ELSEVIER

Journal of Chromatography A, 802 (1998) 297–305

JOURNAL OF CHROMATOGRAPHY A

Determination of paclitaxel and related diterpenoids in plant extracts by high-performance liquid chromatography with UV detection in high-performance liquid chromatography–mass spectrometry

G. Theodoridis[a,1], G. Laskaris[a], C.F. de Jong[a,2], A.J.P. Hofte[b], R. Verpoorte[a,*]

[a]*Division of Pharmacognosy, Leiden/Amsterdam Center for Drug Research, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands*

[b]*Division of Analytical Chemistry, Leiden/Amsterdam Center for Drug Research, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands*

Received 12 May 1997; received in revised form 18 November 1997; accepted 18 November 1997

Abstract

Reversed-phase HPLC was used for the determination of paclitaxel and related compounds in both isocratic and gradient mode, using four different HPLC columns. With the selected acetonitrile–0.05 *M* ammonium acetate gradient, 13 taxanes of a standard mixture were clearly separated on a phenyl column within 30 min. The system was tested with plant extracts and unknown taxanes were identified by LC–MS with an electrospray interface. A specialty 'taxane' column also achieved a baseline resolution of the 13 taxanes, but in 55 min. The developed methods were applied to the analysis of plant extracts.

Keywords: Paclitaxel; Diterpenoids; Taxanes; Cephalomannine

1. Introduction

Paclitaxel is a diterpene amide that shows unique antitumor properties against several types of cancer. It occurs in small amounts in the bark, needles, twigs and roots of many Taxus species like *T. brevifolia*, *T. baccata* and *T. cuspidata*. Currently there is a great interest in the production of taxanes from cell cultures as an alternative source of taxanes.

Reversed-phase column liquid chromatography (RPLC) is mainly used for the determination of taxanes [1] and its use for both plant material and biological fluids was reviewed recently [2]. Analysis of plant material is usually made on phenyl, biphenyl, and pentafluorophenyl phases [3–10], but the use of cyano [3,11], C_{18} [6,12,13] and C_8 [14] phases has also been reported. C_{18} phases are preferred for the analysis of biological samples [2]. The last years new specialty 'taxane' columns, like Phenomenex Curosil [6,8,15–17], Whatman TAC 1,

*Corresponding author.

[1] Present address: Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Greece.

[2] Present address: Department of Phytopathology, Wageningen Agricultural University, Binnenhaven 9, 6709 PD Wageningen, The Netherlands.

0021-9673/98/$19.00
PII S0021-9673(97)01174-6

Metachem Taxil [6,18] and Zorbax SW-Taxane [19] were developed and used in order to tackle problems arising from the complexity of plant extracts. According to the manufacturers, some of these phases are more suitable for bark extracts and some for needle extracts. Ketchum and Gibson [6] discussed briefly the effect of stationary phase selectivity on separation, comparing the suitability of Metachem and Phenomenex Taxol (also known as Curosil) columns for the analysis of taxanes from callus cultures while recent studies report on a new polyfluorinated reversed-phase column that was used for the determination of paclitaxel in injectable formulations [10,20].

Taxanes are detected at 227 nm, and the selectivity of the UV detection is rather low. LC has also been coupled to mass spectrometry (MS) with the use of an ionspray [9], thermospray [11] or electrospray [12] interface. Detection limits were at the level of 100–200 pg [11].

Identification of taxanes in plant or cell culture extracts is often problematic, due to the interference of the complex matrix. The aim of the present study was to develop LC methods applicable to the analysis of plant and cell culture extracts. The developed methods should separate the highest possible number of taxanes in a single and fast run while at the same time allowing coupling to MS. In this context, three general purpose columns, used in our laboratory for the separation of a wide spectrum of secondary metabolites, and one specialty 'taxane' column were tested for their suitability.

2. Experimental

2.1. Chemicals

Paclitaxel and 10-deacetyl baccatin III (10-DAB III) standards were obtained from Sigma (St. Louis, MO, USA). A mixture of 10-DAB III (1), baccatin III (2), 7-xylosyl-10-deacetyltaxol B (3), taxinine M (4), 7-xylosyl-10-deacetyltaxol (5), 7-xylosyl-10-deacetyltaxol C (6), 10-deacetyltaxol (7), 7-xylosyltaxol (8), cephalomannine (9), 7-epi-10-deacetyltaxol (10), paclitaxel (11), taxol C (12) and 7-epi-taxol (13) was generously provided by Dr. Steven Richheimer, Hauser Chemical Research, Boulder CO, USA.

Methanol, ethanol, acetonitrile, chloroform and dichloromethane were all distilled in house prior to use. Tetrahydrofuran (p.a.) was from Janssen (Beerse, Belgium). Ammonium acetate (p.a.) was obtained from Merck (Darmstadt, Germany). Water of Millipore quality was used and all solvents were filtered through a 0.45-μm RC 55 membrane filter (Schleicher and Schuell, Dassel, Germany).

2.2. HPLC columns

The following HPLC columns were evaluated:
– Phenomenex Ultracarb C_{18}, 5 μm, 60 Å, 150×4.6 mm (Torrance, CA, USA)
– Novapak Phenyl, 4 μm, 60 Å, 150×3.9 mm from Waters (Milford, MA, USA)
– Alltech Adsorbospher HS C_{18}, 3 μm, 80 Å, 150×4.6 mm from Alltech (Deerfield, IL, USA)
– Zorbax SW Taxane 10 μm, 60 Å, 250×4.6 mm from BTR Separations (Wilmington, DE, USA).

All columns were protected by a precolumn 20×4.6 mm, filled in house with Lichrosorb RP-18, 10-μm material obtained from Merck.

2.3. Apparatus

Two Waters gradient LC systems were used. The first consisted of a 600 E pump, a WISP 712 autosampler, a Waters 991 photodiode array detector and a 5200 printer/plotter. The second consisted of a 616 pump, a 486 tunable UV detector, a 600 S system controller, a Rheodyne (Rheodyne, Cotati, CA, USA) 7215 injection valve and a HP 600 printer, and was controlled by a computer running the Millennium 2010 software.

LC–MS analysis was performed on a Finnigan MAT (San Jose, CA, USA) TSQ-70 custom made electrospray interface (ESI) with a heated sampling capillary. Sampling capillary and ion source were kept at 250°C and 2017°C respectively. The repeller voltage was optimised for paclitaxel and 10-DAB III. In positive ionisation mode the optimum voltage was 150 V. The optimisation of the mass spectrometer conditions was for paclitaxel and 10-DAB III in the constant infusion mode, with a sheath flow of

methanol:water (80:20 v/v), with 1% acetic acid. Splitting of the flow at 19:1 before the ESI probe, allowed 50 μl/min to be introduced into the mass spectrometer. The rest of the flow was directed to a Waters 440 UV detector operated at 254 nm. For the LC separation two 2150 LKB (Bromma, Sweden) pumps were used to perform the gradient, under the control of a LKB 2152 HPLC controller.

2.4. Sample pretreatment

At present, according to FDA guidelines, paclitaxel is isolated from the bark of *T. brevifolia* while the richest source of its precursor 10-DAB III is the foliage of *T. baccata*. Since a lot of analytical interest has been focused on analyzing taxanes of these two sources and since other plant sources also produce taxanes and could become equally important in the near future, we tested our separation system in other sources, namely the bark of *T. baccata* and the needles of *T. canadensis*.

Dried *T. baccata* bark was ground in the blender and then split in two equal portions and the following extraction methods were tested:

2.4.1. Method 1

The first portion (20 g) was transferred to an Erlenmeyer flask and extracted with 100 ml of methanol by soaking with agitation with a magnetic stirrer for 10 h. The resulting solution was filtered through a glass filter and the residue was reextracted with 50 ml of methanol. The two solutions were combined and evaporated to dryness in a rotary evaporator (40–45°C). The residue was reconstituted with 5 ml of methanol and an aliquot (0.5 ml) was subjected to SPE (extract A): a Supelclean LC-18 SPE cartridge (Supelco, Bellefonte, PA, USA) was conditioned with methanol and water and the 0.5-ml aliquot was brought to 5 ml with water and loaded on the cartridge. The cartridge was washed with 2×2 ml of water, 2 ml of 20% methanol in water and 2 ml of 50% methanol in water. The compounds of interest were eluted with 2 ml of methanol and the collected fraction was evaporated to dryness in a speed vac. The residue was reconstituted in solution with 2×100 μl of acetonitrile. Ten μl of the resulting solution were analysed by HPLC.

2.4.2. Method 2

The second portion (20 g) was homogenised with an Ystral homogeniser with 100 ml of methanol. The homogenate was ultrasonicated in a bath for 10 min, left on a gyratory shaker at 110 rpm for 30 min and subsequently filtered through a glass filter. The residue was reextracted with 50 ml of methanol and the two methanolic extracts were combined and evaporated to dryness in a rotary evaporator (40–45°C). The residue was reconstituted with 5 ml of methanol and 0.5 ml of it was applied to a SPE cartridge as previously (extract B).

Concerning Taxus needles and (or) clippings the pretreatment was the following: 6 grams of *T. canadensis* clippings were ground in the blender and the sample was divided in two equal portions. The first was extracted with 100 ml of methanol and this extract was washed with 50 ml of *n*-hexane. The hexane layer was discarded and the methanolic fraction was evaporated to dryness in a rotary evaporator (40–45°C). The residue was reconstituted in 5 ml of methanol and an aliquot of 0.5 ml was subjected to SPE, as above. The second portion was ultrasonicated in a bath with 100 ml of chloroform–ethanol (1:1) and filtered over a glass filter. The filtrate was evaporated to dryness and the residue was reconstituted in 5 ml of methanol and processed as above.

2.5. Chromatographic conditions

The taxanes of interest can be divided in three groups: the groups of paclitaxel, cephalomannine and 10-DAB III. All groups possess the main taxane ring; paclitaxel and cephalomannine differ in the substitution of the 3′-N atom of the C-13 side chain, whereas 10-DAB III lacks the C-13 side chain (Fig. 1). These differences affect the molecule's polarity, chromatographic behavior and cytotoxic activity.

The LC flow-rate was maintained at 0.8 ml/min and detection was at 227 nm. Spectral data were collected over the 190–400 nm range and for better peak recognition, chromatograms were plotted at two additional wavelengths (215 and 249 nm). Taxanes have their UV absorption maximum at 227 nm and their absorption below this wavelength is lower. The comparison of the absorption at 227 and at 215 nm

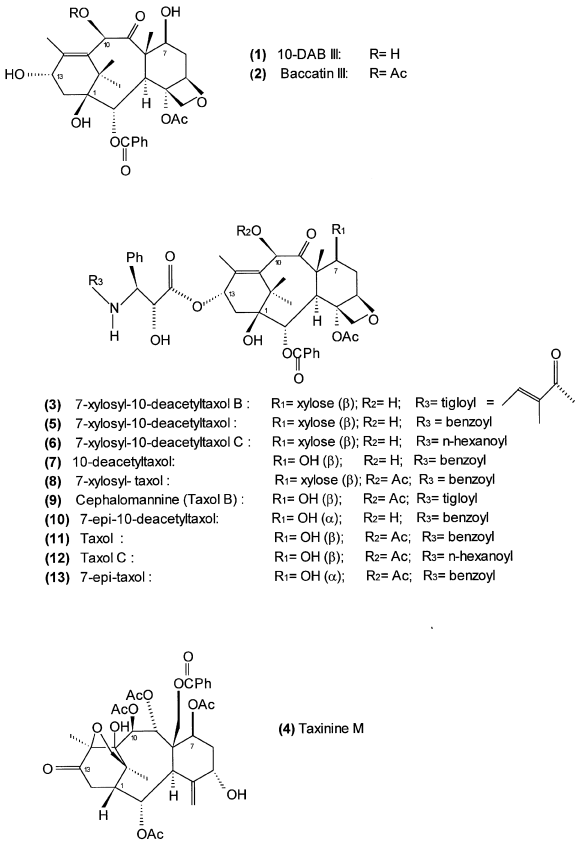

Fig. 1. Molecular structures of the main taxanes.

can selectively differentiate taxanes from other compounds present in the analysed samples.

The injection volume was 10 μl throughout the study. Linearity of the detector response was determined with triplicate analysis of 10-DAB III and paclitaxel standard solutions of 12 concentration points ranging from 0.46 to 166 and 0.83 to 240 μg/ml respectively, using the Phenomenex Ultracarb column and the selected isocratic system (see Table 1).

The evaluation of the SPE clean-up method is described in detail elsewhere [21,22]. It has been developed for cell and tissue cultures and its performance was assessed after spiking callus material of low taxane content with 200 μl of a standard taxane mixture containing 10 μg of paclitaxel and 10 μg of 10-DAB III. Various SPE cartridges were tried and the one with the best performance was Supelclean LC-18 from Supelco (Bellefonte, PA, USA). The extraction recovery was 94% for 10-DAB III and 85% for paclitaxel.

3. Results and discussion

3.1. Sample pretreatment

Concerning sample pretreatment the following remarks can be made: as a general rule methanol is a more potent solvent for extracting taxanes than less polar organic solvents (i.e. dichloromethane) and is capable of efficiently extracting paclitaxel as well as 10-DAB III. Nevertheless a direct extraction of the needles with a mixture of dichloromethane–ethanol (1:1) is as efficient as extraction with methanol.

Table 1
Selected isocratic- and gradient-elution programs

Isocratic elution system 1 (Ultracarb column)			Isocratic elution system 2 (SW Taxane column)		
Mobile phase: Acetonitrile–water–0.05 *M* ammonium acetate 54:4:42, v/v			Mobile phase: A: [0.5 *M* ammonium acetate–acetonitrile, 7:3] B: [methanol–water, 9:1] A–B, 75:25, v/v		
Gradient 1 (Novapak Phenyl column) A: 0.05 *M* ammonium acetate–acetonitrile, 7:3, v/v B: 0.05 *M* ammonium acetate–acetonitrile, 1:9, v/v			Gradient 2 (SW Taxane column) A: 0.05 *M* ammonium acetate–acetonitrile, 9:1, v/v B: methanol–water, 9:1, v/v		
t (min)	A (%)	B (%)	*t* (min)	A (%)	B (%)
0	100	0	0	90	10
30	66	34	30	55	45
32	100	0	32	90	10

Washing with hexane is not necessary since the SPE clean-up step efficiently retains lipophylic interferences (probably as a green fraction which does not elute from the SPE cartridge even after consecutive elution with 2 ml of methanol and 2 ml of chloroform). It is worth mentioning that while trace amounts of 10-DAB III and paclitaxel were found in the needle extract (no LC-MS analysis was carried out) the chromatogram was dominated by unknown taxanes with characteristic taxane UV spectrum (results not shown). Probably they correspond to 9-dihydro taxane structures [23].

Both bark extraction methods were carried out with methanol and it was found that long agitation time with a magnetic stirrer is not as effective as homogenisation with an Ystral homogeniser combined with ultrasonic bath of the resulting solution. With the first method, 8 taxanes were identified (extract A), while with the second (extract B) the higher extraction recovery revealed the presence of three more taxanes in the same bark sample (see Table 2).

3.2. Separation method

With regard to our separation method, isocratic elution could separate up to 11 of the 13 taxanes of the standard mixture on either Novapak Phenyl, Adsorbospher HS and Ultracarb columns. Variations in the nature and content of the organic modifier and salt concentration of the mobile phase did not alter the elution order and only affected band spacing to a small extend. The worst performer was the Adsorbospher column while the best overall isocratic separation was achieved with the Ultracarb column (isocratic system 1, see Table 1). Nine taxanes were baseline resolved while paclitaxel (11) coeluted with 7-epi-10-deacetyltaxol (10) and taxinine-M (4) coeluted with 7-xylosyl-10-deacetyltaxol (5). Baseline separation of all 13 taxanes was achieved with a gradient-elution program of acetonitrile over ammonium acetate solution (Gradient 1, Table 1), run with the Novapak Phenyl column within 30 min (Fig. 2). This system, compared to other systems published, performed better in terms of resolution and efficiency. In addition, this high-performance was achieved without resorting to specialty columns [6,8,15–17] but with a column that is commonly used in the analysis of numerous secondary metabolites. Furthermore it allows coupling to MS.

Quantiation was linear and the calibration curves constructed had R square values close to 1, especially for 10-DAB III which as the first eluted compound afforded a sharper peak. Inter-day reproducibility was evaluated with 12 repeated analyses of three concentration points (5, 50 and 100 μg/ml) and was found satisfactory with R.S.D. values of 3.4% for 10-DAB III and 4.7% for paclitaxel (R.S.D. values

Table 2
Results of the LC–MS analysis of the mixture of taxane standards obtained from Hauser and the *T. baccata* bark extracts

Compound	m/z	Peak	M[a]	t_R[b]	Extract A[c]	Extract B[c]
(1) 10-DAB III	562	$[M+NH_4]^+$	544	4.00	+	+
(2) Baccatin III	604	$[M+NH_4]^+$	586	7.00	+	+
(3) 7-xylosyl-10-deacetyltaxol B	922	$[M+H]^+$	921	11.00	+	+
(4) Taxinine M	704	$[M+H]^+$	703	11:30	+	+
(5) 7-xylosyl-10-deacetyltaxol	944	$[M+H]^+$	943	13:00	+	+
(6) 7-xylosyl-10-deacetyltaxol C	938	$[M+H]^+$	937	14:30	+	+
(7) 10-deacetyltaxol	812	$[M+H]^+$	811	15:00	−	+
(8) 7-xylosyl-taxol	986	$[M+H]^+$	985	16:00	−	+
(9) Cephalomannine	832	$[M+H]^+$	831	18:00	+	+
(10) 7-epi-10-deacetyltaxol	812	$[M+H]^+$	811	19:30	−	−
(11) Paclitaxel	854	$[M+H]^+$	853	20:00	+	+
(12) Taxol C	848	$[M+H]^+$	847	22:00	−	+
(13) 7-epi-taxol	854	$[M+H]^+$	853	25:00	−	−

Chromatographic conditions in Table 1, program 1
[a] Molecular weight.
[b] Retention time (min) obtained in LC–MS analysis.
[c] (+): Detection of the compounds; (−): no detection.

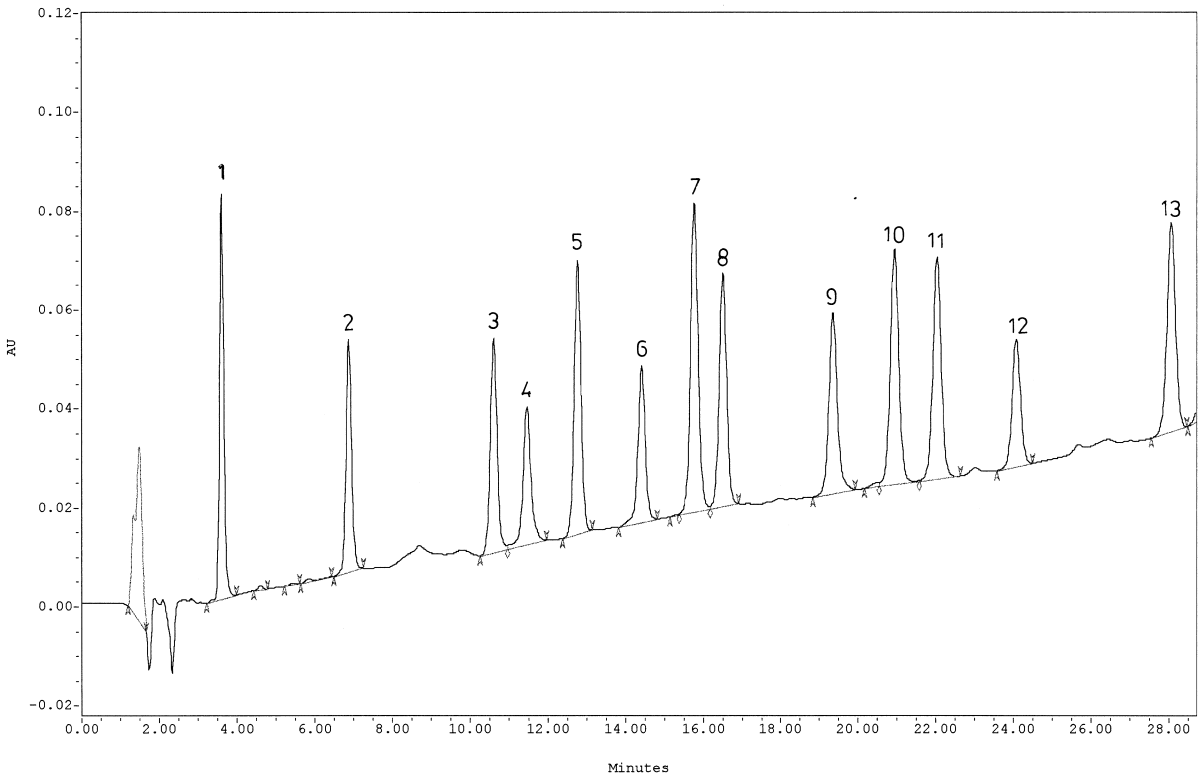

Fig. 2. Gradient elution (program 1, Table 1) of the taxane mixture on the Novapak Phenyl column. For conditions see Table 1. Peak numbering according to the compound assignment in Section 2.

are the average of the three conc. points). Intra-day reproducibility was evaluated by four analysis of the standards at the above mentioned concentrations for a period of 12 consecutive days and was found acceptable (R.S.D.: 4.2% for 10-DAB III and 5.6% for paclitaxel). Retention reproducibility was very satisfactory. Even after months of use and numerous injections of plant and cell culture extracts, retention times were consistent for both isocratic and gradient systems. A refilling of the precolumn after 300–400 injections (depending on the nature of the analysed samples) was sufficient to cope with small increase of the back pressure which occurred after repeated analysis of plant and cell culture samples.

The Zorbax SW Taxane column showed somewhat increased retention for the polar taxanes, but in general the k' distribution range was not as wide as for the other columns tested. It exhibited changes in the elution order compared with the other stationary phases. For instance with the isocratic system 2 (see Table 1), compounds 3 and 4 swap places. With this system, 12 taxanes were separated in 55 min while compound 13 was strongly retained (68 min). Adequate separation of all 13 taxanes was achieved with gradient system 2 (see Table 1) in a long analysis time of 55 min (Fig. 3). While with system 2 the typical elution order (1,2,3, . . . ,13) was observed, peak spacing changed with some other gradients of acetonitrile over 0.05 *M* ammonium acetate, i.e. a switch occurred between compounds 4,5 and 7,8. However the column showed a significant loss of efficiency after a few months, probably as a result of deterioration. A rather dramatic decrease of retention and selectivity for the taxanes necessitated a shifting to weaker and weaker mobile phases in order to get an adequate fingerprint and this caused the expected consequences of peak tailing and loss of resolution. The use of this column was therefore omitted. There is only one report on the use of this column for the preparative separation

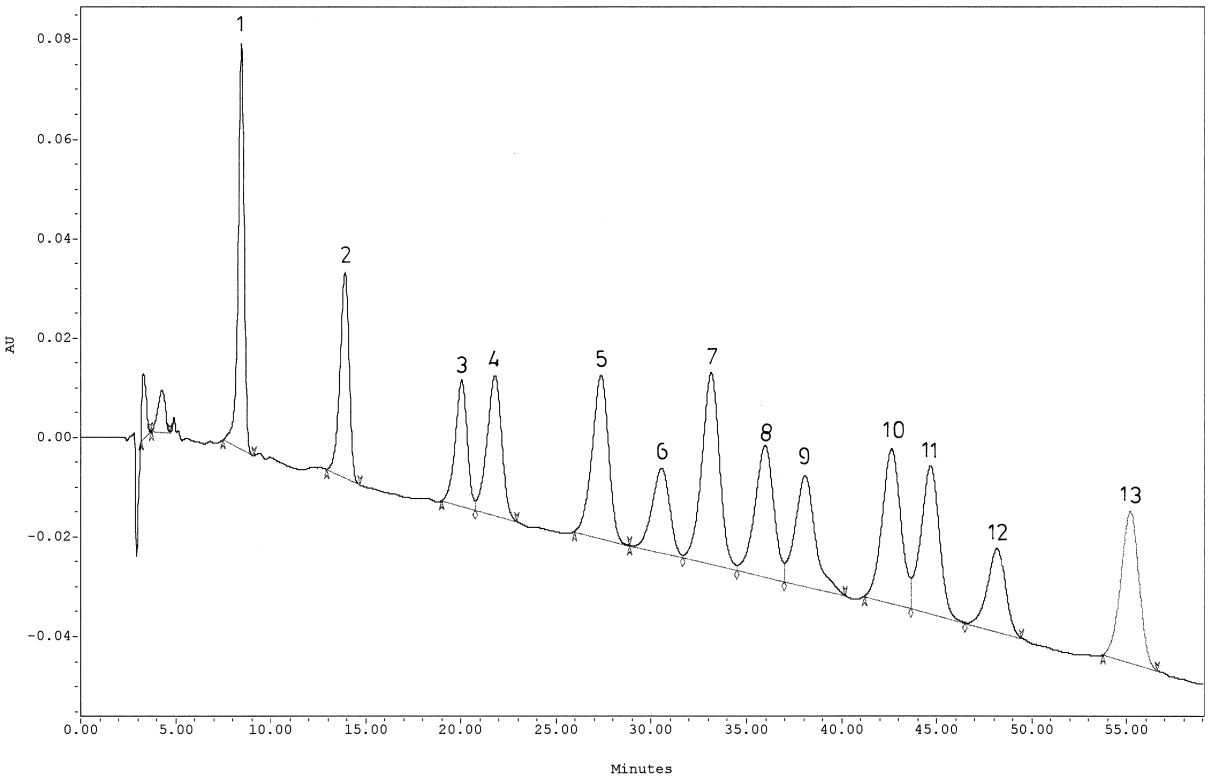

Fig. 3. Gradient elution (program 2, Table 1) of the taxane mixture on the Taxane SW column.

of paclitaxel from *T. canadensis* plant extract, in both normal and reversed-phase operation [19].

No conclusive results were drawn for the taxane composition of *T. canadensis* needles. With regard to *T. baccata* bark, cephalomannine and paclitaxel were present in the extract at concentrations of 3.1×10^{-6} and 7.8×10^{-6}% of dry weight, respectively (peak area measurement). Interferences of other peaks didn't allow quantitation of 10-DAB III although it is obviously by far the most abundant taxane. A typical chromatogram of a bark extract is given at Fig. 4.

3.3. LC–MS analysis

The identification of the peaks of the 13 taxanes present in the 'Hauser' mixture was done by LC–MS. The molecular masses of all compounds were obtained on-line from the full-scan mode. The mass chromatogram of the gradient analysis (Novapak Phenyl column with gradient program 1) is given in Fig. 5. The differences in the retention times between LC–MS and LC–UV runs are due to the differences in instrumentation (tubing, dead volume) and/or the gradient. The already reported LC–MS methods describe the analysis of a limited number of taxanes: 9 and 11 [11] or 2, 7, 9, 11 and deacetylcephalomannine [12]. Kerns et al. [9] report the LC–MS analysis of 18 taxanes in 40 min., but with serious overlapping problems for many pairs: 7, 8 and 10, 11 among them.

The results are shown in Table 2. Taxanes were identified as the protonated molecules, except 10-DAB III and baccatin III which gave the $[M+NH_4]^+$ as the base peak. Another interesting phenomenon was the very high signal/response of taxinine M (m/z 704) in the LC–MS. This compound gave a 15–20 fold higher signal than the other taxanes, although the concentration of all the taxanes in the sample was more or less the same (personal communication with Dr. Steve Richheimer). The same was observed later in the analysis of other taxinines and taxines by LC–UV and LC–MS. Taxines and

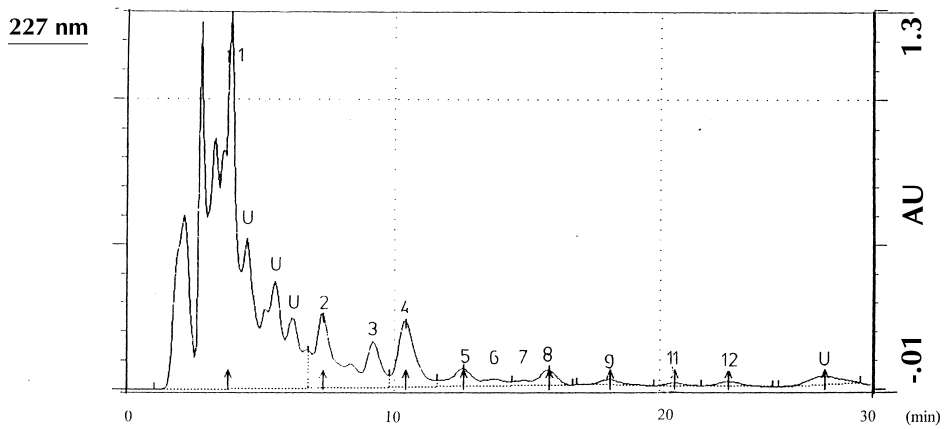

Fig. 4. Chromatographic analysis of a bark extract (extract B). Analysis on the Novapak Phenyl column with the selected gradient program 1 (Table 1). U=unknown. See also Table 2.

especially taxinines have their UV maximum at 280 nm, while their absorption at 227 nm is modest but their signal in the MS is increased compared to the taxanes.

A limited number of Taxus plant extracts were also analysed by LC–MS in the selected-ion monitoring (SIM). LC–MS confirmed the presence of eight in the first (A) and 11 taxanes in the second (B) bark extract, as can be seen in Table 2.

4. Conclusions

From the four columns tested, the Novapak Phenyl

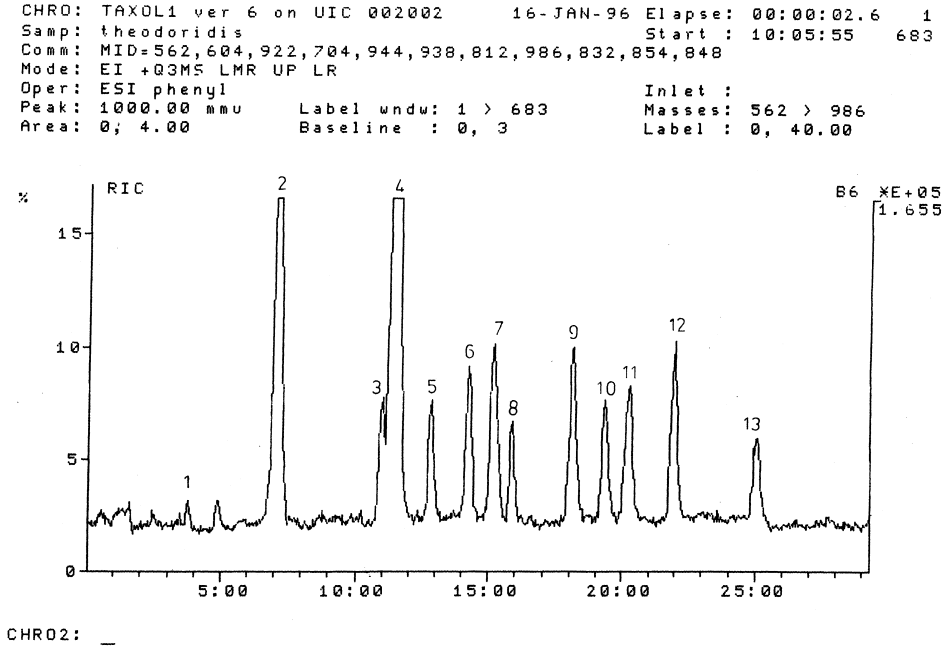

Fig. 5. LC–MS analysis of the taxane mixture. Conditions as in Fig. 4.

column run with a gradient system performed the best and afforded baseline resolution of all 13 taxanes in 30 min while an isocratic system run with the Ultracarb column adequately separated 11 of the 13 taxanes. LC–MS with an electrospray interface, verified the identity of the eluted taxanes of the standard mixture and identified 11 of them in a *T. baccata* bark extract. The use of a specialty column gave the chance to work in a different retention window with satisfactory results in both isocratic and gradient mode, but with longer analysis time. This column had a short life-time.

Acknowledgements

We thank Dr. S. Richheimer (Hauser Laboratories) for the kind gift of taxane standard mixture. G. Theodoridis is grateful to the Committee of the European Union for financial support through the ERBCHBICT9417210 individual fellowship. The work of G. Laskaris was partially supported by a NATO individual research fellowship.

References

[1] K.M. Snader, in: M. Suffness (Ed.), Taxol Science and Applications, CRC Press, Boca Raton, 1995, Ch. 11, p. 277.
[2] G. Theodoridis, R. Verpoorte, Phytochem. Anal. 7 (1996) 169.
[3] K.M. Witherup, S.A. Look, M.W. Stasko, T.G. McCloud, H.J. Issaq, G.M. Muschik, J. Liq. Chromatogr. 12 (1989) 2117.
[4] S.L. Richheimer, D.M. Tinnermeier, D.W. Timmons, Anal. Chem. 64 (1992) 2323.
[5] T.P. Castor, T.A. Tyler, J. Liq. Chromatogr. 16 (1993) 723.
[6] R.E.B. Ketchum, D.M. Gibson, J. Liq. Chromatogr. 16 (1993) 2519.
[7] E.R.M. Wickremesinhe, R.N. Arteca, J. Liq. Chromatogr. 16 (1993) 3263.
[8] W.J. Kopycki, H.N. ElSohly, J.D. McChesney, J. Liq. Chromatogr. 17 (1994) 2569.
[9] E.H. Kerns, K.J. Volk, S.E. Hill, M.S. Lee, J. Nat. Prod. 57 (1994) 1391.
[10] L.K. Shao, D.C. Locke, Anal. Chem. 69 (1997) 2008.
[11] S.O.K. Auriola, A.M. Lepistö, T. Naaranlahti, S.P. Lapinjoki, J. Chromatogr. 594 (1992) 153.
[12] F. Bitsch, W. Ma, F. MacDonald, M. Nieder, C.H.L. Shackleton, J. Chromatogr. 615 (1993) 273.
[13] W. Fang, Y. Wu, J. Zhou, W. Chen, Q. Fang, Phytochem. Anal. 4 (1993) 115.
[14] D.R. Lauren, D.J. Jensen, J.A. Douglas, J. Chromatogr. A 712 (1995) 303.
[15] H.N. ElSohly, E.M. Croom, W.J. Kopycki, A.S. Joshi, M.A. ElSohly, J.D. McChesney, Phytochem. Anal. 6 (1995) 149.
[16] S.S. Kwak, M.S. Choi, Y.G. Park, J.S. Yoo, J.R. Liu, Phytochemistry 40 (1995) 29.
[17] R.E.B. Ketchum, D.M. Gibson, J. Liq. Chromatogr. 18 (1995) 1093.
[18] M.J.I. Mattina, G.J. MacEachern, J. Chromatogr. A 679 (1994) 269.
[19] D.R. Wu, K. Lohse, H.C. Greenblatt, J. Chromatogr. A 702 (1995) 233.
[20] H.Y. Aboul-Enein, V. Serignese, Anal. Chim. Acta 319 (1996) 187.
[21] G. Theodoridis, G. Laskaris, C.F. de Jong, R. Verpoorte, HPLC '96 symposium, San Francisco, June 16–21 1996, Book of Abstracts, p. 233.
[22] G. Theodoridis, C.F. de Jong, G. Laskaris, R. Verpoorte, Chromatographia 47 (1998) 1.
[23] L.O. Zamir, M.E. Nedea, Z.H. Zhou, S. Belair, G. Caron, F. Sauriol, E. Jacqmain, F.I. Jean, F.X. Garneau, O. Mamer, Can. J. Chem. 73 (1995) 655.