%, respectively, n = 4).

The separation efficiency of the Curosil G column was compared to pentafluorophenyl (Taxil) and diphenyl (Supelcosil) bonded silica columns using 3 gradient elution systems.

INTRODUCTION

Taxol, a complex diterpene amide isolated from the bark of the Pacific yew tree *Taxus brevifolia* Nutt. (Taxaceae)[1], has been the subject of intensive research due

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to its unique cancer chemotherapeutic properties [2]. Since taxol occurs with a series of closely related taxanes in various species of *Taxus* [3-6], considerable work has been directed to devising effective methods of analysis and purification.

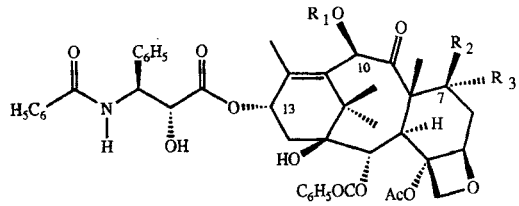
In early work high performance liquid chromatography (HPLC) with UV detection was used for the quantitation of taxol and the other taxanes in plant extracts [7-13], biological samples [14,15], and bulk taxol [16]. The HPLC columns used in these applications included bonded silica-C_{18} and silica-phenyl packing material operated in the reversed phase mode, and -CN columns operated both in the reversed and normal phase modes [7,17]. More recently, better resolution has been achieved with pentafluorophenyl (PFP) bonded silica as the column packing material [16]. There has, however, been a problem when a large number of plant extracts are analyzed with automated systems. After many injections the backpressure increases, resulting in a drastic decrease in column efficiency.

This paper describes a selective, reversed-phase HPLC method for *Taxus* plant extracts utilizing a Curosil G column, with a proprietary bonded phase packing material, for the routine determination of taxol (I) and six closely related taxanes: 10-deacetyl-7-*epi*-taxol (II), cephalomannine (III), 10-deacetyl taxol (IV), baccatin (V), 10-deacetyl-7-*epi*-baccatin III (VI) and 10-deacetyl baccatin III (VII). The backpressure problems were avoided by using a column purging procedure after each run. The separation efficiency of pentafluorophenyl (Taxil) and diphenyl (Supelcosil) columns is also presented and compared to that of the Curosil G column.

EXPERIMENTAL

Materials

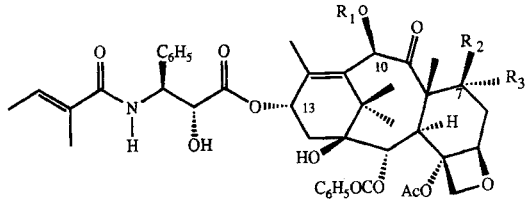
Curosil G HPLC column (4.6 x 250 mm, 6 µm) with a precolumn (4.6 x 30 mm) was purchased from Phenomenex (Torrance, CA). Taxil, a pentafluorophenyl



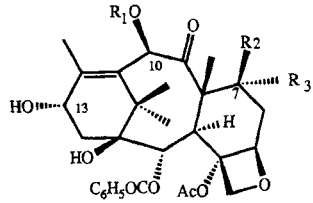
(I) ; R_1=Ac, R_2=OH, R_3=H

(II) ; R_1=Ac, R_2=H, R_3=OH

(IV) ; R_1=H, R_2=OH, R_3=H



(III) ; R_1=Ac, R_2=OH, R_3=H



(V) ; R_1=Ac, R_2=OH, R_3=H

(VI) ; R_1=H, R_2=H, R_3=OH

(VII) ; R_1=H, R_2=OH, R_3=H

(PFP) HPLC column (4.6 x 250 mm, 5 µm), and a precolumn (4.6 x 13 mm) came from Metachem Technologies Inc. (Redondo Beach, CA). Supelcosil LC-DP diphenyl HPLC column (4.6 x 250 mm, 5 µm) was bought from Supelco Inc. (Bellefonte, PA) and used with a New Guard phenyl precolumn (3.2 x 15 mm, 7 µm) purchased from Applied Biosystems Inc. (San Jose, CA). A high pressure column pre-filter (0.5 µm) was purchased from Alltech Associates Inc. (Deerfield, IL) and used in all analyses.

HPLC grade acetonitrile, reagent alcohol, tetrahydrofuran, methyl-*t*-butyl ether and certified ethylene glycol monomethyl ether were purchased from Fisher Scientific Co. (Fair Lawn, NJ). The nanopure water (18 MΩ) was obtained in-house using a Barnstead nanopure purificator. All solvents and samples were filtered through a 0.45 µm nylon membrane (Alltech).

The seven taxane standards (I-VII) were provided by the National Cancer Institute through Dr. Kenneth Snader.

Apparatus

Instrumentation consisted of a Waters 600E Multisolvent Delivery System, Waters 712 WISP Auto sampler, Waters 991 Photodiode Array and NEC Power Mate SX Plus computer for controlling the analytical system and for data processing. The chromatograms and data reports were printed on a Waters 5200 Printer Plotter. Standards were weighed on a Mettler H51AR microbalance.

Methods

Sample preparation and injection

Powdered plant material (1g) was extracted twice with 10 ml of 95% ethanol by soaking with agitation for 16 hours. The combined extracts (25 ml) were evaporated to dryness, and the residue was partitioned 3 times between water (2 ml) and methylene chloride (4 ml). The organic fraction was evaporated and the

residue adsorbed on 1g of celite packed into a small column. The column was washed with 15 ml hexane until the eluate was colorless, followed by 15 ml methylene chloride. The methylene chloride wash was evaporated and dissolved in reagent alcohol to produce a solution containing ~ 20 mg/ml, from which 10 µl volume was injected.

The mixture of standards was prepared in reagent ethanol such that each standard was at a concentration of ~100 µg/ml and ten microliters were injected.

Chromatographic Conditions

Chromatography was performed using the following gradient programs (Table 1):

Gradient System 1. Linear gradient at a flow rate of 1.5 ml/min using a mobile phase starting with a 80:20 mixture of Solvent B (*reagent alcohol:water (6:94) and Solvent C (100% acetonitrile) going to 75:25, 60:40 and 55:45 (Solvent B:Solvent C) in 10, 30 and 40 minutes, respectively, and ending with a 50:50 mixture of (Solvent B:Solvent C) for 45 minutes. The system was purged for 3 minutes with a 50:50 mixture of Solvent A (reagent alcohol:tetrahydrofuran:methyl-*t*-butyl ether, 50:30:20) and Solvent C (100% acetonitrile), followed by 10 minutes equilibration with the initial solvent composition for a total of 1 hour/sample. (*N.B. reagent alcohol consists of a mixture of 90.6% ethanol, 4.5% methanol and 4.9% *iso*-propanol).

Gradient System 2. Linear gradient at a flow rate of 1.2 ml/min using a mobile phase starting with 75:25 mixture of Solvent B (reagent alcohol:water, 6:94) and Solvent C (100% acetonitrile) going to 70:30, 55:45 (Solvent B:Solvent C) in 12 and 35 minutes, respectively; and ending with a 50:50 mixture (Solvent B:Solvent C) for 45 minutes. The system was purged for 3 minutes using the same mobile phase described under Gradient System 1, followed by 10 minutes equilibration with the initial solvent composition for a total of 1 hour/sample.

TABLE 1

Gradient Tables for Gradient Systems 1-3

A. Gradient System 1 (Flow rate maintained at 1.5 ml/minute)

Gradient Time (min.)	Solvent A	Solvent B	Solvent C
0	0	80	20
10	0	75	25
30	0	60	40
40	0	55	45
45	0	50	50
46	50	0	50
49	50	0	50
50	0	80.0	20
60	0	80.0	20

B. Gradient System 2 (Flow rate maintained at 1.2 ml/minute)

0	0	75	25
12	0	70	30
35	0	55	45
45	0	50	50
46	50	0	50
49	50	0	50
50	0	75	25
60	0	75	25

C. Gradient System 3 (Flow rate maintained at 1.1 ml/minute)

0	0	76	24
12	0	70	30
36	0	55	45
45	0	50	50
46	50	0	50
49	50	0	50
50	0	76	24
60	0	76	24

Solvent A =*reagent alcohol:tetrahydrofuran:methyl-*t*-butyl ether (50:30:20)
Solvent B = reagent alcohol:water (6:94) for gradient system1 and 2; ethylene glycol monomethyl ether:water (8:92) for gradient system 3
Solvent C = acetonitrile (100%)

*Fisher Scientific Reagent Alcohol:90.6% ethanol, 4.5% methanol and 4.9% *iso*-propanol

Gradient System 3. Linear gradient at a flow rate of 1.1 ml/min using a mobile phase starting with 76:24 mixture of Solvent B (ethylene glycol monomethyl ether:water 8:92) and Solvent C (100% acetonitrile) going to 70:30, 55:45 (Solvent B:Solvent C) in 12 and 36 minutes, respectively, and ending with a 50:50 mixture (Solvent B:Solvent C) for 46 minutes. The system was purged for 3 minutes using the same mobile phase described under gradient system 1, followed by 10 minutes equilibration with the initial solvent composition for a total of 1 hour/sample.

After the analysis of 10-12 samples the column was flushed with the following regeneration system: starting at the flow rate and mobile phase mixture of Solvent B and Solvent C , and going to 50:50 (Solvent B:Solvent C) in 10 minutes, then a 50:50 mixture of Solvent A (reagent alcohol:tetrahydrofuran:methyl-*t*-butyl ether, 50:30:20) and Solvent C (100% acetonitrile) in 20 minutes,100% Solvent A from 25 to 35 minutes, a 50:50 mixture of Solvent A:Solvent C in 45 minutes, a 50:50 mixture of Solvent B:Solvent C in 50 minutes, and terminating with a starting mixture of Solvent B:Solvent C in 60 minutes.

The spectral data were collected over the 210-300 nm range of the absorption spectrum; chromatograms were plotted at the absorption maxima of taxol 227, and 273 nm.

The linearity of the detector response was determined for taxanes I-VII by injection of a series of standard solutions ranging in concentration from 10-240 µg/ml using the Curosil G column and gradient systems 1 and 3.

RESULTS AND DISCUSSION

A procedure was developed for the analysis of taxol (I) and 6 other related taxanes II-VII: 10-deacetyl-7-*epi*-taxol (II), cephalomannine (III), 10-deacetyl taxol (IV), baccatin III (V), 10-deacetyl-7-*epi*-baccatin III (VI) and 10-deacetyl

baccatin III (VII) in the needles of *Taxus* plants. Three gradient systems were tested, each using 3 different columns: Curosil G, Taxil (pentafluorophenyl, PFP) and Supelcosil (diphenyl, DP). The identity of the peaks was determined using retention times and confirmed by comparison of the UV spectra of peaks of interest with those of standard taxanes (Peak match see Table 3). In addition, the wavelength response ratio (spectral index) obtained for the different taxanes (A_{227}/A_{273}) provided an estimation of peak purity for the taxanes analyzed when compared with the values obtained for the standards I-VII (Table 2).

Since the taxanes studied represent compounds with a relatively wide range of polarity and molecular size, their separation in a single run was a challenging task. In all gradient systems 1-3 (Table 1) when Curosil G, Taxil and Supelcosil columns were used (Figures 1A-8A) a run time of 45 minutes resulted in a baseline separation of standard taxanes I-VII. However, when gradient system 3 and Supelcosil column were used, taxanes I and II appeared on a hump and this might be due to insufficient purging of the column (Figure 9A). The run time of 45 minutes was approximately equal to that obtained with gradient elution methods previously reported for the analysis of multitaxanes in extractives of the bark (7) and needles (8) using a phenyl column.

To avoid back pressure problems associated with multiple injections of plant extracts, the column was purged for 3 minutes after each run with a 50:50 mixture of Solvent A and Solvent C. Solvent A was a mixture of 50 parts reagent alcohol, 30 parts tetrahydrofuran, and 20 parts of methyl-t-butyl ether, while Solvent C was 100% acetonitrile. In addition, after the analysis of 10-12 samples the column was washed with the regeneration gradient system (see experimental). The purging and washing procedures described above allowed for the apparent removal of non-polar components in the plant extracts, the accumulation of which is responsible for the increase of back pressure. In this way, it was possible to perform more than 600 injections on the same column using the processed ethanol extracts.

TABLE 2

Wavelength Response Ratio and Response Factor for the Standards When 3 Different Columns Were Tested Each Using 3 Different Gradient Systems.

Wavelength Response Ratio[1] & Response Factor[2]

Column	Gradient System	DAB (VII)	DEAB (VI)	BIII (V)	DAT (IV)	CPN (III)	DAET (II)	TAX (I)
Curosil G	1	13.32(1.40)	14.23(1.65)	13.65(1.82)	15.49(2.10)	21.51(1.77)	15.91(1.85)	16.37(1.88)
	2	13.13(1.73)	14.18(2.04)	13.90(2.34)	15.60(2.57)	21.97(2.18)	15.51(2.27)	16.08(2.28)
	3	13.50(1.99)	14.57(2.04)	13.90(2.56)	15.38(2.90)	21.30(2.45)	15.84(2.54)	16.56(2.54)
Taxil	1	13.21(1.37)	14.38(1.54)	13.94(1.72)	15.55(1.98)	22.13(1.60)	15.80(1.73)	16.31(1.81)
	2	*	*	13.98(1.95)	14.10(2.20)	14.48(2.49)	21.82(1.99)	15.78(2.14)	15.88(2.25)
	3	13.17(1.86)	14.25(2.22)	13.52(2.49)	15.36(2.95)	22.27(2.32)	15.60(2.49)	15.96(2.52)
Supelcosil	1	12.98(1.44)	14.73(1.64)	13.67(1.82)	15.78(2.05)	22.58(1.93)	15.88(1.79)	15.74(1.80)
	2	13.30(1.72)	14.36(2.04)	13.85(2.25)	15.62(2.54)	22.19(2.39)	15.78(2.21)	15.98(2.20)
	3	13.48(1.90)	14.55(2.21)	14.02(2.48)	15.67(2.86)	22.59(2.69)	16.99(2.17)	14.08(2.77)

[1] wavelength response ratio calculated as the ratio A_{227}/A_{273}

[2] values shown between brackets in (AU*min/µg)*E-2

* peak not detected at 273 nm

Taxanes Code: DAB=10-deacetyl baccatin III, DEAB=10-deacetyl-7-*epi*-baccatin III, BIII=baccatin III, DAT=10-deacetyl taxol, CPN=cephalomannine, DAET=10-deacetyl-7-*epi*-taxol and TAX=taxol.

TABLE 3

Wavelength Response Ratio and Peak Match for the Extracts When 3 Different Columns Were Tested Each Using 3 Different Gradient Systems.

Wavelength Response Ratio[1] & Peak Match[2]

Column	Gradient System	DAB (VII)	DEAB (VI)	BIII (V)	DAT (IV)	CPN (III)	DAET (II)	TAX (I)
Curosil G	1	8.32(870)	2.25(628)	* (769)	18.13(930)	26.00(937)	• (776)	15.40(970)
	2	10.71(857)	2.36(621)	* (798)	18.16(943)	21.42(944)	* (803)	13.73(975)
	3	11.32(880)	• (808)	* (866)	16.70(949)	32.06(934)	• (732)	14.25(969)
Taxil	1	19.74(635)	* (623)	* (764)	15.13(909)	25.38(932)	0.43(353)	1.75(857)
	2	13.00(832)	* (630)	* (811)	14.78(944)	27.67(962)	0.45(378)	1.84(709)
	3	9.00(828)	* (852)	* (907)	15.97(950)	21.63(940)	0.44(375)	1.88(671)
Supelcosil	1	6.28(829)	2.54(675)	5.19(762)	0.31(819)	32.05(901)	* (817)	14.57(968)
	2	* (853)	2.63(648)	4.65(739)	0.43(391)	12.05(932)	• (835)	13.98(968)
	3	12.82(869)	* (695)	• (828)	0.56(430)	28.25(921)	* (812)	17.62(950)

[1] wavelength response ratio (calculated as the ratio A_{227}/A_{273})
[2] values shown between brackets
• peak not detected at 273nm

Taxanes Code: DAB=10-deacetyl baccatin III, DEAB=10-deacetyl-7-*epi*-baccatin III, BIII=baccatin III, DAT=10-deacetyl taxol, CPN=cephalomannine, DAET=10-deacetyl-7-*epi*-taxol and TAX=taxol.

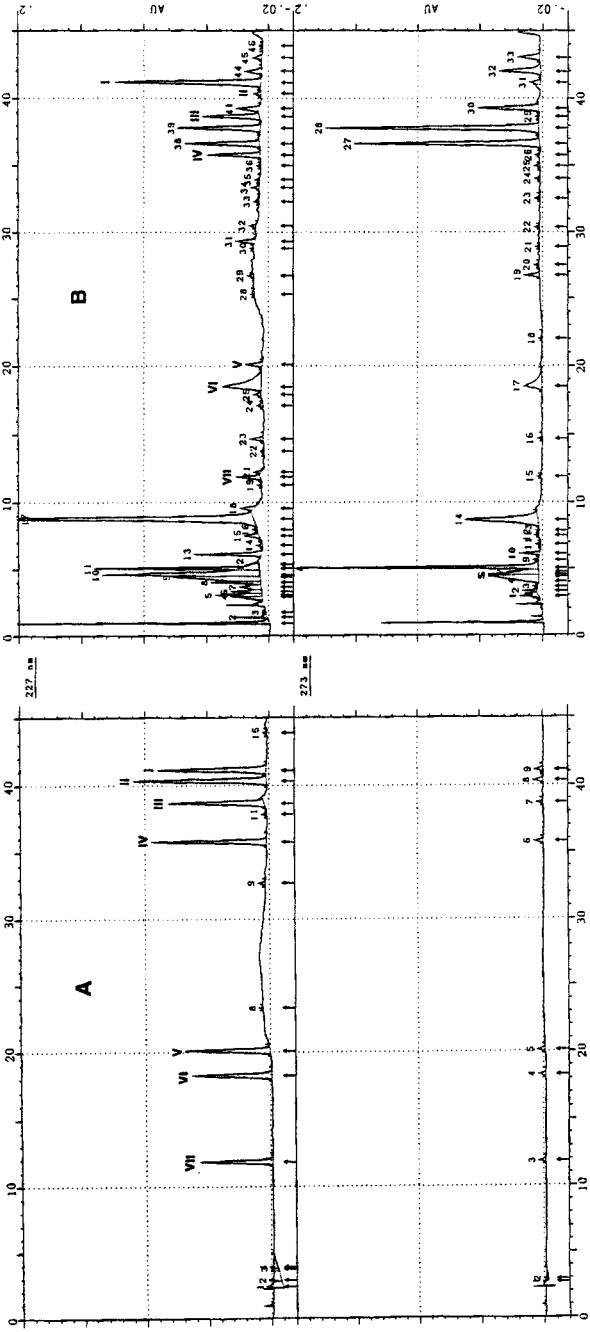


Figure 1. Chromatograms obtained on Curosil G column and gradient system 1.
A. Chromatogram of standards: I=taxol, II=10-deacetyl-7-*epi*-taxol, III=cephalomannine, IV=10-deacetyl taxol, V=baccatin III, VI=10-deacetyl-7-*epi*-baccatin III, VII=10-deacetyl baccatin III
B. Chromatogram of the purified ethanol extract of *T. x media* 'Nigra' needles.

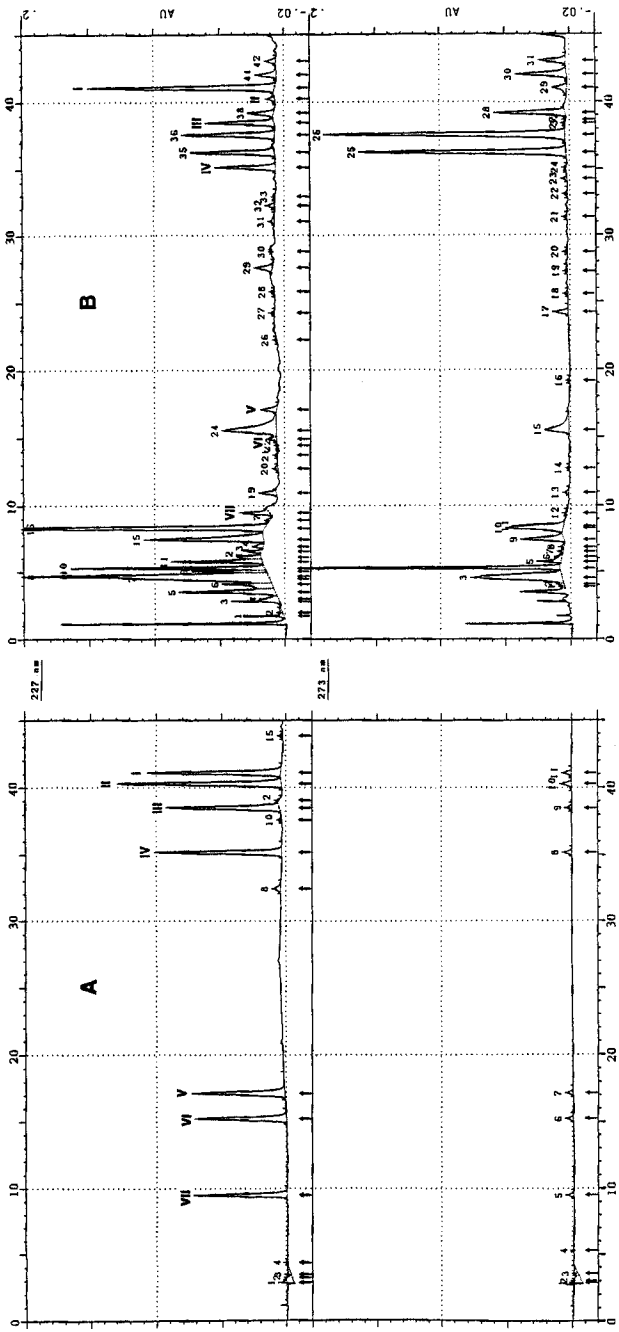


Figure 2. Chromatograms obtained on Curosil G column and gradient system 2.
A. Chromatogram of standards: I=taxol, II=10-deacetyl-7-*epi*-taxol, III=cephalomannine, IV=10-deacetyl taxol, V=baccatin III, VI=10-deacetyl-7-*epi*-baccatin III, VII=10-deacetyl baccatin III
B. Chromatogram of the purified ethanol extract of *T. x media* 'Nigra' needles.

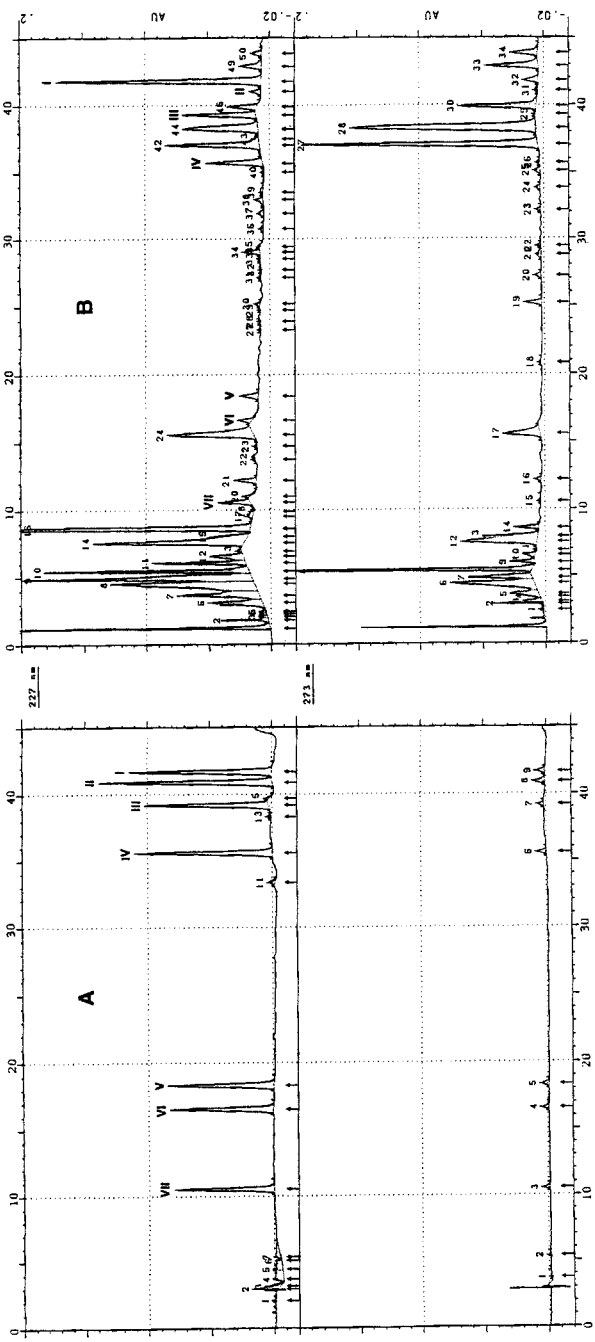


Figure 3. Chromatograms obtained on Curosil G column and gradient system 3.
A. Chromatogram of standards: I=taxol, II=10-deacetyl-7-*epi*-taxol, III=cephalomannine, IV=10-deacetyl taxol, V=baccatin III, VI=10-deacetyl-7-*epi*-baccatin III, VII=10-deacetyl baccatin III
B. Chromatogram of the purified ethanol extract of *T. x media* 'Nigra' needles.

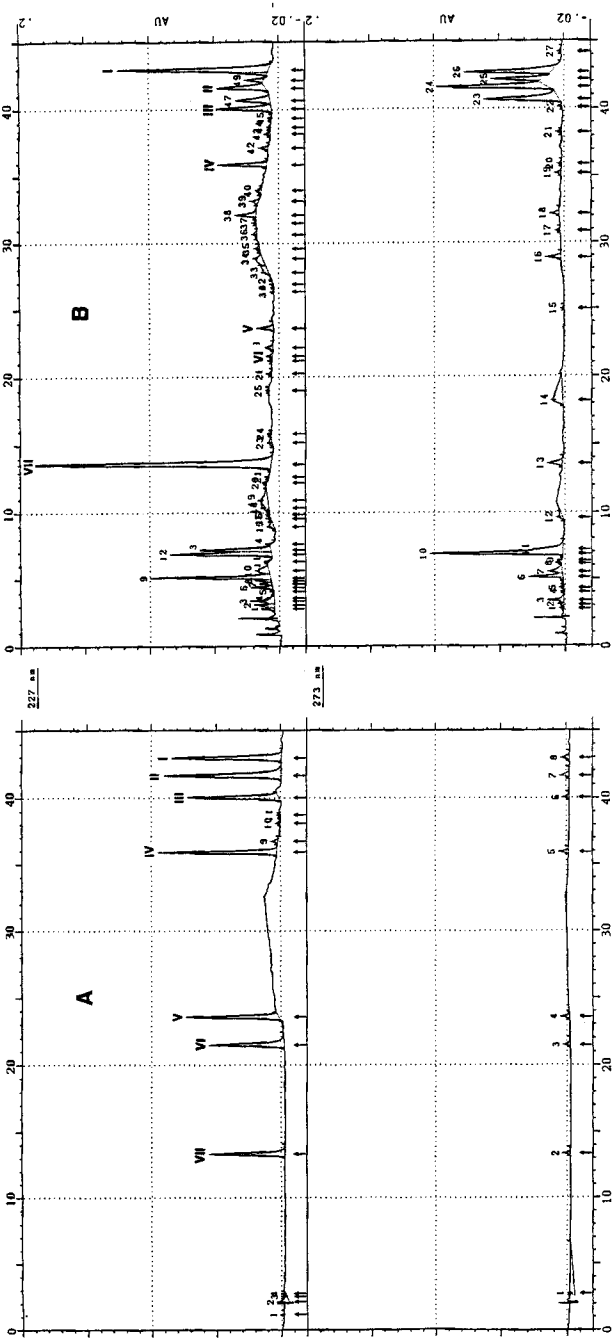


Figure 4. Chromatograms obtained on Taxil column and gradient system 1.
A. Chromatogram of standards: I=taxol, II=10-deacetyl-7-*epi*-taxol, III=cephalomannine, IV=10-deacetyl taxol, V=baccatin III, VI=10-deacetyl-7-*epi*-baccatin III, VII=10-deacetyl baccatin III
B. Chromatogram of the purified ethanol extract of *T. x media* 'Nigra' needles.

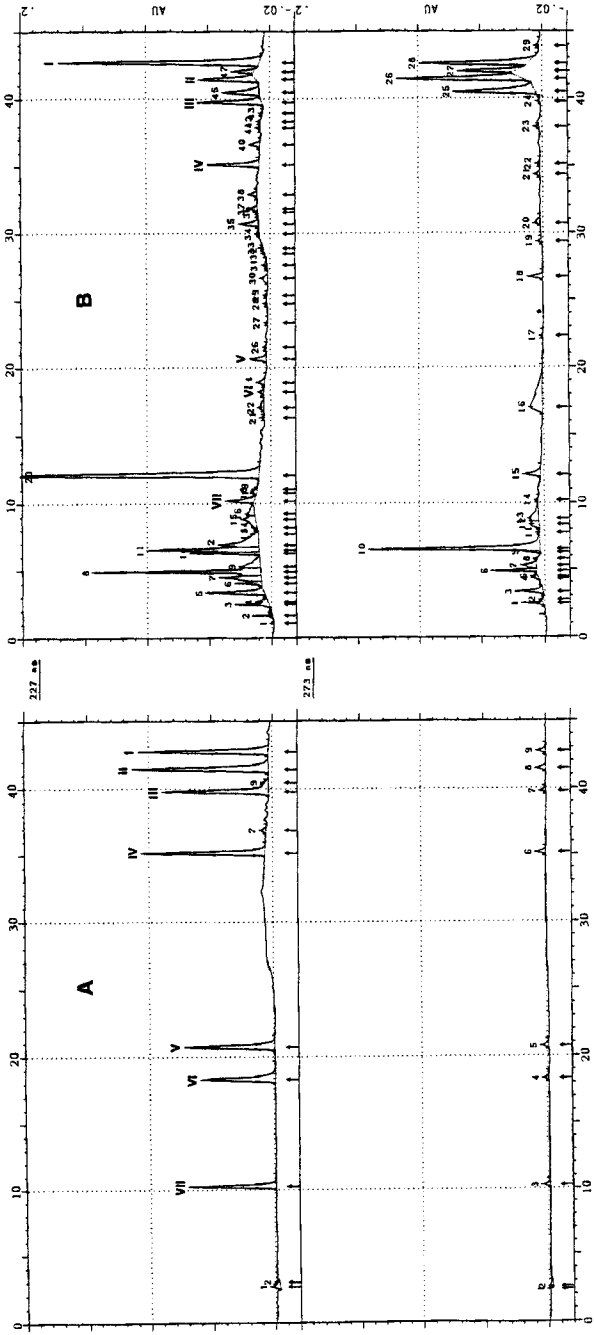


Figure 5. Chromatograms obtained on Taxil column and gradient system 2.
A. Chromatogram of standards: I=taxol, II=10-deacetyl-7-*epi*-taxol, III=cephalomannine, IV=10-deacetyl taxol, V=baccatin III, VI=10-deacetyl-7-*epi*-baccatin III, VII=10-deacetyl baccatin III
B. Chromatogram of the purified ethanol extract of *T. x media* 'Nigra' needles.

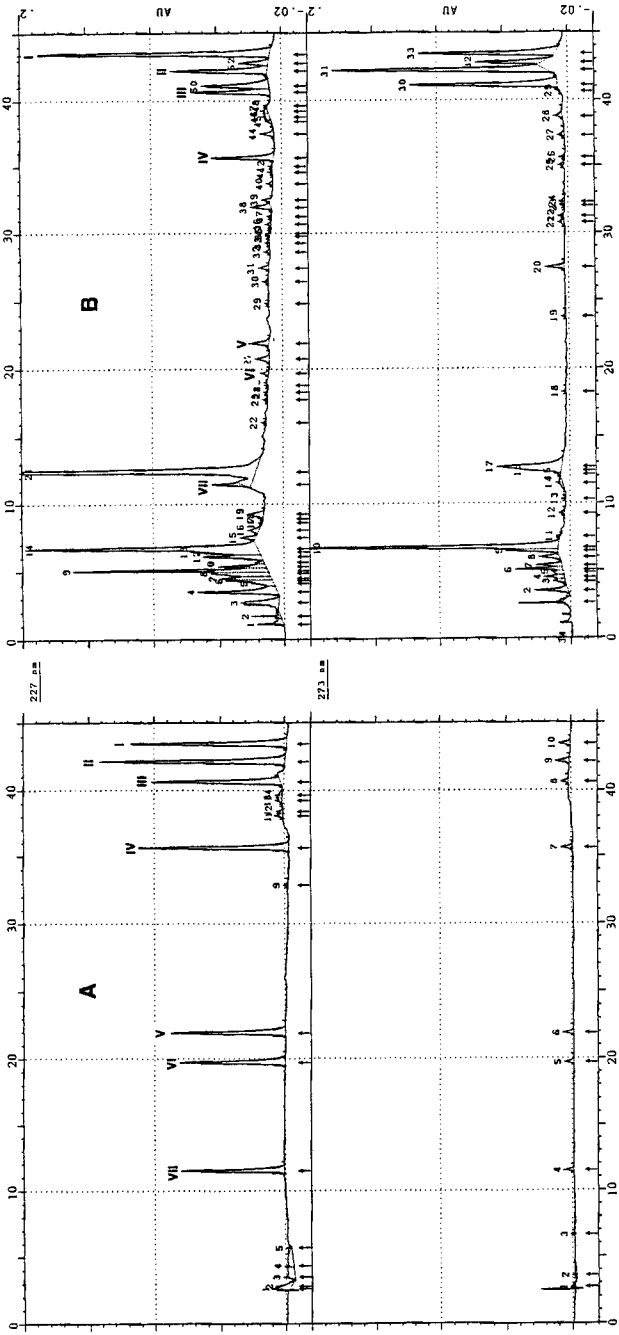


Figure 6. Chromatograms obtained on Taxil column and gradient system 3.
A. Chromatogram of standards: I=taxol, II=10-deacetyl-*7-epi*-taxol, III=cephalomannine, IV=10-deacetyl taxol, V=baccatin III, VI=10-deacetyl-*7-epi*-baccatin III, VII=10-deacetyl baccatin III
B. Chromatogram of the purified ethanol extract of *T. x media* 'Nigra' needles.

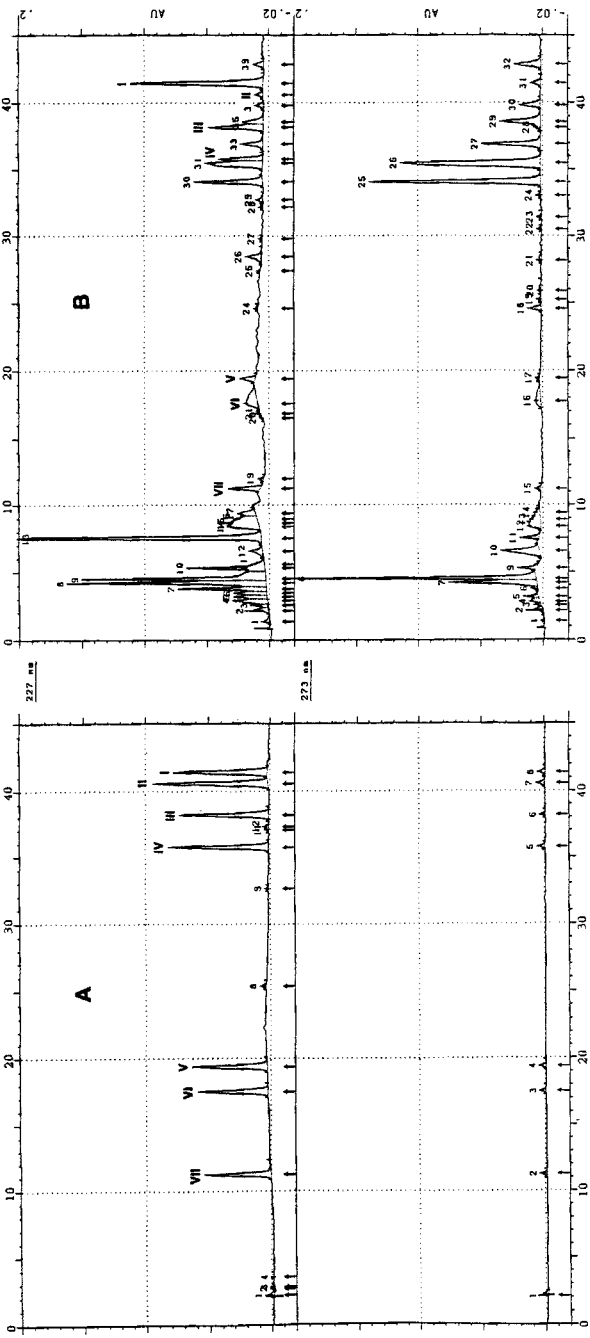


Figure 7. Chromatograms obtained on Supelcosil column and gradient system 1.
A. Chromatogram of standards: I=taxol, II=10-deacetyl-7-*epi*-taxol, III=cephalomannine, IV=10-deacetyl taxol, V=baccatin III, VI=10-deacetyl-7-*epi*-baccatin III, VII=10-deacetyl baccatin III.
B. Chromatogram of the purified ethanol extract of *T. x media* 'Nigra' needles.

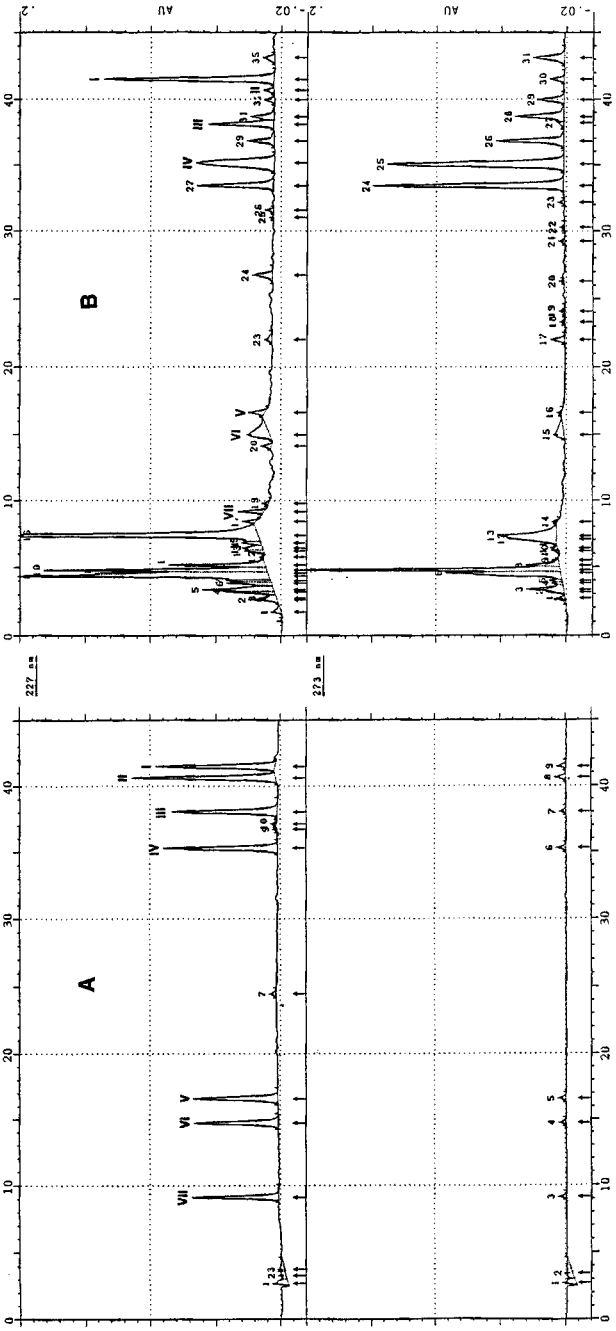


Figure 8. Chromatograms obtained on Supelcosil column and gradient system 2.
A. Chromatogram of standards: I=taxol, II=10-deacetyl-7-*epi*-taxol, III=cephalomannine, IV=10-deacetyl taxol, V=baccatin III, VI=10-deacetyl-7-*epi*-baccatin III, VII=10-deacetyl baccatin III
B. Chromatogram of the purified ethanol extract of *T. x media* 'Nigra' needles.

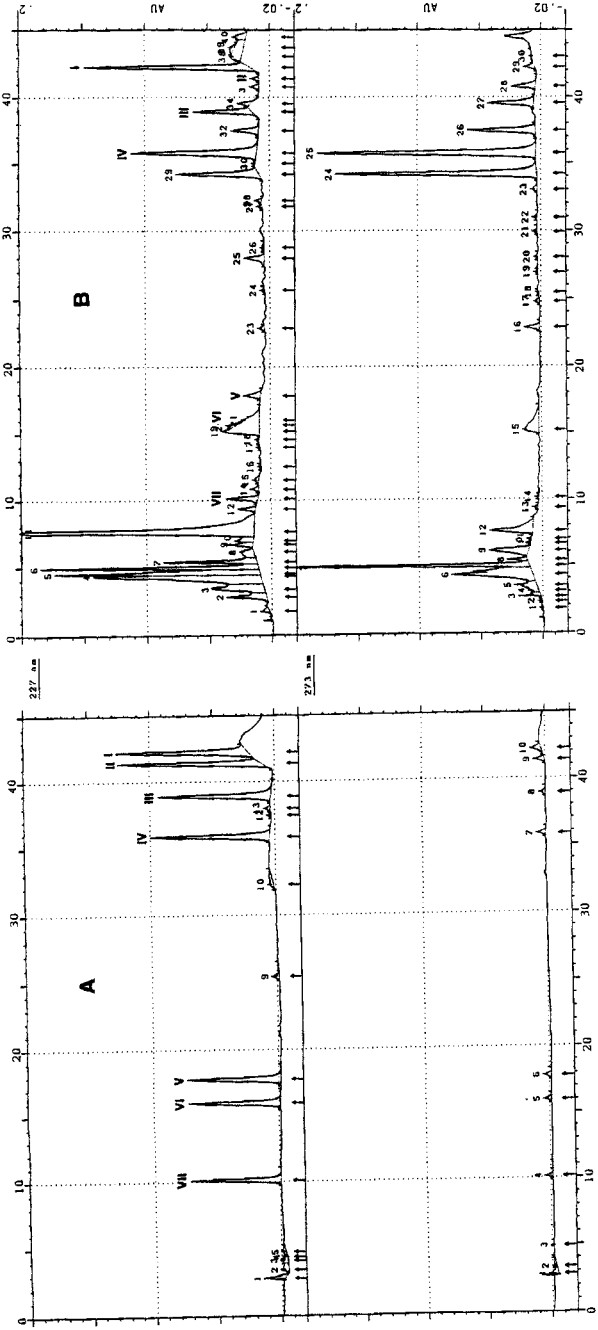


Figure 9. Chromatograms obtained on Supelcosil column and gradient system 3.
A. Chromatogram of standards: I=taxol, II=10-deacetyl-7-*epi*-taxol, III=cephalomannine, IV=10-deacetyl taxol, V=baccatin III, VI=10-deacetyl-7-*epi*-baccatin III, VII=10-deacetyl baccatin III
B. Chromatogram of the purified ethanol extract of *T. x media* 'Nigra' needles.

For HPLC analysis, the ethanolic extracts of *Taxus* needles were partitioned between water and methylene chloride. The methylene chloride phase was further purified by solid phase extraction with celite. Although the ethanol extract could be analyzed directly, it was found that the values of the taxanes studied in these extracts were about 20% higher than those obtained with the solid phase purification step. This apparent reduction in the taxanes content as a result of the celite purification step was found to be due to the removal of interfering substances and not due to incomplete recovery through spiking experiments. Most recently Castor and Tyler [18] reported that the analytical values for taxol in the needles of *T. x media* 'Hicksii' are inflated by 10% due to presence of a co-eluting substance.

The analysis of taxanes I-VII using the purified ethanolic extract of *T. x media* 'Nigra' needles was best achieved on the Curosil G column with all gradient elution systems (Table 3). Satisfactory peak match values (>900) were obtained for 10-deacetyl taxol, cephalomannine and taxol, while lower peak match values were seen for 10-deacetyl-baccatin III (857-880), 10-deacetyl-7-*epi*-baccatin III (621-808) and baccatin III (769-866). The wavelength response ratio values (A_{227}/A_{273}) calculated for standards I-VII and for same in the purified extract of *T. x media* 'Nigra' indicated the presence of small amounts of impurities co-eluting with each of these taxanes.

In general, the use of Taxil column (PFP packing material) resulted in a baseline separation of the polar taxanes, 10-deacetyl baccatin III, baccatin III, 10-deacetyl taxol and cephalomannine. The less polar taxanes, 10-deacetyl-7-*epi*-taxol and taxol, showed partial resolution and interfering peaks, indicated by the values for wavelength response ratio and peak match (Table 3).

Under the conditions of gradient systems 1-3, the Supelcosil column showed the least selectivity in the separation of taxanes III-VII (Table 3). A baseline separation was obtained for 10-deacetyl-7-*epi* taxol and taxol (Figures 7B-8B).

Although, the best separation selectivity for taxanes I-VII was obtained using Curosil G column and gradient system 1, the response factor for each of these taxanes was lower under the conditions of gradient system 1 when compared to that of gradient system 3 (Table 2). The reason for the better response with gradient system 3 may be attributed to the higher concentration of the organic solvent available at the start of the gradient elution which enhances the solubility of these taxanes. The samples analyzed contain a complex mixture of chemical compounds having a wide difference in chemical and physical properties, i.e., polarity, solubility and molecular weight. The use of methanol [7], which has a high solvent strength parameter ε^{o} = 0.95, to effect an increase in the solubility of those compounds resulted in a decrease in the separation selectivity for the structurally related taxanes. The solution of 8% ethylene glycol monomethyl ether (ε^{o} = 0.74) in water 76%, and 24% acetonitrile (ε^{o}= 0.65) at the start of the gradient elution in system 3 enhanced the solubility of sample material without affecting the separation selectivity of taxanes. Hence, a better response factor was attained for taxanes I-VII (Table 2).

The linearity of the detector response was also examined for the Curosil G column and gradient systems 1 and 3. A linear relationship (indicated by the correlation coefficient values shown in Table 4) was observed between the peak areas and the concentration of taxanes I-VII at the concentration range of 10-240 µg/ml. The day-to-day variation in the peak areas of standards I-VII was minimum (C.V.= 9.8%, 9.1%, 5.8%, 7.7%, 7.4%, and 7.8%, respectively, n=12), and the within-day variation was even less (C.V.=1.5%, 1.3%, 1.4%, 1.4%, 1.4%, 1.5%, and 1.5%, respectively, n=4).

In conclusion, a sensitive and reproducible HPLC method was developed for the quantitation of taxol and 6 related taxanes in the needles of *Taxus* plants using a Curosil G column and gradient system 3. The detection limit of the different taxanes

TABLE 4

Comparison of the Correlation Coefficient for the Different Taxanes using Curosil G Column and Two Gradient Systems.

Gradient system	Correlation Coefficient						
	DAB (VII)	DAEB (VI)	BIII (V)	DAT (IV)	CPN (III)	DEAT (II)	TAX (I)
1	0.999	0.998	0.998	0.999	0.999	0.994	0.998
3	0.995	0.999	0.999	0.999	0.999	0.999	0.999

Taxanes Code: DAB=10-deacetyl baccatin III, DAEB=10-deacetyl-7-*epi*-baccatin III, BIII=baccatin III, DAT=10-deacetyl taxol, CPN= cephalomannine, DAET=10-deacetyl-7-*epi*-taxol and TAX=taxol.

was determined to be below 100 ng for 10-deacetyl baccatin III, 10-deacetyl-7-*epi*-baccatin III and baccatin III and 50 ng for 10-deacetyl taxol, cephalomannine, 10-deacetyl-7-*epi*-taxol and taxol.

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