ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
26 (2001) 89–102

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Solid-phase extraction and simplified high-performance liquid chromatographic determination of 10-deacetylbaccatin III and related taxoids in yew species

Tomasz Mroczek, Kazimierz Glowniak *

Department of Pharmacognosy, Medical University, Peowiakow 12 St., 20007 Lublin, Poland

Received 10 November 1999; received in revised form 11 January 2001; accepted 16 January 2001

Abstract

Solid-phase extraction was accomplished with specially prepared cartridges filled with silanised silica gel (RP-2) for the purpose of 10-deacetylbaccatin III (10-DAB III) and related taxoids extracts purification obtained from different yew materials. In the first method, the analysed taxoids eluted in 75% methanol, but in the second method, the preliminary elution with 30% methanol was made. DAB III and its six derivatives were separated from co-extractives in merely acetonitrile-water gradient mode during 25 min on Waters Symmetry C-18 column with photodiode array (PDA) detection. The total recoveries for 10-DAB III and paclitaxel in the first SPE method (all compounds were applied in amounts of 80 μg) were about 98 and 94%, respectively. Almost 100% recoveries for paclitaxel and baseline separation of 10-DAB III and co-extracted compounds were obtained when preliminary elution with 30% methanol was performed. This method can be applied as a routine, inexpensive and uncomplicated procedure for 10-DAB III and related taxoids determination in yew material. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: HPLC gradient separation; Silanised silica gel; Solid-phase extraction; Yew extracts

1. Introduction

Phytochemical investigations on different yew species led to isolation and structural elucidation of more than 100 diterpenoid compounds to have been named taxoids [1]. From the extract of the bark of western yew, *Taxus brevifolia* Nutt., Wani and Wall isolated paclitaxel, the first compounds now known as the anticancer drugs (Taxol®, Yew-taxan®) [2]. It is mainly applied in the treatment of ovarian cancers resistant to other anticancer compounds [3,4] but has also been tried in other cancers [5–7]. Now the semisynthetic procedures have been developed from 10-deacetylbaccatin III (10-DAB III) for obtaining not only paclitaxel [8,9] but also its anticancer analogues such as docetaxel (Taxotere®) [10] (Fig. 1). There are also reports on other taxoids that can be applied in semisynthesis such as 10-deacetyltaxol (10-DAT), 10-deacetylcephalomannine (10-DAC) [11] or 7-xylosyl-10-deacetyltaxol [12].

* Corresponding author. Fax: +48-81-28903.
E-mail address: glowniak@hipokrates.am.lublin.pl (K. Glowniak).

0731-7085/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0731-7085(01)00353-3

Solid-phase extraction of yew extracts was often used for the taxoids determined by HPLC methods. The most popular C_{18} sorbent was reinvestigated, and taxoids (paclitaxel, cephalomannine) were eluted with 80% methanol [13–16] as well as with 70% acetonitrile [17] or with pure methanol [18]. There are also reports on SPE of taxoids on cyano bonded silica phases [19,20], and Van Rozendal et al. applied Extrelut® (E Merck, Darmstadt, Germany) sorbent for yew-extract purification [21].

High-performance liquid chromatography is the analytical method most often used in different taxoid determinations, and a broad range of sorbents and columns have been applied. Using C_{18} packing material usually in isocratic mode, paclitaxel and other less polar taxoids (e.g. cephalomannine) could be resolved in two-component (methanol–water or acetonitrile–water mixtures) [17,22] or three-component (methanol–acetonitrile–water mixtures) [23] mobile phases, but better results were obtained with gradient elution [24]. In biological fluids and tissues, paclitaxel was successfully determined by isocratic HPLC using automated column switching (the clean-up column C_8, the analytical column C_{18}) [25]. Besides C_{18} columns, octyl bonded RP phases were also applied [26,27]. Phenyl bonded silica gel columns in the RP mode and especially with gradient elution shown to be suitable for paclitaxel and related taxoids determination in yew extracts [28,29], whereas pentafluorophenyl (PFP) packing material is especially considered for resolving of taxoids impurities [29,30] or other paclitaxel analogues in bulk drug and injectable dosage form [30,31]. Paclitaxel and its xylosyl derivatives were successfully determined using stationary phases developed specially for paclitaxel (Taxsil®, Taxsol®) [13,32]. Hyphenated LCMS techniques were also applied in paclitaxel and related taxoid determination with a thermospray interface [33] or electrospray ionisation [34]. Selected molecular ions of taxoids could be further analysed by the CID (collision induced dissociation) technique to obtain detailed structural data [35]. Another hyphenated technique — LC/NMR — was successfully applied for the direct analysis of taxoids in the leaf samples of three *Taxus* species [36]. Cao et al. [37] reported very efficient 10-DAB III isolation and purification from the needles of *Taxus chinensis* using a high-speed counter-current chromatography (HSCCC) that was previously used by Vanhaelen-Fastre et al. [23] for the isolation of paclitaxel and cephalomannine from yew samples .

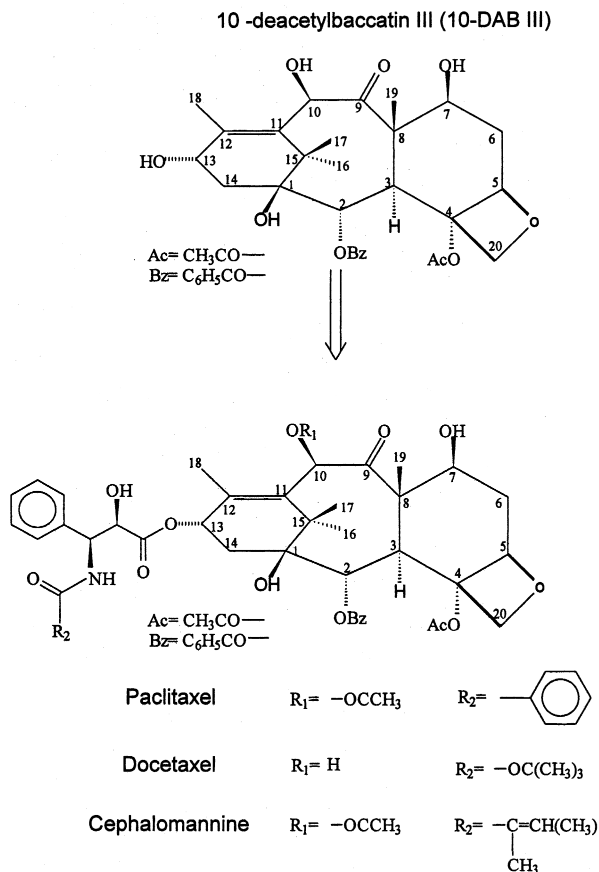

Fig. 1. Chemical structures of 10-deacetylbaccatin III (10-DAB III) and related taxoids.

In this report, for the first time, silanised silica gel cartridges (RP-2 phases) prepared in our laboratory have been developed and reinvestigated for SPE of 10-DAB III and related taxoids, and a new simplified method of gradient elution on a Waters Symmetry® C_{18} column with PDA detection has been elaborated for the purpose of 10-DAB III and its six commonly found in yew extracts derivatives. The recoveries for 10-DAB III and paclitaxel in this SPE-HPLC procedure have been measured.

2. Experimental

2.1. Plant material

Fresh twigs of *Taxus baccata* var. *Elegantissima* and *Taxus baccata* var. *Aurea* were collected from the Botanical Garden of University of Maria Curie-Sklodowska of Lublin in November. The samples were separated into needle and stem fractions. These fractions were cut separately into small parts (2 mm).

2.2. Chemicals and reagents

Standards of 10-deacetylbaccatin III (10-DAB III), baccatin III and paclitaxel were purchased from Sigma (St. Louis, MO), cephalomannine was supplied by the Drug Synthesis and Chemistry Branch of the National Cancer Institute (Bethesda, MD) and 7-xylosyl-10-deacetyltaxol, 7-xylosyl-10-deacetylcephalomannine, 7-xylosyl-10-deacetyltaxol C were donated by Prof. M. Furmanowa [Department of Pharmaceutical Botany, Medical University, Warsaw; the compounds were isolated by Prof. Y. Guo and collaborators (Laboratorie de Pharmacognosie et de Bromatologie, Universite Libre de Bruxelles)]. The taxoid standards were dissolved in methanol to obtain a concentration of 40 μg/ml (1 mg/25 ml). For the optimisation of taxoids separation, a mixed standard solution of 10-DAB III, baccatin III, cephalomannine and paclitaxel at a concentration of about 5 μg/ml, 7-xylosyl-10-deacetylcephalomannine (7-xyl-10-DAC) at a concentration of about 10 μg/ml, 7-xylosyl-10-deacetyltaxol (7-xyl-10-DAT) at a concentration of about 20 μg/ml, and 7-xylosyl-10-deacetyltaxol C (7-xyl-10-DAT C) at a concentration of about 40 μg/ml was prepared.

The solvents used (methanol, acetonitrile) were of chromatographic grade (Baker Chemikalien, Gros-Gerau, Germany). Double-distilled water was used in all experiments.

2.3. Equipment

The HPLC system consisted of a Waters Alliance M2690 chromatograph equipped with a gradient pump, autoinjector, column oven, and Waters 996 photodiode array detector (PDA) and was operated by Millenium 32 software system for controlling the analytical system and data processing.

The analytical column was a 3.5 μm Symmetry C_{18} (150 × 4.6 mm I. D.) (Waters, Milford, MA).

2.4. Extraction

The plant material (5 g of the needles of *Taxus baccata* var. *Elegantissima*) was placed in a 500 ml round-bottom flask and extracted with hot methanol (3 × 100 ml) in a boiling water bath under reflux for 2 h in each case. The second method of extraction was performed for the stem fraction of *Taxus baccata* var. *Aurea*. In this case, 5 g of stems were macerated with 100 ml of methanol on a wrist-action shaker for 16 h.

The combined methanolic extracts were then evaporated to dryness under reduced pressure at 40°C, and the residues were reconstituted in 25 ml of methanol in volumetric flask. Aliquots (1.5 ml) of each extract were transferred to a 50 ml round-bottom flask and then evaporated to dryness under reduced pressure at 40°C. The residues were dissolved in 1 ml of 75% methanol for SPE partitioning.

2.5. SPE partitioning

Two different RP-2 SPE techniques were compared for their partitioning efficiency of the crude extracts and co-extractives removing. In both methods, C_2 columns were prepared in our laboratory by packing 1000 mg of C_2 sorbent (Kieselgel 60 H silanisiert, E Merck, Darmstadt, Germany) into a 6 ml disposable polypropylene filtration column (J.T. Baker, Phillipsburg, NJ). At the top and bottom of the sorbent bed, 20 μm polyethylene frits were fitted.

Four millilitres of the mixture of paclitaxel and 10-DAB III in the equal ratio (both standards were at the concentration of 40 μg/ml) were evaporated to dryness under vacuum and then dissolved in 1 ml of 75% methanol for the recovery tests in both SPE-HPLC methods.

2.5.1. Method 1

The cartridge was conditioned with 10 ml of methanol, 10 ml of double-distilled water and 10 ml of 75% methanol. Before transferring the analysed sample into the cartridge, a 3 mm layer of the last solvent had to remain. After transfer of 1 ml of the standards solution or crude extract, the cartridge was eluted with 8 ml of 75% methanol. About 9 ml of purified sample solution were obtained, and another volume of 75% methanol was added to the cartridge, so that 10 ml of the total sample solution could be obtained accurately.

2.5.2. Method 2

The cartridge was conditioned with 10 ml of methanol, 10 ml of double-distilled water and 10 ml of 30% methanol. Once again, a 3 mm layer of the last solvent had to remain above the top frit at the time of addition of the standards solution or crude extract to the cartridge. First, the cartridge was eluted with 5 ml of 30% methanol and then with 8 ml of 75% methanol. Again, about 9 ml of purified sample solution were obtained, and another volume of 75% methanol was added to the cartridge, so that 10 ml of the total sample solution could be obtained accurately.

2.6. HPLC analysis

The analytical column was held at 25°C, and the mobile phase flow rate was 0.75 ml/min. A two-pump gradient program was applied: reservoir A contained 100% acetonitrile, and reservoir B contained 100% double-distilled water. None of the other solutions to reservoirs were added. The injection volume was 10 μl, and the following linear gradient program was used:

0–8 min: isocratic at 25% A
8–16 min: jump to 44% A
16–20 min: isocratic at 44% A
20–45 min: jump to 80% A
45–50 min: jump to 25% A.

A 10 min delay was maintained for equilibration of the column and stabilisation the baseline. The peaks were recorded at 200 and 233 nm (for quantitation), and for co-extractives, detection LC trace at 280 nm was also measured. The scanning of UV spectrum was performed from 200 to 380 nm at 2 nm intervals. The total time of analysis was 50 min. Quantitation was by peak area relative to a mixed external standard (10-DAB III and paclitaxel) for each component in 75% methanol. The calibration curves for both standards at the concentrations 4–40 g/ml (five-point calibration) were linear with the correlation coefficient 0.9949 for DAB III and 0.9962 for paclitaxel.

3. Results and discussion

Important tasks of taxoid determination in different yew materials are the choice of suitable solvent and sample clean up due to the presence in yew extracts both non-polar compounds (e.g. waxes, chlorophylls) and polar compounds. Earlier investigations suggested that methanol as a solvent permitted not only good recoveries for taxoids with paclitaxel-like structure but also poor non-polar compound extraction [16]. Our attempt at one part of plant material extraction comprised the method with hot methanol in a boiling water bath, which has already been published by us [17,38,39] and was found to be appropriate for rapid taxoids extraction. Methanol maceration was also performed [26,40], and such a process, despite being time-consuming, can be considered for reliable taxoid extraction as high yields of taxoids were measured in some cases [40]. For this reason, the stem fraction of *T. baccata* var. *Aurea* was macerated with methanol according to the procedure applied in our earlier work [40].

Crude methanolic extracts contain plenty of ballast components that not only can have a considerable influence on increasing the background baseline absorbance in HPLC determination, but also can be strongly retained on the column and shorten its lifetime. For these reasons, liquid–liquid extraction (LLE) was often applied [24,26,39] typically with n-hexane wash to remove non-polar compounds such as waxes and some chlorophylls, and then with dichloromethane extraction for taxoids from prepurified extracts. However, LLE has several disadvantages: it is solvent- and time-consuming,

Table 1
Recoveries (%) for 10-DAB III and paclitaxel after SPE partitioning using different methods of cleaning up[a]

Compound	Method 1	Method 2
10-DAB III	97.6 ± 0.2 ($n = 3$)	82.4 ± 3.7 ($n = 3$)
paclitaxel	93.5 ± 0.9 ($n = 3$)	101.7 ± 4.0 ($n = 3$)

[a] One millilitre of the mixed standard solutions (each compound was at concentration 80 μg/ml) in 75% methanol was processed through different SPE partitioning methods (1 and 2) and then made up in 10 ml of 75% methanol for HPLC analysis. The expected concentration of these standards was 8 μg/ml (100%). The injection volume was 10 μl (this means 80 ng of each standard per injection). The total recoveries were measured by calculation of 10-DAB III or paclitaxel concentrations based on measurements of the areas of peaks of these standards and the areas of the corresponding peaks of mixed standard solution in 75% methanol, which was directly analysed by HPLC without SPE partitioning.

and it results in emulsion creation and some loss of target compounds.

Our attempts were made to reinvestigate of specially prepared in our laboratory cartridges filled with silanised silica gel (C_2) sorbent for yew-sample clean-up and partitioning efficiency. This sorbent is cheaper than other RP sorbents and has not yet been applied in SPE of taxoids. Two different methods of SPE partitioning have been performed and compared, and recoveries for 10-DAB III and paclitaxel (from the mixed standard solution in 75% methanol, both compounds were at the amount of 80 μg) measured. When the cartridge with the standards absorbed on the C_2 sorbent was directly washed with 75% methanol (Method 1), the recoveries ranged from about 94% (paclitaxel) to 98% (10-DAB III) (Table 1). If the pre-elution step with 30% methanol was carried out (Method 2), the recoveries for 10-DAB III decreased to about 82% because about 16% of 10-DAB III applied was eluted from the sorbent with 30% methanol but increased to about 100% for paclitaxel. These results suggested that 10-DAB III could be removed from C_2 sorbent in more than 10% when a pre-wash with 30% methanol was applied, but some increase in paclitaxel efficiency could be observed.

A number of gradient conditions and alternative isocratic options were performed to optimise the HPLC separation, especially of the major taxoids (most often found in yew extracts), 10-DAB III, baccatin III, cephalomannine and paclitaxel. The mobile phase composition has been simplified by using merely acetonitrile–water mixtures without buffer or acid addition. The elaborated method was also suitable for satisfactory separation of minor taxoids with an additional 7-xylosyl side chain. Typical separation of the mixture of these seven standards could be achieved during 25 min (Fig. 2). As is common for other C_{18} columns, 10-DAB III eluted first [13,28,29] because it lacks neither a 10-acetyl substituent nor a side chain at the C_{13} position. Baccatin III (10-acetyl derivatives) eluted after 10-DAB III, and then 7-xylosyl-10-deacetyl derivatives of cephalomannine, paclitaxel and taxol C in the order dependent on hydrophobicity of the *N*-acyl substituent at the C_{13} position (*N*-tigloyl, *N*-benzoyl, and *N*-hexanoyl respectively). A similar elution order of 7-xylosyl-10-deacetyltaxol and 7-xylosyl-10-deacetyltaxol C was observed on the pentafluorophenyl stationary phase (PFP) [30]. Cephalomannine and paclitaxel with a 10-acetyl group and with an acyl side chain at C_{13} and free from 7-xylosyl moiety were eluted at the end.

The same conditions of gradient separation were routinely applied in major taxoids determination in the extracts purified by SPE using methods 1 and 2 (the needles of *T. baccata* var. *Elegantissima*) and then in stem extracts from *T. baccata* var. *Aurea* purified by Method 2. As shown in Fig. 3, the peaks of 10-DAB III together with cephalomannine and paclitaxel have been detected. The qualitative determination is based on a comparison of t_R of the compound in the extract with t_R of the component in the mixed standard sample as well as by analysis of UV spectra recorded by PDA detector. DAB III was well separated from closely eluted compound ($t_R \approx 5.5$ min), but the application of the sample SPE with pre-washing with 30% methanol (Method 2) caused a lowering of background baseline absorbance especially for the compounds eluted during the first 8 min (Fig. 4) so that 10-DAB III quantification could be made more accurately. The separation of paclitaxel from the

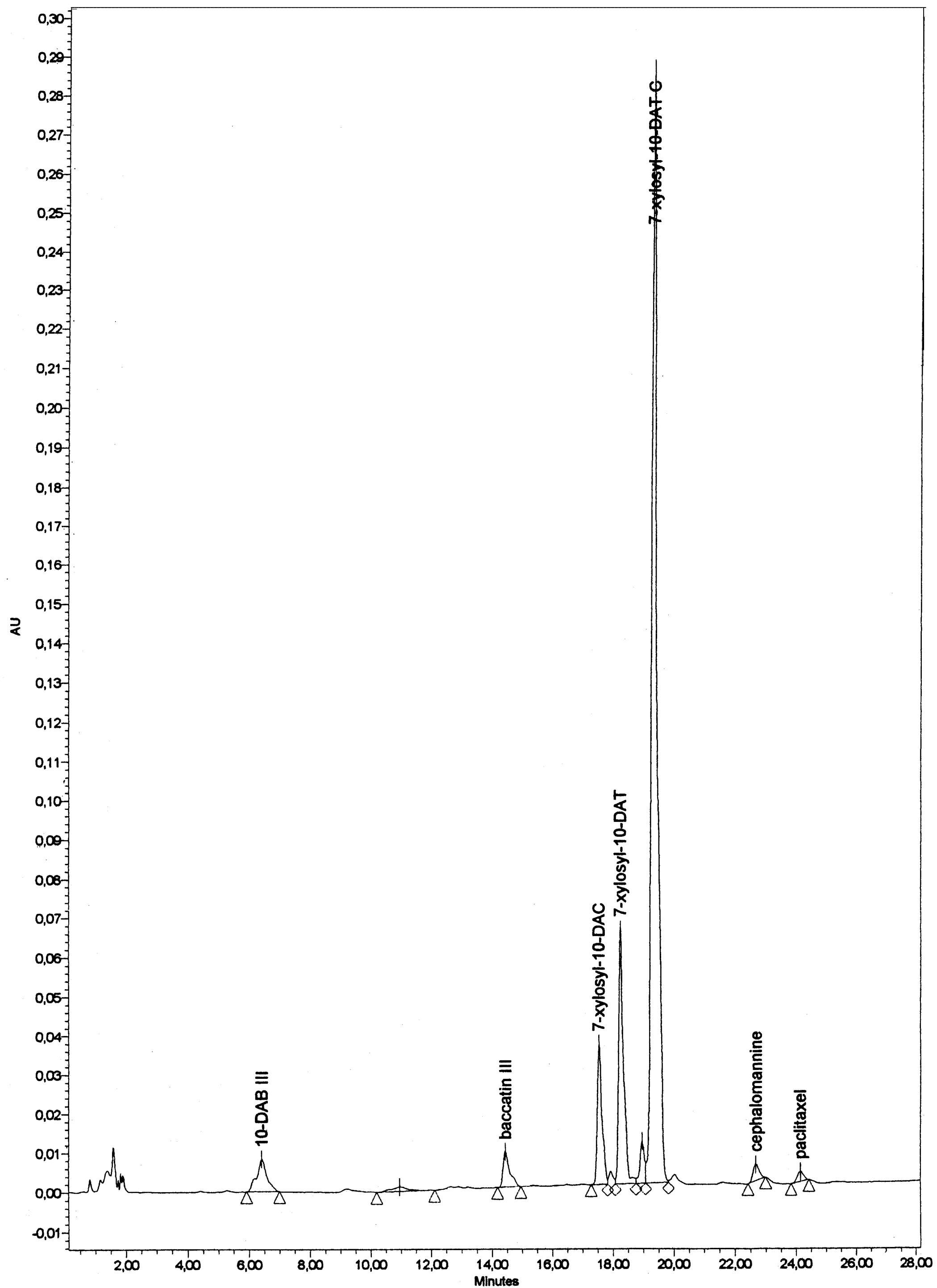

Fig. 2. HPLC gradient separation of the mixture of taxoids on Waters Symmetry C_{18} column. LC trace recorded at 233 nm. The mixed standard solution contained 10-DAB III, baccatin III, cephalomannine and paclitaxel at concentrations of about 5 μg/ml, 7-xylosyl-10-deacetyl cephalomannine (7-xylosyl-10-DAC) at concentrations of about 10 μg/ml, 7-xylosyl-10-deacetyltaxol (7-xylosyl-10-DAT) at concentrations of about 20 μg/ml, and 7-xylosyl-10-deacetyltaxol C (7-xylosyl-10-DAT C) at concentrations of about 40 μg/ml.

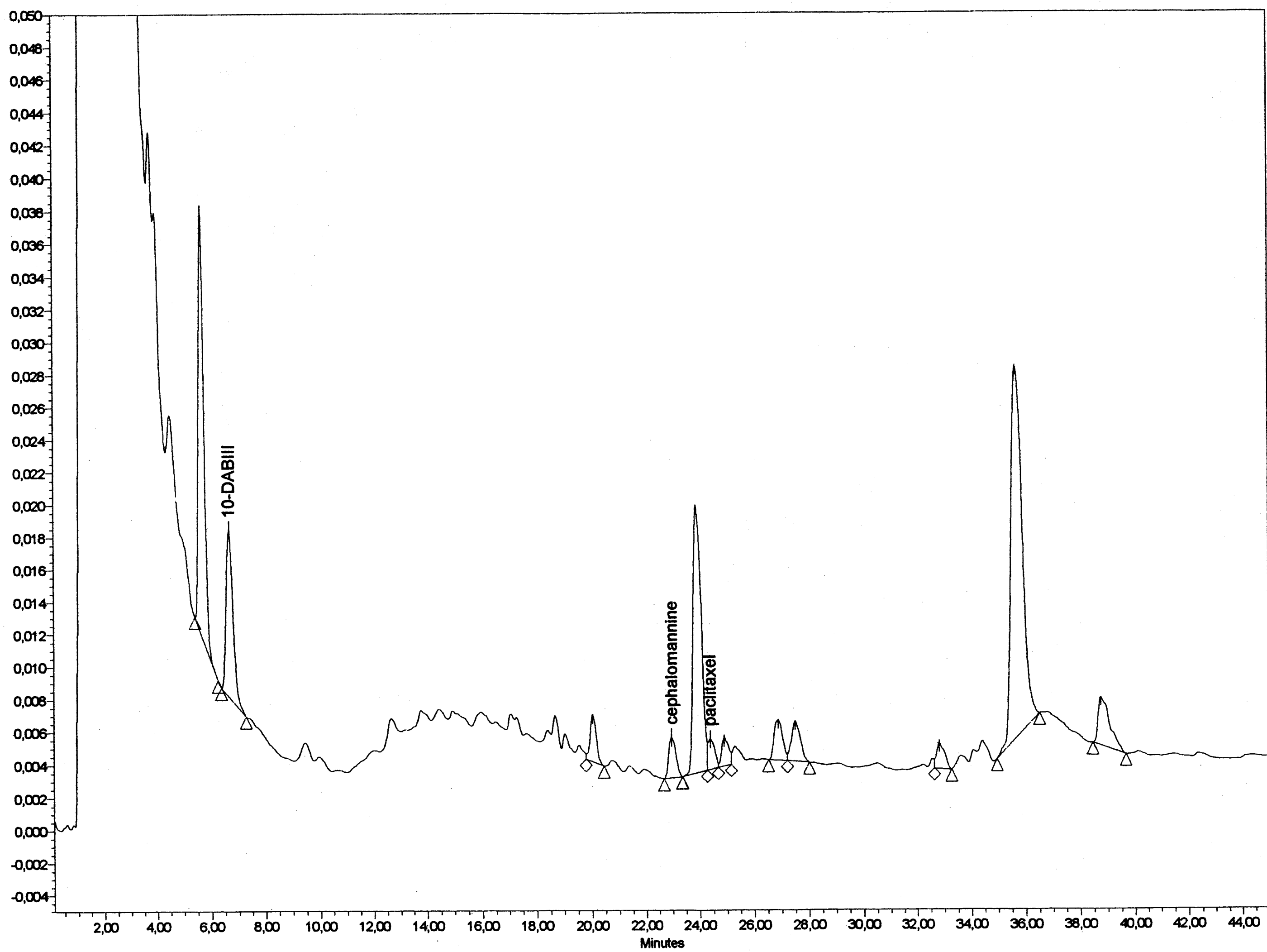

Fig. 3. HPLC gradient separation of the extract from the needles of *T. baccata* var. *Elegantissima* after SPE partitioning using Method 1. LC trace recorded at 233 nm.

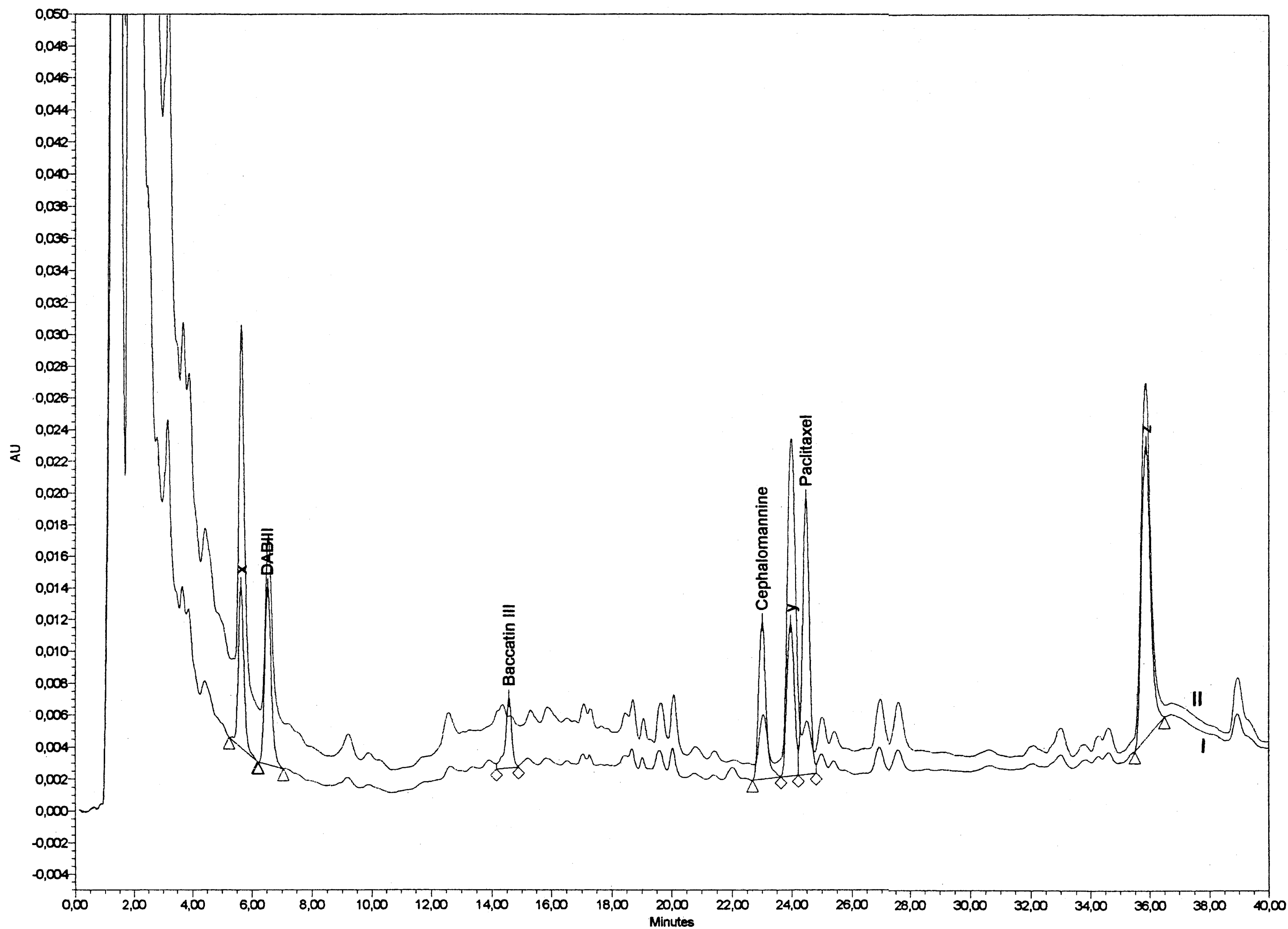

Fig. 4. HPLC chromatograms of yew extract from the needles of *T. baccata* var. *Elegantissima* purified by SPE (Method 2, II) and the extract spiked with mixed standard as overlay (I) showing 10-DAB III, baccatin III, cephalomannine and paclitaxel. LC trace recorded at 233 nm; x–z: unidentified major compounds in the extract.

closely eluted co-extractive compound (t_R = 23.99) was slightly better. The peaks of 10-DAB III, cephalomannine, paclitaxel and small amounts of baccatin III were additionally confirmed in the extract in overlaid chromatograms of purified yew extract and the extract spiked with four standards mixture (Fig. 4). From Fig. 5, where a chromatogram of the extract spiked with standard mixture and then purified is illustrated again, the corresponding UV spectra of main compounds are shown. Typically, 10-DAB III and baccatin III lacking a side chain at C_{13} had the first maximum at 232–233 nm and the second at about 276 nm, whereas cephalomannine and paclitaxel had the first maximum at 229–230 nm. The spectra of other unidentified major compounds (x–z) in the extract could suggest a taxoid-like structure for compound z and the co-extractive one (compound y) with strong absorption at 280 nm. Scanning at 280 nm was earlier used in co-extractives detection during taxoid analysis [13,26], and such an attempt seems to be a reliable approach in taxoid-separation verification. In Fig. 6, where LC traces recorded at 233 nm (for taxoids) and at 280 nm (for co-extractives) are overlain, strongly absorbing at 280 nm, co-extractives are detected out of retention times of the target compounds. Another conclusion is that the higher the wavelength of taxoids scanning (i.e. 250 nm), the greater the decrease of background baseline absorbance but the lower the detection limit. Opposite trends have been found when LC trace (higher UV absorption of taxoids of interest) has been recorded at 200 nm, and a high baseline absorbance can be observed. Our earlier investigations indicated that scanning at 200 nm could be appropriate for analysing the taxoid fraction preliminarily separated by preparative TLC [17,39].

Pre-elution of the extract from the needles of *T. baccata* var. *Elegantissima* with 30% methanol removed about 11% of 10-DAB III (Table 2) together with some amounts of closely eluted compound, but considerable quantities of polar compounds influencing on baseline absorption levels could be lost also. In a similar fraction from the extract from the stems of *T. baccata* var. *Aurea*, 10-DAB III has not been detected. In the chromatogram recorded at 233 nm for the last fraction of this extract eluted with 75% methanol (Fig. 7; 233 nm), some qualitative and quantitative differences in composition are observed, with one dominating unidentified compound, eluted closely with 10-DAB III. This suggested that the ratio of concentration of this compound and 10-DAB III could influence the amounts of 10-DAB III eluted with 30% methanol. Thus, for a pure standard mixture, the loss of 10-DAB III is the highest ($>10\%$), but if the closely eluted compound at a t_R of about 5.54 is strongly dominating (mainly in extracts obtained by maceration where the ratio of the amounts of 10-DAB III and that compound is $<1:4$), 10-DAB III can be

Table 2
Estimated 10-DAB III concentrations in different yew samples purified by SPE[a]

Yew species (plant material section)	Estimated concentration (%) per dry weight	Lost concentration after pre-elution with 30% methanol (%) per dry weight
T. baccata var. *Elegantissima* (needles)	0.0566 ± 0.0009 ($n = 3$)	0.0071 ± 0.0007 ($n = 3$)
T. baccata var. *Aurea* (stems)	0.0695 ± 0.0004 ($n = 3$)	n.d.[b]

[a] An aliquot (1.5 ml) of each extract (at the concentration of 300 mg fresh wt./ml) evaporated to dryness was reconstituted in 1 ml of 75% methanol and then was processed by SPE using Method 2 (with pre-elution with 30% methanol) and further qualitatively and quantitatively analysed by HPLC.

[b] Not detected at the detection limit.

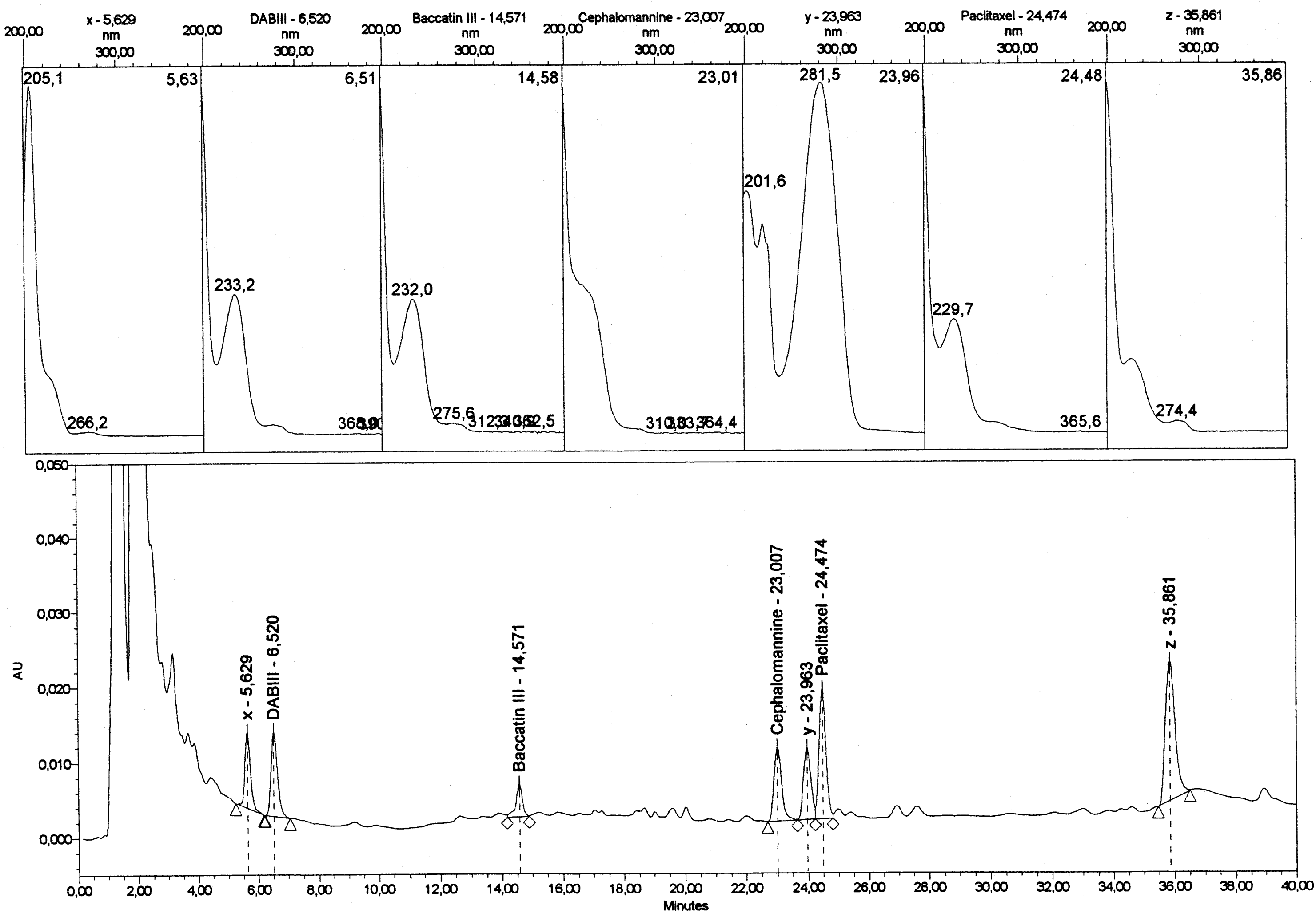

Fig. 5. HPLC chromatogram of yew extract from the needles of *T. baccata* var. *Elegantissima* spiked with mixed standard and purified by SPE (Method 2) showing 10-DAB III, baccatin III, cephalomannine and paclitaxel and their corresponding UV spectra. LC trace recorded at 233 nm; x–z: unidentified major compounds in the extract.

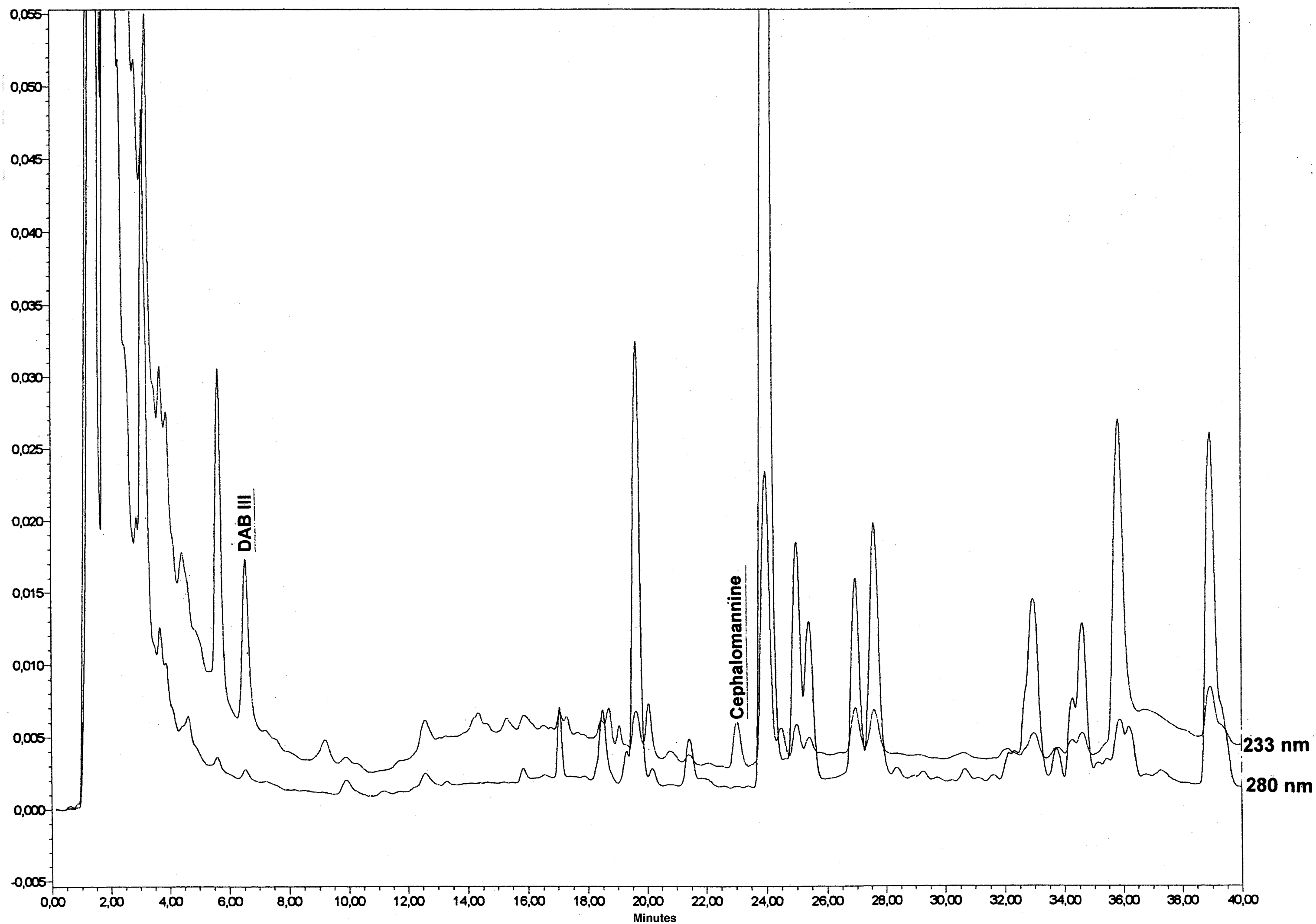

Fig. 6. HPLC chromatograms of yew extract from the needles of *T. baccata* var. *Elegantissima* purified by SPE (Method 2). LC trace recorded at 233 nm (upper) for taxoids was overlaied with the trace recorded at 280 nm (lower) for co-extractives.

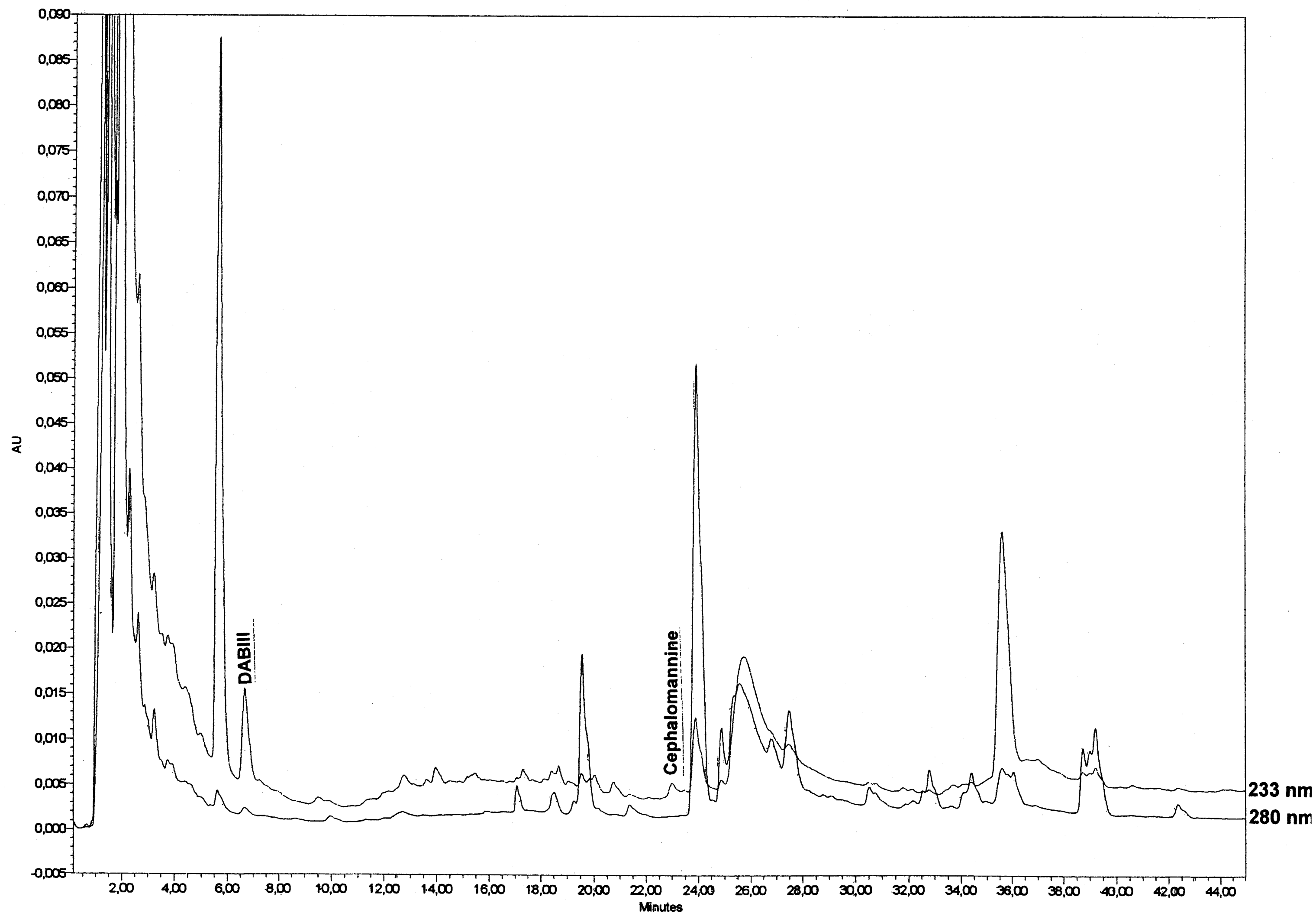

Fig. 7. HPLC chromatograms of yew extract from the stems of *T. baccata* var. *Aurea* purified by SPE (Method 2). The LC trace recorded at 233 nm (upper) for taxoids was overlain with the trace recorded at 280 nm (lower) for co-extractives.

poorly removed or does not elute at all with 5 ml of 30% methanol. This observation makes the pre-washing step very useful for pre-purifying yew samples after maceration as a lowering of background baseline absorbance can be achieved in this way. Several differences have been observed in the composition of UV-absorbing compounds at 280 nm for the stem fraction from *T. baccata* var. *Aurea* (Fig. 7; 280 nm) in comparison with the extract from *T. baccata* var. *Elegantissima* (Fig. 6; 280 nm), but these co-extracted compounds usually did not interfere with the taxoids analysed.

The detection limits for standards were estimated as 5 ng per injection (0.5 g/ml in the final solution before injection into HPLC column) at a signal-to-noise ratio of 3:1. This value is equivalent to 0.23 mg/kg of yew material. Standard deviations were routinely below 10% of the value measured.

In the samples from *T. baccata* var. *Elegantissima* and *T. baccata* var. *Aurea*, relatively high concentrations of the most important semisynthesis taxoid, 10-DAB III, were detected ($>0.06\%$; Table 2). The obtained 10-DAB III levels in the stem fraction from *T. baccata* var. *Aurea* now were found to be about 1.5 higher than the values determined previously by us in this yew sample according to the SPE–TLC–HPLC procedure [40].

4. Conclusions

The comparison of two methods of SPE partitioning of yew samples specially prepared in our laboratory cartridges filled with silanised silica gel indicated that the pre-elution step with 30% methanol permitted not only a lowering of background baseline absorbance but also a better separation of less polar taxoids (paclitaxel, cephalomannine) from their co-extractives. The levels of 10-DAB III eluted with 30% methanol depend on the ratio of the concentrations of 10-DAB III and its closely eluted compound. If this compound is strongly dominating (more than four times abundant from 10-DAB III), 10-DAB III does not elute at all. Higher concentration of polar co-extractives in the extracts obtained after maceration of yew material were also observed in comparison with the extracts obtained by a typical extraction with hot methanol [40]. For these reasons, such a pre-washing step seems to be necessary for these samples because a lowering of background baseline absorbance can be achieved without any considerable loss of 10-DAB III amounts. However, when yew material is rapidly extracted with hot methanol (a lower concentration of polar components is observed), a relatively good separation of 10-DAB III and its closely eluted co-extractive together with high recoveries for 10-DAB III (98%) can be obtained without a pre-elution step of SPE on silanised silica gel cartridges.

The described simplified method of gradient elution elaborated especially for 10-DAB III determination also enabled screening for other major taxoids (cephalomannine, paclitaxel and baccatin III) in yew extracts. Recently published method of 10-DAB III quantification [41] on the Nova-Pak C_{18} column with MeOH–H_2O–AcOH gradient elution made possible almost baseline 10-DAB III separation but in more than 10 min. In our method, a similar separation was achieved in less than 7 min, which makes this method suitable for rapid 10-DAB III analysis, and due to high recoveries and low detection limits and by the use of C_2 sorbent for SPE, this procedure can be applied in a routine 10-DAB III and related major taxoid determination in yew material.

Acknowledgements

We would like to thank Waters Company especially Mr. Staszek Kalinka for supporting of Waters Alliance M2690 chromatograph together with the Symmetry C_{18} column.

References

[1] D.G.I. Kingston, G. Samanarayake, C.A. Ivey, J. Nat. Prod. 53 (1990) 1–12.

[2] M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, A.T. McPhail, J. Am. Chem. Soc. 93 (1971) 2325–2327.

[3] K.D. Swenerton, Indian J. Med. Paediat. Oncol. 15 (1994) 20–27.
[4] P. Potemski, A. Pluzanska, Wiad. Ziel. 6 (1997) 18–19 in Polish.
[5] R.C. Donehower, E.K. Rowinsky, L.B. Grahow, S.M. Longnecker, D.S. Ettinger, Cancer Treat. Rep. 71 (1987) 1171–1177.
[6] J.L. Grem, K.D. Tutsch, K.J. Simon, D.B. Alberti, I.V.K. Wilson, D.C. Tormey, S. Swaminathan, D.L. Trump, Cancer Treat. Rep. 71 (1987) 1179–1184.
[7] E.K. Rowinsky, L.A. Cazenawe, R.C. Donehower, J. Natl. Cancer Inst. 82 (1990) 1247–1259.
[8] J.N. Denis, A.E. Greene, D. Guenard, F. Gueritte-Voegelein, L. Mangatal, P. Potier, J. Am. Chem. Soc. 110 (1988) 5917–5919.
[9] R.A. Holton, H.B. Kim, C. Somoza, F. Liang, R.J. Biediger, P.D. Boatman, M. Shindo, C.C. Smith, S. Kim, H. Nadizadeh, Y. Suzuki, C. Tao, P. Vu, S. Tang, P. Zhang, K.K Murthi, L.V. Gentile, J.H. Liu, J. Am. Chem. Soc. 116 (1994) 1599–1600.
[10] R.A. Holton, J.H. Liu, L.V. Gentile, R.J. Biediger, Presented at the 2nd National Cancer Institute Workshop on Taxol and Taxus, Session III, 1992.
[11] M. Furmanowa, L. Rapczewska, Wiad. Ziel. 6 (1995) 1–4 in Polish.
[12] K.V. Rao, Presented at the International Research Congress on Natural Products, Halifax, Nova Scotia, Canada, July 31–August 4, 1994.
[13] M.J.I. Mattina, G.J. MacEachern, J. Chromatogr. A. 679 (1994) 269–275.
[14] M. Furmanowa, K. Glowniak, A. Zobel, J. Guzewska, G. Zgorka, L. Rapczewska, A. Jozewczyk, Med. Fac. Landbouww. Univ. Gent. 60 (1995) 2115–2118.
[15] G.I. Georg, S.R. Gollapudi, G.L. Grunewald, C.W. Gunn, R.H. Himes, B.K. Rao, X.Z. Liang, Y.W. Mirhom, L.A. Mitscher, D.G. Vander Velde, Q.M.A. Ye, Biorg. Med. Chem. Lett. 1345–1348 (1993).
[16] W. Fang, Y. Wu, J. Zhou, W. Chen, Q. Fang, Phytochem. Anal. 4 (1993) 115–119.
[17] K. Glowniak, G. Zgorka, A. Jozewczyk, M. Furmanowa, J. Pharm. Biomed. Anal. 14 (1996) 1215–1220.
[18] R.E. Schutzki, A. Chandra, M.G. Nair, Phytochemistry 37 (1994) 405–408.
[19] A. Sparreboom, O. Van Tellingen, W.J. Nooijen, J.H. Beijen, J. Chromatogr. B. 664 (1995) 383–391.
[20] M.T. Huizing, H. Rosing, F. Koopman, A.C. Keung, H.M. Pinodo, J.H. Beijen, J. Chromatogr. B. 664 (1995) 373–382.
[21] H.L.M. Van Rozendaal, G.P. Lelyveld, T.A. Van Beek, Phytochem. Anal. 8 (1997) 286–293.
[22] R.G. Kelsey, N.C. Vance, Phytochemistry 55 (1992) 912–917.
[23] R. Vanhaelen-Fastre, B. Diallo, M. Jaziri, M.L. Faes, J. Homes, M. Vanhaelen, J. Liq. Chromatogr. 15 (1992) 697–706.
[24] N.C. Vance, R.G. Kelsey, T.E. Sabin, Phytochemistry 36 (1994) 1241–1244.
[25] D. Song, J.L.S. Au, J. Chromatogr. B. 663 (1995) 337–344.
[26] D.R. Lauren, D.J. Jensen, J.A. Douglas, J. Chromatogr. A. 712 (1995) 303–309.
[27] K.V. Rao, R.S. Bhakuni, J. Jachum, R.M. Davies, J. Liq. Chromatogr. Relat. Techn. 19 (1996) 227–247.
[28] K.M. Witherup, S.A. Look, M.W. Stasko, T.G. Mac Cloud, H.J. Issaq, G.M. Muschik, J. Liq. Chromatogr. 12 (1989) 2117–2132.
[29] S.L. Richheimer, D.M. Tinnermeier, D.W. Timmous, Anal. Chem. 64 (1992) 2323–2326.
[30] L.K. Shao, D.C. Locke, Anal. Chem. 69 (1997) 2008–2016.
[31] H.Y. Aboul-Enein, V. Serignese, Anal. Chim. Acta 319 (1996) 187–190.
[32] R.E.B. Ketchum, D.M. Gibson, J. Liq. Chromatogr. 16 (1993) 2519–2530.
[33] S.O.K. Auriola, A.M. Lepistö, T. Naaranlanthi, J. Chromatogr. 594 (1992) 153–158.
[34] F. Bitsch, J. Chromatogr. 615 (1993) 273–280.
[35] E.H. Kerns, K.J. Volk, S.E. Hill, J. Nat. Prod. 57 (1994) 1391–1403.
[36] B. Schneider, Y. Zhao, T. Blitzke, B. Schmitt, A. Nookandeh, X. Sun, J. Stöckight, Phytochem. Anal. 9 (1998) 237–244.
[37] X. Cao, Y. Thian, T.Y. Zhang, Y. Ito, J. Chromatogr. A 813 (1998) 397–401.
[38] K. Glowniak, T. Mroczek, A.M. Zobel, Phytomedicine 6 (1999) 67–72.
[39] K. Glowniak, T. Mroczek, J. Liq. Chromatogr. Relat. Techn. 22 (1999) 2483–2502.
[40] K. Glowniak, T. Mroczek, M. Furmanowa, M. Hajnos, Presented at the 7th Conference on the Application of Chromatographic Methods in Phytochemical & Biomedical Analysis, Lublin, Poland, 25–27 June, poster P-48, 1998.
[41] M.T. Adeline, X.P. Wang, C. Poupat, A. Ahond, P. Potier, J. Liq. Chromatogr. Relat. Techn. 20 (1997) 3135–3145.