Post-print: Analysis of 10-DAB III and Paclitaxel in Taxus yunnanensis by MSPD-HPLC

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Abstract

To establish analytical methods for 10-deacetylbaccatin III (10-DAB III) and paclitaxel (Taxol) in Taxus wallichiana var. chinensis, this study employed matrix solid-phase dispersion-high performance liquid chromatography (MSPD-HPLC) for quantitative analysis of 10-DAB III and Taxol in Yunnan yew. The investigation examined 12 sample pretreatment dispersants including silica gel, Florisil, acidic alumina, neutral alumina, basic alumina, C18, C18-ME, C18-G1, C18-HC, Diol, Xamide, and Xion, as well as the effects of various dispersant masses, eluent types, eluent concentrations, and eluent volumes on the analysis of the two components. The optimized conditions were subjected to methodological validation and compared with ultrasonic extraction and heat reflux extraction pretreatment methods to demonstrate effectiveness. The results indicated: (1) Among the 12 solid-phase dispersants examined, basic alumina exhibited favorable extraction and detection amounts for the target compounds, and when the mass ratio of basic alumina to sample was 3:1 with 6 mL methanol as eluent, the extraction and detection amounts for 10-DAB III and Taxol were relatively high. (2) The established analytical method for 10-DAB III and Taxol in yew branches and leaves demonstrated good linearity ($r \geq 0.9999$). The limits of detection and quantification for 10-DAB III and Taxol ranged between 0.0239~0.0301 $g \cdot mL^{-1}$ and 0.142~0.178 $g \cdot mL^{-1}$, respectively; spiked recovery tests at three standard substance concentration levels showed average spiked recoveries for each target analyte between 93.6%~109.0%. (3) Comparison of this method with ultrasonic extraction and heat reflux extraction pretreatment analysis results demonstrated that the three methods showed essentially no difference in extraction and detection amounts for the two taxanes; however, the MSPD method features low solvent consumption, simple operation, short analysis time, and high purification efficiency, making it applicable for rapid analysis of yew raw materials. This study applied basic alumina as a dispersant for extraction of taxanes from Yunnan yew, establishing a rapid and efficient analytical method for 10-DAB III and paclitaxel in yew, providing a basis for quantitative analysis

of taxanes in Taxus.

Full Text

Preamble

Determination of 10-DAB III and Taxol in *Taxus yunnanensis* by MSPD-HPLC

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Abstract: To establish an analytical method for 10-deacetylbaccatin III (10-DAB III) and taxol in *Taxus yunnanensis*, this study employed matrix solid-phase dispersion-high performance liquid chromatography (MSPD-HPLC) for quantitative analysis. The effects of various parameters were investigated, including twelve types of solid-phase dispersants (silica gel, florisil, acidic alumina, neutral alumina, alkaline alumina, C18, C18-ME, C18-G1, C18-HC, Diol, Xamide, Xion), dispersant mass, eluent type, eluent concentration, and eluent volume on the extraction of the two target compounds. After optimization, the methodology was validated and compared with conventional ultrasonic extraction and hot reflux extraction pretreatment methods to demonstrate its effectiveness. Results indicated that: (1) Among the twelve dispersants examined, alkaline alumina provided superior extraction yields for the target compounds when used at a 1:3 sample-to-dispersant mass ratio with 6 mL methanol as eluent. (2) The developed method exhibited excellent linearity ($r \geq 0.9999$), with detection limits and quantification limits for 10-DAB III and taxol ranging from 0.0239–0.0301 $g \cdot mL^{-1}$ and 0.142–0.178 $g \cdot mL^{-1}$, respectively. Average recoveries of target analytes at three spiked concentration levels ranged from 93.6% to 109.0%. (3) Comparative analysis revealed no significant differences in extraction yields of the two taxanes among the three methods; however, the MSPD method consumed less solvent, was simpler to operate, required shorter analysis time, and offered higher purification efficiency, making it suitable for rapid analysis of *Taxus* raw materials. This study establishes a rapid and efficient analytical method for 10-DAB III and taxol in *Taxus yunnanensis* using alkaline alumina as dispersant, providing a reliable basis for quantitative analysis of taxanes in *Taxus* species.

Keywords: *Taxus yunnanensis*, 10-deacetylbaccatin III, taxol, matrix solid-phase dispersion, high-performance liquid chromatography

Background

Taxus wallichiana var. *chinensis* (Chinese yew) belongs to the family Taxaceae and genus *Taxus*, with approximately eleven species worldwide. The primary active constituents of *Taxus* are taxane compounds, among which taxol and

10-DAB III are the most well-known. Taxol demonstrates significant therapeutic efficacy against various cancers including uterine and ovarian cancer, while 10-DAB III not only exhibits tumor-inhibiting properties but also serves as an important precursor for semi-synthetic anticancer drugs such as taxol and docetaxel. Therefore, rapid and accurate analysis of these two components in *Taxus* is of considerable practical importance.

Current analytical methods for taxanes in *Taxus* primarily employ HPLC, UPLC, HPLC-MS, and UPLC-MS. Quantification of multiple taxanes including 10-DAB III, cephalomannine, and taxol in different *Taxus* species is typically performed using HPLC with C18 or pentafluorophenyl columns. While UPLC and LC-MS/MS methods have also been developed for analyzing taxanes, conventional sample pretreatment methods such as reflux extraction or ultrasonic-assisted extraction combined with liquid-liquid extraction or SPE purification suffer from drawbacks including large sample requirements, high consumption of toxic organic solvents, and lengthy pretreatment times.

Matrix solid-phase dispersion (MSPD), first introduced by Professor Staren Barker, is a sample pretreatment method suitable for solid, semi-solid, or viscous samples that offers purification and enrichment of target compounds with minimal time consumption and simple operation. The principle involves grinding and mixing the sample with a dispersant in a mortar based on the molecular structural characteristics of analytes and properties of the dispersant, followed by elution and quantitative analysis. MSPD has been widely applied in food and drug residue determination, natural product extraction, and pharmaceutical analysis. This study focuses on *Taxus yunnanensis* branches and leaves, developing a rapid MSPD-HPLC method for analyzing 10-DAB III and taxol content. By investigating and optimizing factors affecting extraction yield and comparing with ultrasonic and hot reflux extraction methods, this research addresses: (1) selection and optimization of dispersants for MSPD-HPLC analysis of 10-DAB III and taxol in *Taxus*; (2) optimization of extraction conditions for the selected dispersant; (3) methodological validation and applicability of the newly developed method; and (4) comparative analysis to validate the effectiveness of MSPD against conventional pretreatment methods.

1 Materials and Methods

1.1 Instruments and Reagents

The HPLC system consisted of an AC Chrom S6000 analyzer (Beijing Huapu Xinchuang Technology Co., Ltd.). A 12 mL solid-phase extraction column (Hubei Sulin Technology Co., Ltd.), 600Y multifunctional grinder (Guangzhou Xulang Equipment Co., Ltd.), and Bmnson 3800 ultrasonic cleaner (Gongyi Yingyu High-tech Instrument Factory) were used for sample preparation. Standards of 10-DAB III (99%) and taxol (99%) were purchased from Xi'an Tianfeng Biotechnology Co., Ltd. Silica gel and florisil (200–300 mesh), C18, C18-ME, C18-G1, C18-HC, Diol, Xamide, and Xion (10 m particle size) were obtained

from Beijing Huapu Xinchuang Technology Co., Ltd. Alkaline, acidic, and neutral alumina (200–300 mesh) were from Shanghai Ludu Chemical Reagent Factory. HPLC-grade methanol and acetonitrile were from Tianjin Kermel Chemical Reagent Co., Ltd., while analytical-grade methanol, acetonitrile, ethanol, and acetone were from Tianjin Concord Technology Co., Ltd. *Taxus yunnanensis* branches and leaves were collected from domesticated cultivars in Baoshan, Yunnan Province.

1.2 Methods

1.2.1 Sample Preparation *Taxus yunnanensis* branches and leaves were pulverized using a grinder, passed through a 40-mesh sieve, dried to constant weight in a 50 °C oven, and stored in a glass desiccator for subsequent use.

1.2.2 Standard Solution Preparation Appropriate amounts of reference standards were dissolved in methanol to prepare mixed standard solutions of 10-DAB III and taxol at 0.1 $mg \cdot mL^{-1}$ each. Solutions were filtered through 0.22 m microporous membranes before HPLC analysis.

1.2.3 MSPD Procedure A 0.25 g sample of *Taxus* powder was mixed with various dispersants at specified ratios, including normal-phase chromatographic fillers (silica gel, florisil, alkaline alumina, acidic alumina, neutral alumina), reversed-phase fillers (C18-HC, C18-G1, C18, C18-ME), and HILIC fillers (Xion, Xamide, Diol). The mixture was thoroughly ground in a mortar, transferred to an extraction column with frits placed at both ends, and compacted. Methanol was added to elute the target compounds under atmospheric pressure, and the eluate was collected in a 25 mL volumetric flask, diluted to volume with methanol, filtered through a 0.22 m membrane, and subjected to quantitative analysis. All experiments were performed in triplicate.

1.2.4 Comparison of Pretreatment Methods Three pretreatment methods were compared: (1) Ultrasonic extraction: 0.25 g *Taxus* sample was extracted in 20 mL methanol for 1 h, diluted to 25 mL, mixed, filtered, and analyzed; (2) Hot reflux extraction: 0.25 g sample was placed in a 100 mL conical flask with 25 mL methanol, refluxed for 1 h (repeated three times), filtered, concentrated, transferred to a 25 mL volumetric flask, filtered, and analyzed; (3) MSPD method: After optimization, MSPD extraction yields and purification efficiency were compared using the procedure described in section 1.2.3.

1.2.5 Chromatographic Conditions Separation was performed on a C18 column (4.6 mm × 250 mm, 5 m) using a mobile phase of acetonitrile (A)-water (B) with gradient elution: 0–20 min, 15% A–50% A; 20–60 min, 50%–100% A; 60–65 min, 100%–10% A; 65–67 min, 10% A–15% A. Flow rate was 0.8 $mL \cdot min^{-1}$, detection wavelength 227 nm, column temperature 30 °C, and injection volume 10 L.

2 Results and Discussion

2.1 Optimization of MSPD Conditions

2.1.1 Selection of Dispersant The solid-phase dispersant is a critical factor affecting analytical accuracy in MSPD. Twelve dispersants were evaluated: normal-phase fillers (silica gel, florisil, alkaline alumina, acidic alumina, neutral alumina), reversed-phase fillers (C18-HC, C18-G1, C18, C18-ME), and HILIC fillers (Xion, Xamide, Diol). As shown in [Figure 1: see original paper], all twelve dispersants provided similar extraction yields, though HILIC fillers exhibited slightly lower yields for taxanes, likely because HILIC fillers adsorb polar compounds through hydrogen bonding or dipole-dipole interactions, resulting in poorer adsorption of relatively nonpolar 10-DAB III and taxol. While adsorption performance differences were minimal among dispersants, normal-phase fillers showed higher adsorption capacity for target compounds, with alkaline alumina providing the highest extraction yield. This may be attributed to alkaline alumina' s ability to reactively adsorb polar compounds while preserving nonpolar taxanes, making it suitable for purification and enrichment of relatively weakly polar taxanes. Therefore, alkaline alumina was selected as the optimal dispersant.

2.1.2 Effect of Sample-to-Dispersant Mass Ratio The influence of sample-to-alkaline alumina mass ratios (2:1, 1:1, 1:2, 1:3, 1:4, 1:5) on extraction yield was investigated. As illustrated in [Figure 2: see original paper], extraction yields for both 10-DAB III and taxol increased significantly as the ratio changed from 2:1 to 1:5, reaching an optimum at 1:3. Increased dispersant mass expands the interfacial area between sample components and dispersant, enhancing extraction efficiency. However, excessive dispersant mass hinders complete elution of target analytes, reducing yields. Additionally, larger packing amounts may lead to non-uniform dispersion. Consequently, a 1:3 sample-to-alkaline alumina mass ratio was selected.

2.1.3 Effect of Eluent Type Polar eluents facilitate desorption of analytes from dispersants. Four solvents—methanol, ethanol, acetonitrile, and acetone —were evaluated for their elution capacity. Results demonstrated that all four eluents could elute the target compounds, but methanol provided significantly higher extraction yields for both analytes compared to the other three solvents, indicating its superior ability to desorb 10-DAB III and taxol from alkaline alumina. Therefore, methanol was selected as the eluent.

2.1.4 Effect of Eluent Concentration Eluent concentration affects extraction yield. Different methanol concentrations (20%, 40%, 60%, 80%, 100%) were examined. As shown in [Figure 4: see original paper], extraction yields increased with methanol concentration up to 40%, after which further concentration increases produced minimal yield changes. However, lower methanol concentrations increased elution time, likely due to increased viscosity from wa-

ter content. Considering equivalent elution efficacy, 100% methanol was selected to minimize elution time.

2.1.5 Effect of Eluent Volume Eluent volume is a key parameter in MSPD separation, where minimal volume should be used while ensuring complete extraction. Methanol volumes of 2, 4, 6, 8, 10, and 12 mL were tested. As depicted in [Figure 5: see original paper], target compound yields increased as methanol volume increased from 2 to 6 mL, reaching maximum at 6 mL, with no significant improvement at larger volumes. Therefore, 6 mL was selected as the optimal eluent volume.

2.2 Method Validation

2.2.1 Linearity, Limit of Detection, and Limit of Quantification Aliquots (1.00, 0.50, 0.30, 0.20, 0.10 mL) of the mixed standard solution were diluted to 10 mL with methanol to prepare five concentration gradients. After filtration, samples were injected under the conditions described in section 1.2.5. Calibration curves were constructed using chromatographic peak area (y) versus concentration (x). The limits of detection (LOD) and quantification (LOQ) were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively. All compounds showed correlation coefficients greater than 0.9999, demonstrating excellent linearity. Detailed parameters are presented in .

2.2.2 Precision and Stability Intra-day precision was assessed by analyzing the same MSPD sample at 0, 2, 4, 6, 8, and 10 h, while inter-day precision was evaluated at 0, 24, and 48 h under the conditions in section 1.2.5. Relative standard deviations (RSD) for intra-day precision were 1.3% and 1.5%, and for inter-day precision were 1.1% and 1.4% for 10-DAB III and taxol, respectively, confirming good precision and stability.

2.2.3 Spike Recovery Spike recovery tests were conducted at three concentration levels (50%, 100%, 150%). Recoveries for 10-DAB III and taxol ranged from 93.6% to 109.0% with RSD values between 1.2% and 3.2%, as summarized in .

2.3 Comparison of Pretreatment Methods

Optimized MSPD was compared with hot reflux and ultrasonic extraction methods using *Taxus yunnanensis* samples. As shown in , no significant differences in extraction yields for 10-DAB III and taxol were observed among the three methods. However, MSPD required only 6 mL methanol and could be completed within 20 min, demonstrating simpler operation, lower solvent consumption, shorter analysis time, and promising application potential compared to conventional methods.

3 Discussion and Conclusion

Sample pretreatment is crucial for accurate quantification of natural products. Reported pretreatment methods for taxanes in *Taxus* include ultrasonic extraction and conventional solvent extraction, which suffer from high organic solvent consumption, large sample requirements, and low extraction efficiency, making them unsuitable for rapid quantitative analysis. MSPD offers advantages of simplicity, speed, and environmental friendliness for analyzing trace components in complex matrices.

The dispersant significantly affects extraction accuracy in MSPD. Common dispersants include normal-phase (silica gel, alumina, florisil), reversed-phase (C18-based), and novel dispersants, each with different adsorption mechanisms. Normal-phase fillers provided higher yields than reversed-phase fillers, possibly due to hydrogen bonding between Si−OH groups and polar functional groups (−OH, −NH) in the compounds. HILIC fillers yielded lower taxane recoveries than normal-phase fillers, likely because their strong adsorption of highly polar groups through multiple interactions (dipole, hydrogen bonding) is unsuitable for weakly polar taxanes, though the mechanism requires further investigation. Reversed-phase C18 is commonly used for pesticide residue and environmental sample analysis, with adsorption properties adjustable through water content or pH. In this study, alkaline alumina outperformed other normal-phase dispersants for 10-DAB III and taxol, possibly because it reactively adsorbs acidic phenolic impurities while preserving taxanes, providing clear extracts. Alkaline alumina is rarely reported as a dispersant for *Taxus* extraction, and these results offer valuable reference for taxane quantification.

The sample-to-dispersant mass ratio is an important factor requiring optimization based on extraction column dimensions. For example, Chu et al. (2017) achieved maximum lignan recovery from *Schisandra chinensis* at a 1:2 sample-to-dispersant ratio with methanol elution. In this study, 0.25 g sample with 0.75 g alkaline alumina provided optimal dispersion and elution.

Eluent selection in MSPD must consider analyte-dispersant interactions, using single or mixed organic solvents or even water. Methanol, acetonitrile, acetone, and ethanol were evaluated, with methanol proving most suitable for taxane elution from alkaline alumina. Eluent volume and concentration also affect yields; literature reports effective elution with 4–25 mL volumes. Salemi et al. (2012) achieved high pesticide recoveries from soil using 8 mL chloroform-hexane with 10% C18 silica. This study obtained high taxane yields with 6 mL methanol, saving solvent while maintaining efficiency.

In conclusion, using alkaline alumina as dispersant, 6 mL methanol as eluent, and a 1:3 sample-to-dispersant mass ratio, this study established an MSPD-HPLC method for simultaneous determination of 10-DAB III and taxol in *Taxus yunnanensis*. Comparative analysis demonstrated that alkaline alumina effectively purifies and enriches taxanes during MSPD. Methodological validation confirmed high recovery, good reproducibility, and excellent linearity within

the tested range, making this method suitable for rapid analysis of taxanes in *Taxus yunnanensis*.

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Note: Figure translations are in progress. See original paper for figures.