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Determination of paclitaxel and other six taxoids in *Taxus* species by high-performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

A method of high-performance liquid chromatography–tandem mass spectrometry (LC–MS-MS) has been developed for the trace analysis of paclitaxel and other six taxoids in three Taxus species including *Taxus cuspidata*, *Taxus media* and *Taxus chinensis var. mairiei*. Seven taxoids were separated using a gradient mode on an Eclipse XDB-C18 column (4.6 × 150 mm; i.d., 5 μm) at 20 °C. The compound separations were detected by an API 3000 mass spectrometer equipped with a TurboIonSpray™ interface. The compounds were detected using electrospray ionization (ESI) in positive-ion mode and quantified by multiple-reaction monitoring (MRM) mode using the transition mass of *m/z* 567.4 → 445.4, 609.3 → 549.5, 944.9 → 286.4, 812.6 → 286.1, 832.8 → 264.1, 854.4 → 286.1, and 812.6 → 286.1 for 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, and 7-epi-10-DAT, respectively. The ranges of limit of quantitation (LOQ) for taxoids were 32, 14, 26, 14, 20, 32, and 26 ng/mL for 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, and 7-epi-10-DAT, respectively. Linearity was confirmed over the whole calibration range (0.07–45, 0.058–37.5, 0.058–37.5, 0.056–36.3, 0.053–33.8, 0.057–37.5, and 0.06–38.8 μg/mL for 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, and 7-epi-10-DAT, respectively) with coefficients higher than 0.9903. The inter- and intra-day precision of taxoids ranged from 2.86% to 6.31% for retention time and ranged from 3.91% to 7.33% for peak area. The recovery rates of this method were higher than 94.32% for 10-DAB III, 94.68% for baccatin III, 93.65% for 7-xyl-10-DAT, 93.29% for 10-DAT, 92.91% for cephalomannine, 93.41% for paclitaxel, and 93.06% for 7-epi-10-DAT, respectively.

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1. Introduction

Paclitaxel (Fig. 1), a naturally occurring diterpene alkaloid, was originally isolated from the bark of *Taxus brevifolia*. It is regarded as one of the most useful anticancer drugs of plant origin available today. It is effective in the treatment of many tumor types, such as ovarian, breast, melanoma, head, neck, bladder, gastrointestinal, lung cancer, and non-small-cell lung cancer [1–3], as well as for AIDS-related Kaposi's sarcoma [4–6]. Its fundamental anticancer mechanism is unique [7]. In contrast to *Vinca* alkaloids, colchicine, podophyllotoxin, vinblastine, and other antitumor agents that act to prevent the polymerization of tubulin into microtubule, paclitaxel promotes microtubule assembly and suppresses its depolymerization.

Yew trees grow extremely slowly, and thus the yields of paclitaxel are too low to supply sufficient quantities for clinical use [6–8]. Consequently, alternative methods have been extensively investigated [8]. Semi-synthesis has proven to be an appropriate method for commercial use. Currently, paclitaxel and its analogue docetaxel are produced semi-synthetically by the coupling reaction of 10-deacetylbaccatin III (10-DAB III) with an appropriately protected side chain that can be prepared synthetically [9,10]. In addition, other related taxoids, including baccatin III, cephalomannine, 7-xylosyl-10-deacetyl paclitaxel (7-xyl-10-DAT), 7-epi-10-deacetylpaclitaxel (7-epi-10-DAT), 10-deacetylpaclitaxel (10-DAT) and others, can be used for the semi-synthesis of paclitaxel and docetaxel. These taxoid compounds are obtained from the bark, needles, twigs, and roots of many *Taxus* species [2,3,8,11–20].

Currently, several analytical methods exist for quantifying taxoids in plant extracts. The majority of them involve high-performance liquid chromatography (HPLC) with UV detection [21–23]. Enzyme-linked immunosorbent assays (ELISA) have also been used to screen plant extracts for taxoids [24]. In addition, high-speed countercurrent chromatography (HSCCC) was also used

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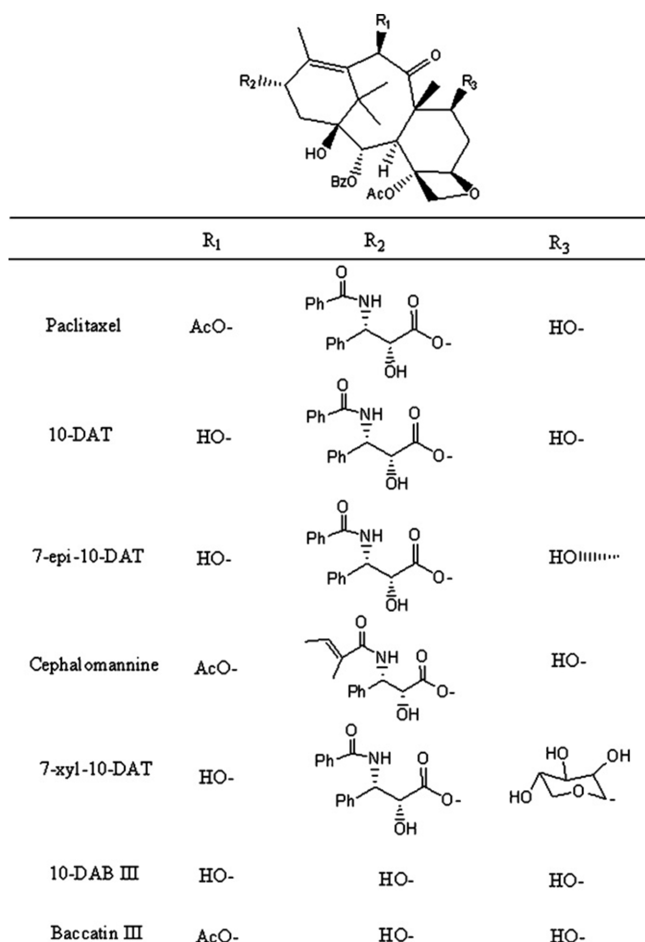

Fig. 1. Structures of seven taxoids: 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, and 7-epi-10-DAT.

to analyze taxoids [25]. Due to the very low content of taxoids in yews, however, the feasibility of these methods is restricted by a low sensitivity.

As a result of recent advances in electrospray ionization (ESI) [1,26] and atmospheric pressure ionization (API) techniques, liquid chromatography coupled with tandem mass spectrometry (LC–MS-MS) has emerged as an efficient analytical tool with improved sensitivity, selectivity, and specificity. The usefulness of this technique has been demonstrated for a wide range of applications in bioanalytical, environmental, and pharmaceutical research [27–31]. It is also employed to identify and then determine amounts of paclitaxel. Some reports of LC–MS-MS methods have described the analysis of only paclitaxel or docetaxel in human or animal's plasma [27,32–34], but only a few studies have attempted to identify the structure of taxoids in extracts from yew plants [32,35,36]. In addition, the number of taxoids detected by using LC–MS-MS methods were limited to paclitaxel, baccatin III, cephalomannine, and 10-DAB III [36–38]. So far, studies seeking to detect 7-xyl-10-DAT, 10-DAT, and 7-epi-10-DAT in yews by using LC–MS-MS methods with multiple-reaction monitoring (MRM) mode have not been undertaken.

In the present assay, we have developed and validated a rapid and sensitive LC–MS-MS method to quantitatively determine the presence of seven taxoids in three yews. This method relies on HPLC with an Eclipse XDB-C18 column followed by positive electrospray ionisation-tandem mass spectrometry detection. A multiple-reaction monitoring (MRM) mode was utilized to optimize sensitivity and accuracy. The method is rapid, sensitive, and specific for quantifying seven taxoids in yew extracts.

2. Experimental

2.1. Reagents and standards

Paclitaxel, 10-DAB III, baccatin III, and cephalomannine were purchased from Sigma (St. Louis, MO, USA); 7-xyl-10-DAT, 10-DAT and 7-epi-10-DAT were obtained from Shanghai Jinhe Bio-Technology Co., Ltd. (Shanghai, China).

For standard solutions, the standard taxoids were dissolved in acetonitrile to obtain a concentration of 45, 37.5, 37.5, 36.25, 33.75, 38.75, and 37.5 μg/mL for 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, 7-epi-10-DAT, and paclitaxel, respectively. To optimize taxoid separation and determine recovered amounts, a mixed standard solution of 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, 7-epi-10-DAT, and paclitaxel at a concentration of 6.43, 5.36, 5.36, 5.18, 4.82, 5.54, and 5.36 μg/mL, respectively, was prepared.

The solvent acetonitrile and methanol were of chromatographic grade (J & K Chemical Ltd., China). Ethanol obtained from Beijing Chemical Reagents Co. (Beijing, China) was of analytical grade. Double-distilled water was used in all experiments. All samples prepared for HPLC were filtered through a membrane filter (0.45 μm pore size) before use.

2.2. Chromatographic conditions

The liquid chromatography (LC) system consisted of an Agilent 1100 series equipped with G1379A Degasser and G1312A Binpump (Agilent, USA). A 4.6 mm × 150 mm; i.d., 5 μm, Eclipse XDB-C18 column (Agilent, USA) was utilized for chromatographic separation. In the course of the experiments, several systems, including methanol–water, acetonitrile–water, and methanol–acetonitrile–water, were examined in different ratios. The elution mode including ordinary elution and gradient elution were also optimized. The LC flow rate was 1 mL/min. The compounds were eluted using a mobile phase of 60:40 (acetonitrile:water) held isocratically for 36 min, followed by a 1-min linear gradient to 40:60 (acetonitrile:water), and then by a 7-min isocratic elution before returning to the initial conditions in 1 min. After 15 min of re-equilibration, the column was ready for a new injection. Separation was performed at 20 °C.

2.3. MS-MS conditions

An API 3000 Triple tandem quadrupole mass spectrometry from Applied Biosystems (USA) with a TurboIon–Spray interface in the positive electrospray ionization mode (ESI$^+$) was used for detection. The mass spectrometer was operated in MRM mode so as to enhance the selectivity and specificity of low content compounds in *Taxus* species.

Analytes were ionized in positive-ionization mode under ESI conditions as follows: the ion source was operated at a temperature of 350 °C. Nitrogen was used as the curtain gas (CUR), nebulizing gas (NEB), auxiliary gas (AUX), and collision gas. The ion spray voltage (IS) was set at 5500 V. The declustering potential (DP), entrance potential (EP), and focusing potential (FP) voltages were set at 80, 10, and 400, respectively. The collision energies (CE) were optimized for each compound., Following HPLC separation, the peak area corresponding to the transitions of m/z 567.4 → 445.4, 609.3 → 549.5, 944.9 → 286.4, 812.6 → 286.1, 832.8 → 264.1, 854.4 → 286.1, and 812.6 → 286.1 reaction (dwell time 200 ms) for 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT,

cephalomannine, paclitaxel, and 7-epi-10-DAT were measured, respectively. Peak areas obtained from the MRM were used to quantify seven taxoids and the analyst software Version 1.4 was utilized for acquiring and processing the data.

2.4. Preparation of analytical samples

Fresh twigs of *Taxus cuspidata*, *Taxus chinensis*, and *Taxus media* were obtained from the Botanical Garden of the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin, China, in April 2005. The plant specimens were identified by Professor Nie Shaoquan (Botanical Garden of the Key Laboratory of Forest Plant Ecology, Harbin, China). Voucher specimens of yews were kept in the same Key Laboratory. The twigs were separated into needle and stem fractions, and the needle fraction was then separately cut into small parts (2 mm).

For maceration, 2 g of fresh yew needles was weighed accurately and put into a beaker with 40 mL of 80% ethanol. The beaker was placed at room temperature for 10 h. Then the filtered aqueous solution was concentrated to dryness in a rotary evaporator (Shanghai Qingpu Huxi Instrument Factory, China) at 50 °C. Before being injected into HPLC, the residues were dissolved in 5 mL methanol and degreased by adding 2.5 mL water for 8 h. After filtration, the sample was analyzed by HPLC–MS-MS.

For heat reflux, 2 g of fresh yew needles was weighed accurately and put into a round-bottom flask with 20 mL of 80% ethanol. The flask was refluxed at 80 °C for 2 h. Then the filtered aqueous solution was concentrated to dryness. The subsequent process was the same as for maceration.

For diacolation, 2 g of fresh yew needles was weighed accurately and put into a columned tundish. Then a piece of gauze was put on the top of the needles and 40 mL of 80% ethanol was added into the tundish gradually to permeate the material. The dropping rate was 0.11 mL/min and the extravasate was collected in an Erlenmeyer flask. The subsequent processes were same as for maceration.

For ultrasonic extraction, 2 g of fresh needles from each yew was weighed accurately and put into an Erlenmeyer flask with 20 mL of 80% ethanol. Then the flask was placed in an ultrasonication bath (KQ-250DB, Kunshan Ultrasonic Instrument Co., Ltd., China) and extracted at 40 °C for 60 min. The subsequent processes were the same as for maceration.

2.5. Method validation

2.5.1. Calibration curves

Calibration curves of eight concentrations of 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, and 7-epi-10-DAT ranging from 0.070 to 45.0, 0.058 to 37.5, 0.058 to 37.5, 0.056 to 36.3, 0.053 to 33.8, 0.060 to 38.8, and 0.058 to 37.5 μg/mL, respectively, were assayed according to the conditions described in Sections 2.2 and 2.3. The limit of detection (LOD) and the limit of quantification (LOQ) were determined as the concentrations at signal-to-noise ratios of 3 and 10, respectively.

2.5.2. Precision

The precision of the assay was determined from the standard samples by replicate analyses ($n = 6$). Intra- and inter-day reproducibility and standard deviations were also assessed by repeated analyses on three consecutive days ($n = 15$). The calibration of the method was determined to demonstrate accuracy. The concentration of each sample was determined using the calibration curve prepared and analyzed on the same batch.

2.5.3. Recovery

The recovery tests for the HPLC–MS-MS method were carried out by adding the standards to the raw materials. Three initial concentrations were prepared to estimate the recovery. Then the materials mixed with standards were prepared using ultrasonic extraction according to the procedure described in Section 2.4. The samples were then analyzed by HPLC–MS-MS according to the conditions described in Sections 2.2 and 2.3.

3. Results and discussion

3.1. Optimization of the extraction method

In order to quantify the extracted amounts, the variables involved in the procedure such as extraction methods, solvent, and extraction times were optimized. Maceration, heat reflux, diacolation, and ultrasonic extraction were performed to extract seven taxoids from *Taxus* needles. The results are shown in Fig. 2.

Fig. 2 shows that the extraction yields by maceration were clearly lower than those obtained by heat reflux, diacolation, or ultrasonication. While the extraction completeness by maceration and diacolation depended to a large extent on the extraction time, the extraction completeness of ultrasonication was nearly time-independent. In addition, ultrasonic extraction gave much better results within 60 min than heat reflux and diacolation extraction within 120 and 350 min. Fig. 2 also shows that there were some differences in the extraction yields between the three species. However, the trends for the relation between extraction time and extraction yield of different extraction methods were similar. The extraction yields of each taxoid compound from three *Taxus* species were also different among the three extraction methods. In our

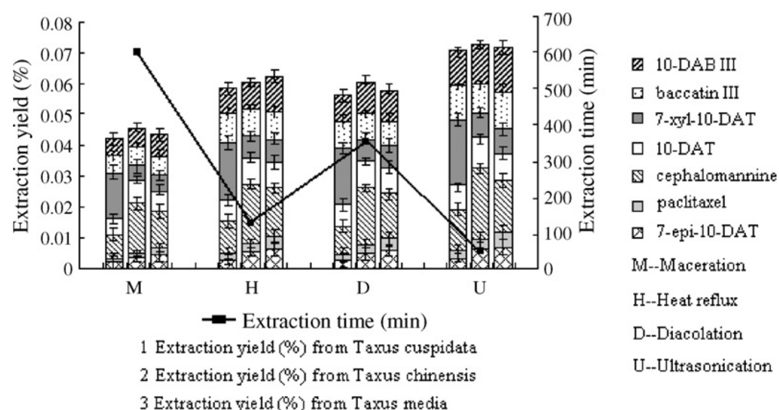

Fig. 2. Effect of different extraction methods on the yields of seven compounds derived from three *Taxus* species needles.

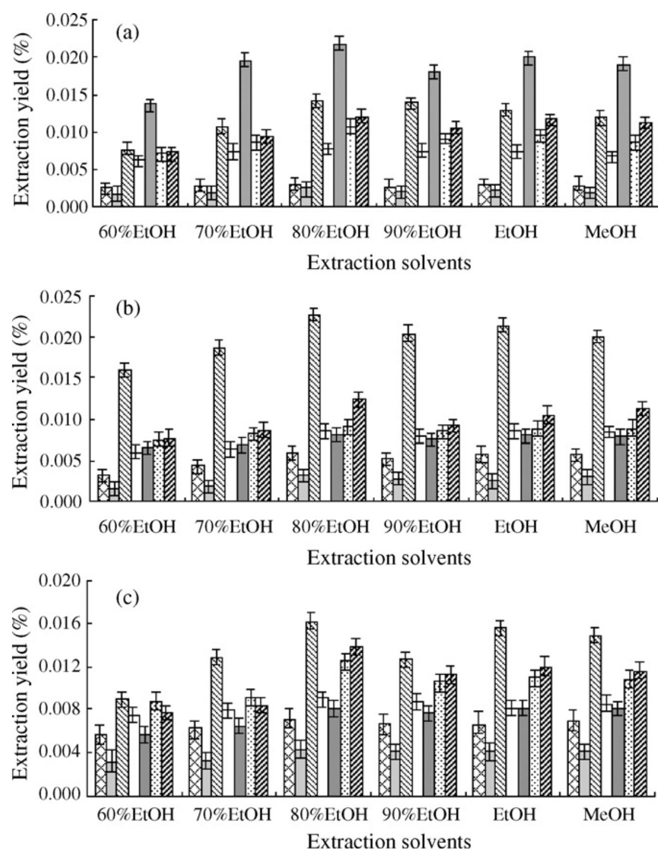

Fig. 3. Effect of different extraction solvent on the yields of seven compounds derived from the needles of *Taxus cuspidata* (a), *Taxus chinensis* (b) and *Taxus media* (c). 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, 7-epi-10-DAT.

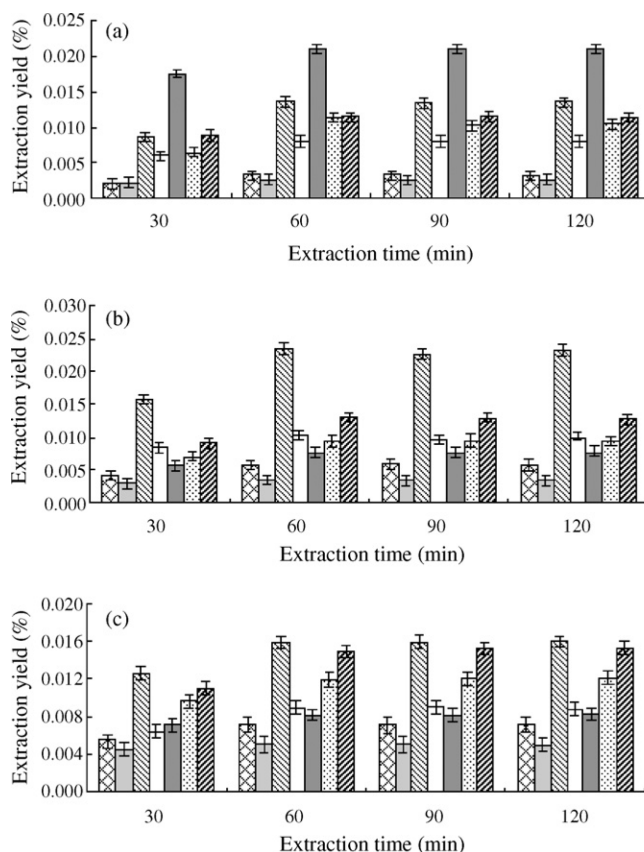

Fig. 4. Effect of different extraction time on the yields of seven compounds derived from the needles of *Taxus cuspidata* (a), *Taxus chinensis* (b) and *Taxus media* (c). 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, 7-epi-10-DAT.

study, the ultrasonic method was considered to be effective for extracting taxoids from *Taxus* needles.

To optimize the extraction solvent, ethanol and methanol solution were tested as extraction solvents. The best solvent was 80% ethanol, with which high yields of all the taxoids could be extracted, while pure ethanol and methanol could not efficiently extract the taxoids (Fig. 3). On this basis, extraction times were also investigated. Fresh needles (2 g) were extracted with 20 mL 80% EtOH for 30, 60, 90, 120 min, respectively. All the taxoids were almost completely extracted within 60 min, whereas longer times did not further improve the extraction efficacy (Fig. 4).

3.2. Optimization of chromatographic conditions

It is crucial to optimize the chromatographic conditions before applying MS-MS because impurities in the samples can greatly decrease the sensitivity of determination by affecting the ionization of the compounds of interest.

A number of gradient conditions and alternative options were implemented to optimize the HPLC separation for seven taxoids. In the course of the experiments, several systems such as methanol–water, acetonitrile–water, and methanol–acetonitrile–water performed in different ratios were examined. Finally, in consideration of economy and simplicity, the mobile phase composition was simplified by using acetonitrile–water mixtures. Comparisons between ordinary elution and gradient elution showed that the latter possessed a distinct advantage for separating the seven compounds and a high sensitivity for determination by using MS-MS. Representative separations of these taxoids from the mixture were achieved after 45 min. The optimal separation condition was as follows: The compounds were eluted isocratically at 1 mL/min with acetonitrile–water (60:40, v/v) for 36 min, then washed with a linear gradient program of acetonitrile–water from 60:40 (v/v) to 40:60 (v/v) within 1 min, and maintained as an isocratic elution for 7 min. After changing back to 60% acetonitrile within 1 min, the re-equilibration was maintained for 15 min.

3.3. Optimization of MS-MS conditions

Electrospray ionization (ESI) was tested in both positive- and negative-ion modes. Taxoids showed much higher response signals using positive-mode ESI than in the negative mode. Therefore, the ESI source in positive mode was chosen for taxoid detection. The optimal ionization conditions were tuned by infusing the standard mixture of paclitaxel and six other taxoids in methanol with a syringe pump at a flow rate of 0.5 μL/min (Harvard Apparatus Inc., Holliston, USA). Before injection, the ion source was thermally stabilized for 60 min.

Firstly, a standard solution of each taxoid was chosen to obtain a constant signal in the Q1 scan mode. Full-scan spectra were acquired over the *m/z* range of 10–1000 amu with a dwell time of 1.5 s and a step size of 0.1 amu. The declustering potential (DP) was optimized using the quantitative optimization function of Analyst 1.4 to achieve the highest signal response. The adduct patterns of different compounds are listed in Table 1. The main ions were observed at *m/z* 567.4 (Na^+ adduct), 609.3 (Na^+ adduct), 944.9 (H^+ adduct), 812.6 (H^+ adduct), 832.8 (H^+ adduct), 854.4 (H^+ adduct), and 812.6 (H^+ adduct) for 10-DAB III,

Table 1
Parameters of the LC–MS-MS analysis for taxoids.

Compound	M[a]	m/z	Peak	CE (V)	MRM (m/z)	t_R[b] (min)
10-DAB III	544	567.4	$[M+Na]^+$	40	567.4 → 445.4	2.57
Baccatin III	586	609.3	$[M+Na]^+$	40	609.3 → 549.5	5.36
7-xyl-10-DAT	944	944.9	$[M+H]^+$	20	944.9 → 286.4	7.11
10-DAT	811	812.6	$[M+H]^+$	15	812.6 → 286.1	13.77
Cephalomannine	832	832.8	$[M+H]^+$	30	832.8 → 264.1	25.24
Paclitaxel	853	854.4	$[M+H]^+$	40	854.4 → 286.1	31.56
7-epi-10-DAT	811	812.6	$[M+H]^+$	35	812.6 → 286.1	34.16

[a] Molecular weight.
[b] Retention time obtained in LC–MS-MS analysis.

Table 2
Calibration analysis of taxoid standards.

Compound	Linearity range (μg/mL)	Calibration equation[a]	LOD[b] (ng/mL)	LOQ[b] (ng/mL)	Correlation factor (R^2)
10-DAB III	0.070–45.0	373.58C + 558.06	10	32	0.9903
Baccatin III	0.058–37.5	168361C + 10618	4	14	0.9914
7-xyl-10-DAT	0.058–37.5	764.68C + 1144.5	8	26	0.9925
10-DAT	0.056–36.3	13284C + 52915	4	14	0.9905
Cephalomannine	0.053–33.8	3450.7C + 7698.9	6	20	0.9916
Paclitaxel	0.060–38.8	729.12C + 8311.3	8	26	0.9937
7-epi-10-DAT	0.058–37.5	239.21C + 595.71	10	32	0.9941

C: the concentration of taxoids standard (μg/mL).
[a] Eight data points (n = 3).
[b] LOD = limit of detection; LOQ = limit of quantification.

baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, and 7-epi-10-DAT, respectively, were chosen as precursor ions of the taxoids.

To optimize the MRM mode, standard solutions of seven taxoids in methanol were infused into the electrospray ion source with a syringe pump at a flow rate of 0.5 μL/min. By means of the software interface, it was possible to tune the instrument automatically within a few minutes. We used product ion scans to search for the most abundant product ion. The intensity of ion transitions was optimized by tuning the value of collision energy (CE) range from 10 to 50 V. The intensity of ion transitions was greatly influenced by collision energy. Thus, optimization of collision energy (CE) played a significant role for generating the final MRM mode. The optimal collision energies (CE) of the taxoids were different. For 10-DAB III, baccatin III, and paclitaxel, the precursor ions at m/z 567.4 (Na^+ adduct), 609.3 (Na^+ adduct) and 854.4 (H^+ adduct) were observed as the main ions if the collision energy (CE) was under 40 V. When the CE was above 40 V, unordered product ions with smaller signals were obtained. While the CE was set at 40 V, product ions at m/z 445.4, 549.5 and 286.1 were obtained at high intensity. Accordingly, optimal values of collision energy were 20 V for 7-xyl-10-DAT; 15 V for 10-DAT; 30 V for cephalomannine, and 35 V for 7-epi-10-DAT, respectively. Fig. 5 summarizes the mass spectrometry parameters required to achieve maximum MRM transition sensitivity and the cracking way. As they were intense and most abundant, ions at m/z 445.4, 549.5, 286.4, 286.1, 264.1, 286.1 and 286.1 were chosen as the product ion for 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, and 7-epi-10-DAT, respectively.

Finally, according to above results, the most intensive precursors for product ion transitions of m/z 567.4 → 445.4, 609.3 → 549.5, 944.9 → 286.4, 812.6 → 286.1, 832.8 → 264.1, 854.4 → 286.1, and 812.6 → 286.1 for 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, and 7-epi-10-DAT, respectively, were chosen for the MRM scan and defined as quantifier and qualifier. MRM was performed with a 200-ms dwell time. Peak areas obtained from the MRM of standards were utilized for the quantitative determination of seven taxoids. Sample solutions prepared in Section 2.4 were injected, directly separated, and detected under the optimal conditions mentioned earlier (Section 2.3). The MRM chromatogram of seven taxoid standards and samples extracted from *Taxus* species are shown in Fig. 6. The retention times of seven compounds were 2.57, 5.36, 7.11, 13.77, 25.24, 31.56, and 34.16 min, respectively (Fig. 6). The parameters of the LC–MS-MS analysis for taxoids are listed in Table 1.

3.4. Method validation

Linear regression analyses for the seven compounds were performed by using the external standard method. The calibration curves of the individual taxoids were constructed using a range of 8 concentrations of the standard. The peak area values were the average values of three replicate injections. The results of calibration are summarized in Table 2. All taxoid compounds showed

Table 3
Results of precision analysis for seven taxoids (n = 6).

Compounds	Intra-day R.S.D. for retention time (%)	Intra-day R.S.D. for peak area (%)	Inter-day R.S.D. for retention time (%)	Inter-day R.S.D. for peak area (%)
10-DAB III	3.09	3.91	5.16	7.12
Baccatin III	2.86	4.06	4.67	5.82
7-xyl-10-DAT	3.07	4.39	6.31	6.34
10-DAT	3.25	4.28	4.54	4.69
Cephalomannine	3.59	5.37	4.61	7.33
Paclitaxel	3.67	4.93	5.28	6.08
7-epi-10-DAT	3.71	5.03	5.43	6.99

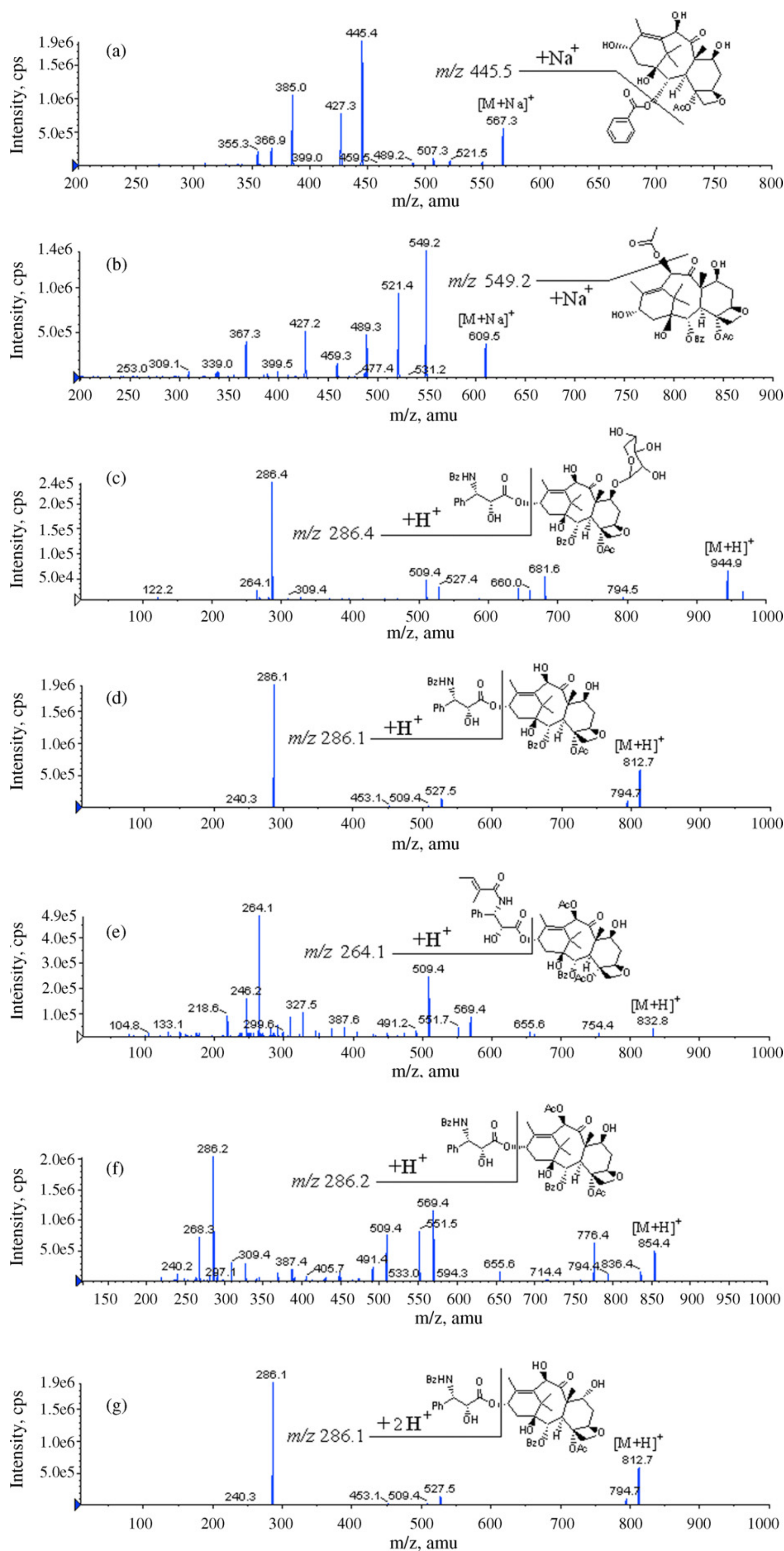
(a)
m/z 445.5
+Na+
[M+Na]+
567.3
(b)
m/z 549.2
+Na+
[M+Na]+
609.5
(c)
m/z 286.4
+H+
[M+H]+
944.9
(d)
m/z 286.1
+H+
[M+H]+
812.7
(e)
m/z 264.1
+H+
[M+H]+
832.8
(f)
m/z 286.2
+H+
[M+H]+
854.4
(g)
m/z 286.1
+2H+
[M+H]+
812.7
Intensity, cps
m/z, amu

Table 4
Recovery rates of the taxoids for the HPLC–MS-MS ($n = 6$).

Compounds		Amount added (μg)	Recovery (%)	R.S.D. (%)
10-DAB III	1	12.86	94.32	5.16
	2	25.72	95.11	5.47
	3	38.58	94.67	4.84
Baccatin III	1	10.72	95.24	5.22
	2	21.44	94.68	5.71
	3	32.16	94.96	6.07
7-xyl-10-DAT	1	10.72	93.84	4.67
	2	21.44	94.79	4.37
	3	32.16	93.65	5.26
10-DAT	1	10.36	94.55	4.39
	2	20.72	95.81	4.96
	3	31.08	93.29	5.48
Cephalomannine	1	9.64	92.91	5.91
	2	19.28	93.64	6.18
	3	28.92	93.48	6.06
Paclitaxel	1	11.08	94.74	5.74
	2	22.16	93.41	5.91
	3	33.24	94.56	5.83
7-epi-10-DAT	1	10.72	93.06	5.31
	2	21.44	94.15	5.78
	3	32.16	94.20	6.39

Table 5
Contents of four taxoids in the needles of different Taxus species (mg/g, $n = 3$).

		Species		
		T. cuspidata	*T. chinensis*	*T. media*
10-DAB III	Mean value	0.0304	0.0604	0.0725
	Intra-day R.S.D. (%)	3.39	4.47	5.09
	Inter-day R.S.D. (%)	5.27	5.16	6.66
Baccatin III	Mean value	0.0267	0.0342	0.0504
	Intra-day R.S.D. (%)	5.95	4.84	6.03
	Inter-day R.S.D. (%)	6.67	5.01	7.24
7-xyl-10-DAT	Mean value	0.1436	0.2316	0.1632
	Intra-day R.S.D. (%)	5.34	4.83	5.04
	Inter-day R.S.D. (%)	6.16	5.94	5.73
10-DAT	Mean value	0.0854	0.1034	0.0954
	Intra-day R.S.D. (%)	5.94	4.89	4.44
	Inter-day R.S.D. (%)	7.78	5.93	6.24
Cephalomannine	Mean value	0.2158	0.0783	0.0814
	Intra-day R.S.D. (%)	3.59	5.06	3.96
	Inter-day R.S.D. (%)	4.39	6.67	4.13
Paclitaxel	Mean value	0.1104	0.0904	0.1243
	Intra-day R.S.D. (%)	4.67	4.16	6.87
	Inter-day R.S.D. (%)	5.37	5.91	7.21
7-epi-10-DAT	Mean value	0.1127	0.1316	0.1420
	Intra-day R.S.D. (%)	5.26	6.11	5.13
	Inter-day R.S.D. (%)	5.93	6.18	6.07

good linearity ($R^2 \geq 0.9903$) in a relatively wide concentration range.

In our work, detection and quantification limits were estimated by successively decreasing the concentration of the prepared standards, down to the smallest detectable peak. This concentration was multiplied by 3 and 10 to obtain the detection and quantification limits, respectively. Table 2 summarizes LOD and LOQ values of individual compounds and clearly indicates that the analytical method has excellent sensitivity.

The precision test was carried out by injecting the same sample solution 6 times for retention time and peak area of each taxoid compound. The relative standard deviations (RSD) of intra-day measurements were between 2.86% and 3.67% for retention time and 3.91% and 5.37% for peak area. RSD values of inter-day

Fig. 5. Mass spectrum and product ion spectrum of seven taxoids. (a) 10-DAB III, (b) baccatin III, (c) 7-xyl-10-DAT, (d)10-DAT, (e) cephalomannine, (f) paclitaxel, and (g) 7-epi-10-DAT. The optimal collision energy (CE) of taxoids was 40 V for 10-DAB III, baccatin III and paclitaxel; 20 V for 7-xyl-10-DAT; 15 V for 10-DAT; 30 V for cephalomannine, and 35 V for 7-epi-10-DAT, respectively. The product ion spectra were m/z 445.4, 549.5, 286.4, 286.1, 264.1, 286.1, and 286.1 for 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, and 7-epi-10-DAT, respectively.

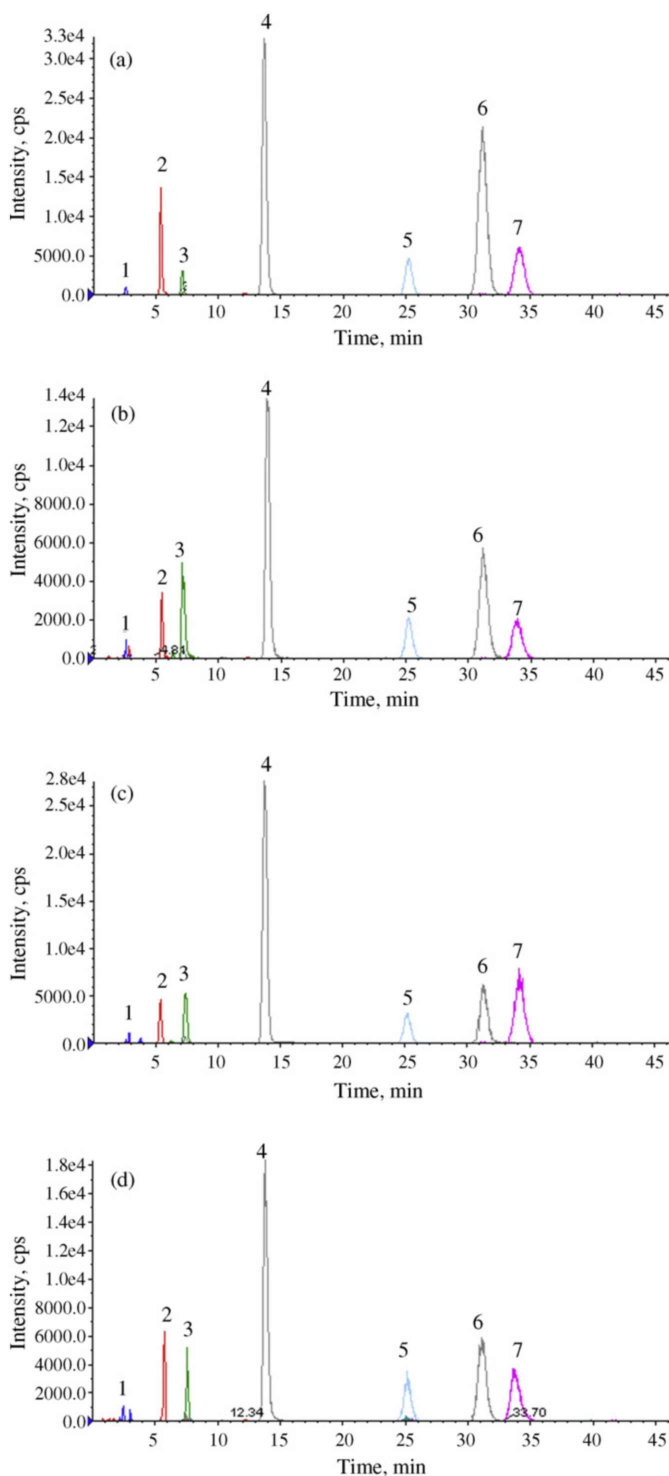

Fig. 6. LC–MS-MS total ion chromatogram (MRM mode) of seven taxoid standards (a) and samples from *Taxus cuspidata* (b), *Taxus chinensis* (c), and *Taxus media* (d). Peaks: 1 = 10-DAB III (*m*/*z* 567.4 → 445.4), 2 = baccatin III (*m*/*z* 609.3 → 549.5), 3 = 7-xyl-10-DAT (*m*/*z* 944.9 → 286.4), 4 = 10-DAT (*m*/*z* 812.6 → 286.1), 5 = cephalomannine (*m*/*z* 832.8 → 264.1), 6 = paclitaxel (*m*/*z* 854.4 → 286.1), and 7 = 7-epi-10-DAT (*m*/*z* 812.6 → 286.1). MRM was performed with 200 ms dwell time. The retention time of seven compounds was approximately 2.57, 5.36, 7.11, 13.77, 25.24, 31.56, 34.16 min, respectively.

measurements were between 4.54% and 6.31% for retention time, 4.69% and 7.33% for peak area (Table 3).

Recovery was determined for the HPLC–MS-MS method. The average recovery rates of the seven taxoids determined at three concentrations were higher than 94.32% for 10-DAB III, 94.68% for baccatin III, 93.65% for 7-xyl-10-DAT, 93.29% for 10-DAT, 92.91% for cephalomannine, 93.41% for paclitaxel, and 93.06% for 7-epi-10-DAT, respectively (Table 4).

3.5. *Content of taxoids in three taxus species*

The method was developed and applied to identify seven taxoids in *T. cuspidata*, *T. chinensis*, and *T. media* in this assay. The calculated contents of the seven taxoids are given in Table 5, in which each result is the average value of three replicate injections. Analyses of test samples of three Chinese yews have shown variations in concentrations of 10-DAB III, baccatin III, 7-xyl-10-DAT, 10-DAT, cephalomannine, paclitaxel, and 7-epi-10-DAT in the ranges of 0.0304–0.0725, 0.0267–0.0504, 0.1436–0.2316, 0.0854–0.1034, 0.0783–0.2158, 0.0904–0.1243, and 0.1127–0.1420 mg/g, respectively. Table 5 showed that 7-xyl-10-DAT and cephalomannine were more abundant than other taxoids in three yews. The contents of 7-xyl-10-DAT were highest in *Taxus chinensis* and *Taxus media.*

4. Conclusion

In this study, a LC–MS-MS method has been developed that offers a feasible and reliable means of detecting paclitaxel, 10-DAB III, baccatin III, cephalomannine, 7-xyl-10-DAT, 10-DAT, and 7-epi-10-DAT in the needles of three yews. ESI in positive-ion mode and a MRM mode were utilized to optimize sensitivity and accuracy. Validation statistics showed that this method possesses good sensitivity, precision, and repeatability. Linearity was confirmed over a wide calibration range with coefficients higher than 0.9903. The inter- and intra-day precision of seven taxoids ranged from 2.86% to 6.31% for retention time and ranged from 3.91% to 7.33% for peak area.

The assay method was applied to three *Taxus* species (*T. cuspidata*, *T. chinensis* and *T. media*). The contents of different taxoids compounds were distinct in the three *Taxus* species. The content of 7-xyl-10-DAT and cephalomannine was more abundant than other taxoids in *T. cuspidata* while the maximum amounts of 7-xyl-10-DAT and 7-epi-10-DAT appeared in *T. chinensis* and *T. media*. The successful detection of seven taxoids showed that this method is suitable for the determining amounts of paclitaxel and other taxoids.

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