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# Non-chromatographic method for the purification of high purity paclitaxel from Taxus chinensis using tandem cavitation fractional precipitation

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**Research Article**

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# Non-chromatographic method for the purification of high purity paclitaxel from *Taxus chinensis* using tandem cavitation fractional precipitation

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**Abstract**

In this study, a non-chromatographic method using tandem cavitation fractional precipitation was developed for the purification of high-purity paclitaxel. Paclitaxel was purified with a purity of 98.8% and an overall yield of 91.1% through a three-step fractional precipitation using acetone/pentane (1:9, v/v), methanol/water (61.5:38.5, v/v), and acetone/water (1:2, v/v) systems. In addition, the precipitation characteristics and step-by-step impurity removal behaviors were observed using RP-HPLC, and the structure of the paclitaxel purified by tandem cavitation fractional precipitation was identified using FT-IR spectroscopy.

**Keywords**: Paclitaxel, Purification, Non-chromatographic Process, Tandem Cavitation Fractional Precipitation, Solvent Polarity Index

## INTRODUCTION

Paclitaxel (Fig. 1) is a diterpenoid alkaloid isolated from the epidermis of *Taxus brevifolia* that induces the inhibition of mitosis by stabilizing microtubules to induce cancer cell death [1,2]. Because of its unique anticancer mechanism, paclitaxel is a representative chemotherapy agent that has been proven effective against various cancers such as ovarian cancer, cervical cancer, and breast cancer [3-5]. With the expansion of clinical trials and the diversification of indications, the demand for paclitaxel is expected to increase [6-8]. The global market for paclitaxel as a drug substance was $117.4 million in 2020, and it is expected to reach $193.6 million in 2026 with a CAGR of 8.7% [9]. Paclitaxel can be produced through extraction, semi-synthesis, total synthesis, and cell culture. Among these methods, cell culture is recognized as a stable and promising long-term alternative [10-12].

Paclitaxel produced by plant cell culture is mostly accumulated in plant cells as an intracellular product. To use paclitaxel as an active pharmaceutical ingredient (API), high purity (>98%) separation and purification are essential [13]. However, the separation and purification processes for intracellular products account for more than 50% of the total manufacturing cost, so downstream processes must be designed well [14,15]. Paclitaxel is produced through final purification via biomass extraction followed by pretreatment using an organic solvent. In earlier attempts, paclitaxel was extracted using chromatography as a pretreatment for purification or immediately purified crude paclitaxel (purity: ~0.5%) was extracted without pretreatment using high performance liquid chromatography (HPLC), which made it difficult to commercially mass-produce paclitaxel due to the high a quantity of organic solvent used and the short column lifetime [14,16]. Subsequently, a variety of pretreatment methods such as liquid-liquid extraction [13,17], adsorption [13,18], low-pressure liquid chromatography (LPLC) [19,20], and precipitation [13,21] were introduced to the process in order to enhance the final purification efficiency using chromatography [14]. To obtain high purity (>98%) products, multi-step chromatography (octadecylsilylate (ODS C18)-HPLC, silica-HPLC) or an integrated purification process of chromatography and crystallization is still needed [14,19,22,23]. In addition, by introducing a continuous purification process using simulated moving bed (SMB) chromatography, the purification efficiency in terms of improved separation performance and reduced organic solvent use was improved to some extent [24-26]. Among downstream processes, selective purification by chromatography accounts for more than 70% of the downstream cost due to packing material cost, a long operation time, and

limited capacity, which are main limiting factors in process [27,28]. Therefore, the development of an efficient purification process that can purify paclitaxel to a high purity of 98% or more without using chromatography in the purification step, while considering yield and operating time, is urgently needed.

Studies have focused on fractional precipitation as a purification process for paclitaxel. Fractional precipitation is a highly effective and simple purification method based on solubility differences [13,29]. To shorten the long precipitation time (~3 days) of conventional fractional precipitation, studies have attempted to improve the precipitation efficiency using surface area increasing materials, but the precipitation still takes a long time, which limits its application for mass production [30,31]. Recently, cavitation-assisted fractional precipitation, which can dramatically decrease the precipitation time by using negative pressure and ultrasound-induced cavitation, has been developed [13,28,32]. In the case of ultrasonic cavitation fractional precipitation (precipitation solution: methanol/water ratio = 61.5:38.5, v/v), high purity (~74%) paclitaxel can be obtained in a short working time (<10 min) [32]. Additionally, equipment commonly used in the chemical industry, such as stirred-tank reactors, can be easily applied for fractional precipitation. However, the process cannot obtain high-purity (>98%) paclitaxel with only one step of fractional precipitation, which is a major limitation. Therefore, based on previous studies, this study investigated the fractional precipitation efficiency of paclitaxel according to the change in polarity index of the precipitation solution by varying the solvent composition in various precipitation solvent systems (acetone-pentane, methanol-water, and acetone-water). This process confirmed the possibility of selective purification by changing the polarity index of the precipitation solution. In addition, a non-chromatographic purification technology was developed to obtain high-purity (>98%) paclitaxel by performing two- and three-step tandem cavitation fractional precipitations by appropriately combining three types of optimized precipitation solvent systems. This is similar to the principle in which components are selectively separated by interactions with the stationary phase and the mobile phase in chromatography. Furthermore, the structure of paclitaxel (purity>98%) purified by tandem cavitation fractional precipitation was identified using FT-IR spectroscopy.

## MATERIALS AND METHODS

### 1. Sample Preparation

After culturing *Taxus chinensis* plant cells in modified Gamborg's B5 medium (24 °C, dark conditions, 150 rpm), the plant cells (biomass) were recovered using a centrifuge [13]. The sample was prepared via biomass extraction using methanol, liquid-liquid extraction using methylene chloride, adsorbent treatment using adsorbent Sylopute, low-pressure liquid chromatography using a silica-gel 60N column (Timely, Japan), and hexane precipitation. The pretreated sample (purity: 63.6%) was used in this study after vacuum drying at 35 °C for 24 hr. The detailed procedure was reported in a previous study [33].

**2. Cavitation Fractional Precipitation**

Cavitation fractional precipitation was performed using the precipitation solvent systems acetone-pentane, methanol-water, and acetone-water obtained in previous studies [34,35]. The schematic diagram of ultrasonic cavitation fractional precipitation is presented in Fig. 2. In the case of acetone-pentane fractional precipitation (Fig. 2A), the sample (purity: 63.6%) was dissolved in acetone (16.7 g crude paclitaxel/mL acetone), and n-pentane (acetone/n-pentane=1:7, v/v) was added drop by drop to induce precipitation. In the case of methanol-water fractional precipitation (Fig. 2B), the sample was dissolved in methanol (pure paclitaxel contents in methanol: 0.5%, w/v), and distilled water (methanol/water=61.5:38.5, v/v) was added dropwise to induce precipitation. In the case of acetone-water fractional precipitation (Fig. 2C), the sample was dissolved in acetone (pure paclitaxel contents in acetone: 0.5%, w/v) and then distilled water (acetone/water=1:4, v/v) was added drop by drop to induce precipitation. The fractional precipitations were performed using a 40 kHz ultrasonic bath (UC-10, Jeio Tech, Korea) under 250 W of ultrasonic power in the precipitator at 5 °C and an agitation speed of 335 rpm for 30 min. To obtain high-purity (>98%) paclitaxel, two-step and three-step fractional precipitations were performed by changing the composition of the precipitation solvent. The acetone/pentane ratios were 1:6, 1:7, 1:8, 1:9, 1:10, and 1:11 (v/v), the methanol/water ratios were 55:45, 60:40, 61.5:38.5, 65:35, 70:30, and 75:25 (v/v), and the acetone/water ratios were 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6 (v/v), respectively. After precipitation, the precipitate was obtained through filtration (150 mm, Whatman, Buckinghamshire, UK), and dried in a vacuum drying oven (UP-2000, EYELA, Japan) at 40 °C for 24 hr.

**3. Paclitaxel Analysis**

The paclitaxel content was analyzed using an HPLC system (SCL-10AVP, Shimadzu, Japan) and a C18 column (250 × 4.6 mm, Shiseido, Japan). The mobile phase, distilled water and acetonitrile (65/35~35/65, v/v, gradient mode) were made flow at a flow rate of 1.0 mL/min. The sample injection amount was 10 μL and was detected by ultraviolet (UV) at 227 nm [9]. Authentic paclitaxel (purity: 99%) was purchased from Samyang Biopharm Co., Ltd. and used as a standard.

### 4. Identification of Paclitaxel

The structure of the purified paclitaxel was identified using Fourier-transform infrared (FT-IR) spectroscopy (Spectrum 100, Perkinelmer, UK). The paclitaxel (purity: 99%) purchased from Samyang Biopharm Co., Ltd. as a standard sample and the paclitaxel (purity > 98%) obtained through the three-step fractional precipitation were analyzed in the transmission mode using KBr pellets at the wavelength range of 500-4000 $cm^{-1}$.

## RESULTS AND DISCUSSION

### 1. Two-Step Tandem System

In previous studies, it was possible to purify high purity (~85%) paclitaxel in high yield (~95%) with fractional precipitation solvent systems such as acetone-pentane (1:7, v/v), methanol-water (61.5:38.5, v/v), and acetone-water (1:4, v/v) [34,35]. In addition, by introducing ultrasonic cavitation into the fractional precipitation solution, the time required for precipitation could be drastically reduced (30-192 times). In this study, a two-step tandem cavitation fractional precipitation was performed using the three optimized solvent systems (Fig. 3). When acetone-pentane fractional precipitation (Fig. 3(A)) was the first step, the purity of paclitaxel was 88.5%, and when the second step was also acetone-pentane fractional precipitation, the purity was 92.9%. On the other hand, when methanol-water and acetone-water fractional precipitation were used in the second step, the purities were 95.6% and 94.1%, respectively. Next, when methanol-water fractional precipitation (Fig. 3(B)) was used in the first step, the purity of paclitaxel was 78.3%, and when methanol-water fractional precipitation was also used in the second step, the purity was 84.1%. On the other hand, when acetone-pentane and acetone-water fractional precipitation were used in the second step, the purities were 88.3% and 88.4%, respectively. Lastly, when acetone-water fractional precipitation (Fig.

3(C)) was used in the first step, the purity of paclitaxel was 83.0%, and when acetone-water fractional precipitation was also used in the second step, the purity was 85.6%. On the other hand, when methanol-water and acetone-pentane fractional precipitation were used in the second step, the purities were 88.5% and 89.2%, respectively. Through these results, it was found that the purity increase of paclitaxel was insignificant when the same solvent system was used in both the first and second steps, but the purity increased significantly when different solvent systems were used. This is similar to the precipitation of (+)-dihydromyricetin derived from *Ampelopsis grossedentata*, indicating a decrease in purification efficiency due to the use of the same precipitation solvent [28]. The highest purity (95.6%) and yield (96.2%) of paclitaxel were obtained in the precipitation using acetone-pentane in the first step and methanol-water in the second step. The yield was more than 95.4% under all conditions (data not shown). This pattern is similar to that of continuous cavitation fractional precipitation (acetone/pentane = 1:7, v/v and methanol/water = 61.5:38.5, v/v) for paclitaxel purification [13]. Thus, high purity (>95%) paclitaxel can be purified by two-step tandem cavitation fractional precipitation, which may be able to replace the existing ODS (C18)-HPLC (isocratic elution with methanol and water, methanol/water ratio = 7:3, v/v) purification process [14].

**2. Three-Step Tandem System**

The two-step tandem cavitation fractional precipitation showed an improvement in the purity of paclitaxel. However, to obtain high-purity (>98%) paclitaxel as an active pharmaceutical ingredient (API), three-step fractional precipitation was performed (Fig. 4). The first step solvent system was fixed as acetone-pentane and the second step solvent system was fixed as methanol-water because they showed the highest purities, and the experiment was performed by changing the solvent system for the third step of fractional precipitation. When acetone-pentane, methanol-water, and acetone-water were used as the third step, the purities of paclitaxel were 96.3%, 96.8%, and 97.4%, respectively. The yields were 95.1, 96.1, and 94.9%, respectively (data not shown). The highest purity of paclitaxel could be obtained in fractional precipitation using acetone-pentane in the first step, methanol-water in the second step, and acetone-water in the third step. However, the level of paclitaxel purification (purity >98%) using conventional chromatography (ODS- and silica-HPLC) could not be achieved [14]. To overcome this shortcoming, the possibility of high-purity paclitaxel purification was confirmed by performing fractional precipitation and changing the polarity index (PI) of the precipitation

solution by varying the composition of the precipitation solvent. The polarity index of each solvent mixture according to each composition was calculated through Eq. (1) and shown in Table 1 [28,36].

$$P_m = \varphi_1 P_1 + \varphi_2 P_2 \quad (1)$$

where φ is the volume fraction of solvent and P is the polarity index of solvent. When the acetone/pentane ratios (v/v) were 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, and 1:11, the polarity indices of the solvents were 0.850, 0.729, 0.638, 0.567, 0.510, 0.464, and 0.425, respectively. As the ratio of pentane increased, the polarity index of the precipitate solution decreased. When the methanol/water ratios (v/v) were 55:45, 60:40, 61.5:38.5, 65:35, 70:30, and 75:25, the polarity indices of the solvents were 7.305, 7.060, 6.987, 6.815, 6.570, and 6.325, respectively. As the ratio of methanol increased, the polarity index of the solvent decreased. When the acetone/water ratios (v/v) were 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6, the polarity indices of the solvents were 7.550, 8.367, 8.775, 9.020, 9.183, and 9.300, respectively. As the water ratio increased, the polarity index of the solvent increased. The polarity index of the mixture in the three solvent systems is highest in the acetone-water solution followed by methanol-water and acetone-pentane.

After fixing the solvent systems of the three steps to be acetone-pentane, followed by methanol-water, and acetone-water, fractional precipitation was conducted by changing the solvent composition at each step (Fig. 5). A total of 216 combinations are possible with six solvent compositions for each step in the three-step tandem cavitation fractional precipitation (Fig. 5(A)). When the acetone/pentane ratio was 1:5 (v /v) in the first step, the highest purity obtained was 92.8% at a methanol/water ratio of 61.5:38.5 (v /v) and an acetone/water ratio of 1:2 (v/v). However, purity 98% could not be achieved under all conditions (data not shown). When the acetone/pentane ratio was 1:6 (v/v) (Fig. 5(B)), the highest purity that could be obtained was 96.7%. When the acetone/pentane ratio was 1:7 (v/v) (Fig. 5(C)), it showed a high purity (97.9%). When the acetone/pentane ratio was 1:8 (v/v) (Fig. 5(D)), the purity was more than 98% at a methanol/water ratio of 60:40 (v/v) and acetone/water ratio of 1:2 (v/v), and a methanol/water ratio of 61.5:38.5 (v/v) and acetone/water ratio of 1:1-1:4 (v/v). Also, with a methanol/water ratio of 65:35 (v/v) and acetone/water ratio of 1:1-1:5 (v/v), and methanol/water ratio of 70:30 (v/v) and acetone/water ratio of 1:2 (v/v), the purity of paclitaxel was more than 98%. Particularly, the highest purity (98.6%) could be obtained at a

methanol/water ratio of 65:35 (v/v) and acetone/water ratio of 1:2 (v/v). On the other hand, with the methanol/water ratios of 70:30 (v/v) and 75:25 (v/v), the purity was less than 98%. The purity of paclitaxel was more than 98% when the acetone/pentane ratio was 1:9 (v/v) (Fig. 5(E)) at the methanol/water ratio of 55:45 (v/v) and the acetone/water ratio of 1:2 (v/v), and the methanol/water ratio of 60:40 (v/v) and the acetone/water ratio of 1:1-1:3 (v/v). In addition, the purity of paclitaxel was found to be more than 98% when the methanol/water ratio was 61.5:38.5-65:35 (v/v) under all conditions except at the acetone/water ratio of 1:6 (v/v). In particular, the highest purity (98.8%) was obtained at a methanol/water ratio of 61.5:38.5 (v/v) and acetone/water ratio of 1:2 (v/v). When the methanol/water ratio was 70:30 (v/v), the purity was higher than 98% at the acetone/water ratio of 1:2 (v/v), and the purity decreased as the methanol ratio increased. When the acetone/pentane ratio was 1:10 (v/v) (Fig. 5(F)), the purity was 98.3% at the methanol/water ratio of 60:40 (v/v) and the acetone/water ratio of 1:2 (v/v). With the acetone/water ratio of 1:2-1:4 (v/v) at the methanol/water ratio of 61.5:38.5 (v/v) and at the acetone/water ratio of 1:1-1:2(v/v) at the methanol/water ratio of 65:35 (v/v), the paclitaxel purity was more than 98%. When the acetone/pentane ratio was 1:11 (v/v) (Fig. 5(G)) at the methanol/water ratio of 65:35 (v/v) and the acetone/water ratio of 1:2 (v/v), the highest purity obtained was 97.5%. The overall yield of paclitaxel in all the above conditions was more than 90% (data not shown).

Table 2 summarizes the purities, step yields, and overall yields in the three-step tandem cavitation fractional precipitations that could obtain high-purity (>98%) paclitaxel. High purity (>98%) paclitaxel was obtained in high yield (>90%) from a total of 32 combinations including 11 conditions at an acetone/pentane ratio of 1:8 (v/v), 15 conditions at an acetone/pentane ratio of 1:9 (v/v), and 6 conditions at an acetone/pentane ratio of 1:10 (v/v). This is considered to be able to replace the existing ODS- and silica-HPLC or chromatography-crystallization hybrid processes for the purification of high purity (>98%) paclitaxel [14,19,22,23]. Particularly, the acetone/pentane ratio of 1:9 (v/v) in step 1, methanol/water ratio of 61.5:38.5 (v/v) in step 2, and acetone/water ratio of 1:2 (v/v) in step 3 was the optimal tandem system to obtain the highest purity (98.8%) of paclitaxel. The impurities included in the sample might have been selectively removed according to the polarity of the solvent used for precipitation.

### 3. Impurities Removal Behavior According to Polarity Index of the Solvent

During paclitaxel purification using conventional chromatography, polar impurities in the sample are selectively removed through an HPLC system equipped with an ODS-column (polar solvent mobile phase: methanol/water = 7:3 (v/v), polarity index = 6.570) and non-polar impurities with a silica-column (non-polar solvent mobile phase: methylene chloride/methanol=99:1 (v/v), polarity index=3.120) [14,20]. To compare the impurity removal behaviors according to the polarity index of the cavitation fractional precipitation solvent, the chromatogram was analyzed by reverse phase-high performance liquid chromatography at each fractional precipitation step as shown in Fig. 6. The chromatogram according to the acetone/pentane ratio in the first-stage fractional precipitation (Fig. 6(A)) shows that impurity A (retention time: 22.5-23.9 min) and B (retention time: 29.4 min) were present in relatively non-polar taxanes (weak polar taxanes) compared to paclitaxel (retention time: 20.2 min). Impurity B, which is more non-polar than impurity A, gradually decreased as the pentane ratio increased and was completely removed at the acetone and pentane ratio of 1:7 (v/v) [PI=0.638]. On the other hand, impurity A decreased as the pentane ratio increased, showing the smallest peak at the acetone and pentane ratio of 1:9 (v/v) [PI=0.510], and then increased again. Impurity A obtained through plant cell cultures of *Taxus chinensis* is representative of taxol C and yunnanxane, and impurity B is taxuyunnanin C [37]. These results indicated that impurities were selectively removed according to the polarity index of the fractional precipitation solvent and affected the purity of paclitaxel. This tendency was similar to the removal of non-polar impurities in the conventional silica-HPLC step for paclitaxel purification [14,20]. When the stationary phase was a normal phase (silica), the mobile phase was a non-polar solvent, and the polarity of the solvent strength was 0.1-5.1, so the non-polar impurities were selectively removed [38]. In the chromatogram for the methanol/water ratio in the second step of cavitation fractional precipitation (Fig. 6(B)), impurity D (retention time: 8,5-9.9 min), impurity E (retention time: 11.1-15.1 min), and impurity F (retention time: 17.3 min) were present in taxane (large/strong polar taxane) that had higher polarities than paclitaxel. As the water ratio decreased, impurity F gradually decreased. After being completely removed at the methanol/water ratio of 70:30 (v/v) [PI=6.570], it increased again at 75:25 (v/v) [PI=6.325]. In addition, impurity E, which was more polar than impurity F, gradually decreased as the water ratio decreased, and was mostly removed at methanol/water ratios of 61.5:38.5 (v/v)-65:35 (v/v) [PI=6.987-6.815], but then increased again. Impurity D, which was more polar than impurity E, decreased as the water ratio decreased and then increased again after the methanol/water ratio became 60:40 (v/v) [PI=7.060]. Impurity D obtained from plant cell cultures of *Taxus*

*chinensis* is representative of 10-DAB, taxuspine E, and baccatin III, impurity E is taxauntin E, 10-deacetylpaclitaxel, and 13-deoxybaccatin III, and impurity F is taxcultine [37]. In the chromatogram for the acetone/water ratio in step 3 of cavitation fractional precipitation (Fig. 6(C)), the polar impurity D was completely removed under all conditions. Also, when the acetone/water ratio changed from 1:1 (v/v) [PI=7.550] to 1:2 (v/v) [PI=8.367], impurity C, which was a strong polar impurity (retention time: 1.7-5.6 min) decreased, then increased again after the solvent composition was changed to 1:3 (v/v) [PI=8.775]. Impurity C obtained through plant cell cultures of *Taxus chinensis* included sugars, salts, and amino acids [38]. The precipitation behaviors of the second step methanol-water and third step acetone-water fractional precipitation were similar to the removal of polar impurities in the conventional ODS-HPLC purification step [14,20]. When the stationary phase was a reverse-phase (ODS, C18), the mobile phase was a polar solvent, and the polarity of the solvent strength was 5.1-10.2, so the polar impurities were selectively removed [39]. This impurity removal pattern was related to paclitaxel purity by precipitation condition (Fig. 5). These results indicate that impurities present in the sample were selectively removed according to the polarity index of the precipitation solution. That is, due to the polarity interaction between the precipitation solution and the impurities, the impurities moved to the precipitation solution or to the precipitate, so the impurities were selectively removed through three-step tandem cavitation fractional precipitation. This phenomenon is similar to the principle of selective purification by interactions of the sample with the mobile and stationary phases in traditional chromatographic methods [14,19,23]. The mechanism of cavitation fractional precipitation based on selectivity was compared with the conventional chromatographic purification method, and it is proposed in Fig. 7.

## 4. Identification of Purified Paclitaxel

As shown in Fig. 8, the structure of the standard sample (purity: 99%) and the purified paclitaxel (purity > 98%) by three-step tandem cavitation fractional precipitation were compared using FT-IR spectroscopy. Table 3 summarizes the major infrared absorption frequencies of paclitaxel in the two samples. In the standard paclitaxel (Fig. 8(A)), N-H/O-H, $CH_3$/C-H, C=O, and C-C stretching bands were observed at 3485, 2945, 1735, and 1657 $cm^{-1}$, respectively. A $CH_3$ deformation band appeared at 1371 $cm^{-1}$, and C-N and C-O stretching bands at 1250 and 1073 $cm^{-1}$. C-H in-plane deformation was observed at 979 $cm^{-1}$ and C-H

out-of-plane/C-C=O deformation at 706 $cm^{-1}$. In the paclitaxel purified through three-step tandem cavitation fractional precipitation (Fig. 8(B)), N-H/O-H stretching was confirmed at 3485 $cm^{-1}$, the same as the standard sample, and $CH_3$/C-H, C=O, C-C stretching bands were observed at 2933, 1734, and 1645 $cm^{-1}$. $CH_3$ deformation was confirmed at 1374 $cm^{-1}$, and C-N and C-O stretching bands at 1252 and 1066 $cm^{-1}$. C-H in-plane deformation was observed at 977 $cm^{-1}$ and C-H out-of-plane/C-C=O deformation at 711 $cm^{-1}$. The FT-IR spectra of the standard sample and the paclitaxel purified through the three-step fractional precipitation were similar and no structural differences were observed. These results are also consistent with the FT-IR spectrum results of paclitaxel found in the existing literature [40]. Therefore, it was confirmed that the standard sample and paclitaxel purified through tandem fractional precipitation were the same active substance.

## CONCLUSIONS

In this study, a non-chromatographic method was developed using three-step tandem cavitation fractional precipitation for the purification of high purity (>98%) paclitaxel. Through fractional precipitation with a solvent system of acetone-pentane in step 1, methanol-water in step 2, and acetone-water in step 3, it was possible to purify the paclitaxel to a level (purity > 98%) that could replace the conventional paclitaxel purification process using chromatography (ODS-HPLC and silica-HPLC). Particularly, at an acetone/pentane ratio of 1:9 (v/v) in step 1, a methanol/water ratio of 61.5:38.5 (v/v) in step 2, and an acetone/water ratio of 1:2 (v/v) in step 3, paclitaxel with the highest purity (98.8%) was obtained in high yield (91.1%), making it an optimal tandem system. These results suggested that the polarity interaction between the precipitation solution and the impurities caused the impurities to move to the precipitation solvent or the precipitate, and the impurities were selectively removed through the tandem cavitation fractional precipitation. As a result of comparing the structures of paclitaxel purified through three-step fractional precipitation and the standard sample through FT-IR analysis, the spectra of the two samples matched.

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## REFERENCES


1. H. Yuan, H. Guo, X. Luan, M. He, F. Li, J. Burnett, N. Truchan and D. Sun, *Mol. Pharm.*, **17**, 2275 (2020).

2. H. G. Kim and J. H. Kim, *Biotechnol. Bioprocess Eng.*, **27**, 668 (2020).

3. M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon and A. T. McPhail, *J. Am. Chem. Soc.*, **93**, 2325 (1971).

4. P. B. Schiff, J. Fant and S. B. Horwitz, *Nature*, **277**, 665 (1979).

5. L. Zhu and L. Chen, *Cell. Mol. Biol. Lett.*, **24**, 40 (2019).

6. M. Ghobani, F. Pourjafar, M. Saffari and Y. Asgari, *Meta Gene*, **26**, 100800 (2020).

7. H. S. Min and J. H. Kim, *Biotechnol. Bioprocess Eng.*, **27**, 111 (2022).

8. W. S. Jang and J. H. Kim, *Biotechnol. Bioprocess Eng.*, **24**, 529 (2019).

9. H. Woo and J. H. Kim, *Korean J. Chem. Eng.*, **40**, 1425 (2023).

10. E. J. Shin and J. H. Kim, *Biotechnol. Bioprocess Eng.*, **28**, 336 (2023).

11. A. Escrich, L. Almagro, E. Moyano, R. M. Cusido, M. Bonfill, B. Hosseini and J. Palazon, *Plant Physiol. Biochem.*, **163**, 68 (2021).

12. S. B. Oguzkan, S. Karadeniz, B. Karagul, A. Uzun, E. S. Aksoy, O. O. Guler, U. Cakir and H. I. Ugras, *Int. J. Pharmacol.*, **14**, 835 (2018).

13. H. S. Min and J. H. Kim, *Korean J. Chem. Eng.*, **39**, 3389 (2022).

14. S. -H. Pyo, H. -B. Park, B. -K. Song, B. -H. Han and J. H. Kim, *Process Biochem.*, **39**, 1985 (2004).

15. K. Y. Jeon and J. H. Kim, *Process Biochem.*, **44**, 736 (2009).

16. C. G. Lee and J. H. Kim, *Process Biochem.*, **59**, 216 (2017).

17. T. J. Mcpartland, R. A. Patil, M. F. Malone and S. C. Roberts, *Biotechnol. Prog.*, **28**, 990 (2012).

18. H. -J. Oh, H. R. Jang, K. Y. Jung and J. H. Kim, *Process Biochem.*, **47**, 331 (2012).

19. S. -H. Pyo and H. -J. Choi, *Sep. Purif. Technol.*, **76**, 378 (2011).

20. S. -H. Pyo, H. -J. Choi and B. -H. Han, *J. Chromatogr. A*, **1123**, 15 (2006).

21. S. B. Oguzkan, B. Karagul, A. Uzun, Ö. Ö. Güler and H. I. Ugras, *Int. J. Pharmacol.*, **14**, 76 (2018).

22. F. Wei, L. Shi, Q. Wang and Y. Zhao, *J. Chromatogr. A*, **1564**, 120 (2018).

23. H. Zhang, L. Huang, Y. Wu, Y. Chen, G. Song, J. Liu, C. Zhao, C. Fu and L. Yu, *Process Biochem.*, **131**, 175 (2023).

24. D. -J. Wu, Z. Ma and N. -H. L. Wang, *J. Chromatogr. A*, **855**, 71 (1999).

25. S. Mun and N. -H. L. Wang, *Process Biochem.*, **43**, 1407 (2008).

26. S. H. Kang, J. H. Kim and S. Mun, *Process Biochem.*, **45**, 1468 (2010).

27. A. M. Azevedo, P. A. J. Rosa, I. F. Ferreira and M. R. A. Barros, *Trends Biotechnol.*, **27**, 240 (2009).

28. H. Oh and J. H. Kim, *Process Biochem.*, **130**, 503 (2023).

29. J. H. Kim, I. -S. Kang, H. -K. Choi, S. -S. Hong and H. -S. Lee, *Biotechnol. Lett.*, **22**, 1753 (2000).

30. Y. L. Jeon and J. H. Kim, *Korean J. Chem. Eng.*, **30**, 1954 (2013).

31. H. A. Sim, J. Y. Lee and J. H. Kim, *Sep. Purif. Technol.*, **89**, 112 (2012).

32. H. W. Seo and J. H. Kim, *Process Biochem.*, **87**, 238 (2019).

33. H. J. Kang and J. H. Kim, *Process Biochem.*, **99**, 316 (2020).

34. H. J. Kang and J. H. Kim, *Korean Chem. Eng. Res.*, **59**, 379 (2021).

35. H. S. Min and J. H. Kim, *Biotechnol. Bioprocess Eng.*, **26**, 660 (2021).

36. J. Y. Lee and J. H. Kim, *Korean J. Chem. Eng.*, **28**, 1561 (2011).

37. J. H. Kim, *Korean J. Biotechnol. Bioeng.*, **21**, 1 (2006).

38. T. A. van Beek, K. K. R. Tetala, I. I. Koleva, A. Dapkevicius, V. Exarchou, S. M. F. Jeurissen, F. W. Claassen and E. J. C. van der Klift, *Phytochem. Rev.*, **8**, 387 (2009).

39. E. Lesellier and C. West, *J. Chromatogr. A*, **1382**, 2 (2015).

40. T. S.Renuga and S. Gayathri, *Int. J. Pharm. Sci. Rev. Res.*, **2**, 106 (2010).

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Fig. 5. Strategies (A) and results of the three-step tandem cavitation fractional precipitation with acetone/pentane ratio (1:6, v/v) (B), acetone/pentane ratio (1:7, v/v) (C), acetone/pentane ratio (1:8, v/v) (D), acetone/pentane ratio (1:9, v/v) (E), acetone/pentane ratio (1:10, v/v) (F), and acetone/pentane ratio (1:11, v/v) (G).

Fig. 6. Chromatogram of purification steps analyzed by RP-HPLC. (A) Step 1 with acetone-pentane system, (B) Step 2 with methanol-water system, and (C) Step 3 with acetone-water system.

Fig. 7. Principle of traditional chromatographic method and cavitation fractional precipitation method for the selective purification of paclitaxel.

Fig. 8. Fourier-transform infrared (FT-IR) spectra of standard paclitaxel (A) and purified paclitaxel by three-step tandem cavitation fractional precipitation (B).

**(Table 1)**

| Acetone-Pentane | | | Methanol-Water | | | Acetone-Water | | |
|---|---|---|---|---|---|---|---|---|
| **Solvent ratio (v/v)** | **Percent solvent (%)** | **Polarity Index (-)** | **Solvent ratio (v/v)** | **Percent solvent (%)** | **Polarity Index (-)** | **Solvent ratio (v/v)** | **Percent solvent (%)** | **Polarity Index (-)** |
| 1:0 | 100.0 | 5.100 | 100:0 | 100.0 | 5.100 | 1:0 | 100.0 | 5.100 |
| 1:6 | 14.3 | 0.729 | 55:45 | 55.0 | 7.305 | 1:1 | 50.0 | 7.550 |
| 1:7 | 12.5 | 0.638 | 60:40 | 60.0 | 7.060 | 1:2 | 33.3 | 8.367 |
| 1:8 | 11.1 | 0.567 | 61.5:38.5 | 61.5 | 6.987 | 1:3 | 25.0 | 8.775 |
| 1:9 | 10.0 | 0.510 | 65:35 | 65.0 | 6.815 | 1:4 | 20.0 | 9.020 |
| 1:10 | 9.1 | 0.464 | 70:30 | 70.0 | 6.570 | 1:5 | 16.7 | 9.183 |
| 1:11 | 8.3 | 0.425 | 75:25 | 75.0 | 6.325 | 1:6 | 14.3 | 9.300 |

**(Table 2)**

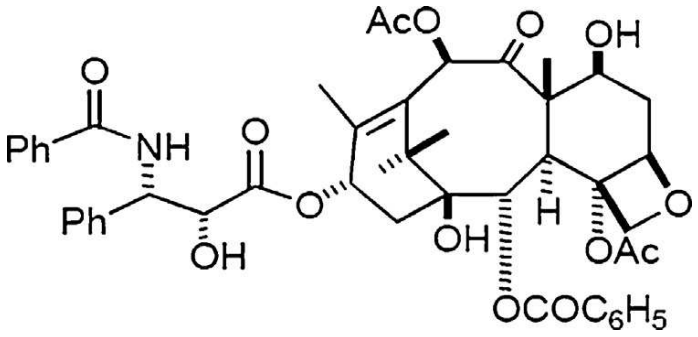
| Three-step fractional precipitation method | | Purity (%) | Step yield (%) | Overall yield (%) |
|---|---|---|---|---|
| | Starting material | 63.60 | - | - |
| Method 1 | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | 91.1±0.7 |
| | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 97.2±0.7 | 97.7±0.6 | |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.8±0.5 | 95.1±1.0 | |
| Method 2 | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | 91.4±0.9 |
| | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 97.2±0.7 | 97.7±0.6 | |
| | Step 3 : Acetone/Water ratio (1:1, v/v) | 98.7±0.8 | 95.4±1.3 | |
| Method 3 | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | 90.3±0.3 |
| | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 97.2±0.7 | 97.7±0.6 | |
| | Step 3 : Acetone/Water ratio (1:3v/v) | 98.7±1.0 | 94.2±0.5 | |
| Method 4 | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | 92.1±0.5 |
| | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 97.2±0.7 | 97.7±0.6 | |
| | Step 3 : Acetone/Water ratio (1:4, v/v) | 98.7±0.2 | 96.1±0.8 | |
| Method 5 | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | 92.3±0.5 |
| | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 97.3±0.3 | 97.0±0.5 | |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.7±0.7 | 97.0±0.9 | |
| Method 6 | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | 94.1±0.5 |
| | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 96.6±0.8 | 98.0±1.5 | |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.6±1.1 | 97.5±0.4 | |
| Method 7 | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | 92.2±0.7 |
| | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 97.3±0.3 | 97.0±0.5 | |
| | Step 3 : Acetone/Water ratio (1:3, v/v) | 98.6±0.7 | 96.9±1.2 | |
| Method 8 | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | 90.7±1.3 |
| | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 96.5±1.5 | 96.1±2.1 | |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.5±0.5 | 95.8±0.8 | |
| Method 9 | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | 94.5±1.1 |
| | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 96.6±0.8 | 98.0±1.5 | |
| | Step 3 : Acetone/Water ratio (1:3, v/v) | 98.5±0.5 | 97.9±0.9 | |
| Method 10 | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | 92.1±0.2 |
| | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 97.3±0.3 | 97.0±0.5 | |
| | Step 3 : Acetone/Water ratio (1:1, v/v) | 98.5±0.1 | 96.8±0.4 | |

| | | | | |
|---|---|---|---|---|
| | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | |
| Method 11 | Step 2 : Methanol/Water ratio (70.0:30.0, v/v) | 95.3±0.6 | 96.8±0.5 | 90.6±0.4 |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.5±0.5 | 95.4±0.8 | |
| | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | |
| Method 12 | Step 2 : Methanol/Water ratio (60.0:40.0, v/v) | 96.7±0.2 | 97.9±0.5 | 92.8±0.1 |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.4±0.8 | 96.6±0.2 | |
| | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | |
| Method 13 | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 96.5±1.5 | 96.1±2.1 | 90.8±1.0 |
| | Step 3 : Acetone/Water ratio (1:3, v/v) | 98.3±1.0 | 95.9±0.6 | |
| | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | |
| Method 14 | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 96.6±0.8 | 98.0±1.5 | 94.0±1.3 |
| | Step 3 : Acetone/Water ratio (1:1, v/v) | 98.3±0.1 | 97.4±1.1 | |
| | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | |
| Method 15 | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 97.3±0.3 | 97.0±0.5 | 91.5±0.6 |
| | Step 3 : Acetone/Water ratio (1:4, v/v) | 98.3±0.5 | 96.2±1.0 | |
| | Step 1 : Acetone/Pentane ratio (1:10, v/v) | 85.3±1.1 | 98.2±1.0 | |
| Method 16 | Step 2 : Methanol/Water ratio (60.0:40.0, v/v) | 96.6±0.8 | 97.4±0.2 | 93.3±0.1 |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.3±2.1 | 97.5±0.5 | |
| | Step 1 : Acetone/Pentane ratio (1:10, v/v) | 85.3±1.1 | 98.2±1.0 | |
| Method 17 | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 96.3±1.1 | 97.0±1.5 | 91.6±1.7 |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.3±1.5 | 96.2±1.1 | |
| | Step 1 : Acetone/Pentane ratio (1:10, v/v) | 85.3±1.1 | 98.2±1.0 | |
| Method 18 | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 96.6±0.8 | 97.4±0.2 | 91.9±1.2 |
| | Step 3 : Acetone/Water ratio (1:3, v/v) | 98.3±0.8 | 96.5±0.8 | |
| | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | |
| Method 19 | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 96.5±1.5 | 96.1±2.1 | 90.1±2.4 |
| | Step 3 : Acetone/Water ratio (1:1, v/v) | 98.2±0.4 | 95.2±1.4 | |
| | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | |
| Method 20 | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 96.6±0.8 | 98.0±1.5 | 93.2±0.6 |
| | Step 3 : Acetone/Water ratio (1:4, v/v) | 98.2±0.6 | 96.5±0.5 | |
| | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | |
| Method 21 | Step 2 : Methanol/Water ratio (60.0:40.0, v/v) | 96.7±0.2 | 97.9±0.5 | 92.2±0.1 |
| | Step 3 : Acetone/Water ratio (1:1, v/v) | 98.2±1.0 | 96.0±0.2 | |
| Method 22 | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | 91.2±0.3 |

| | | | | |
|---|---|---|---|---|
| | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 97.3±0.3 | 97.0±0.5 | |
| | Step 3 : Acetone/Water ratio (1:5, v/v) | 98.2±1.0 | 95.8±0.6 | |
| | Step 1 : Acetone/Pentane ratio (1:10, v/v) | 85.3±1.1 | 98.2±1.0 | |
| Method 23 | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 96.6±0.8 | 97.4±0.2 | 91.3±1.4 |
| | Step 3 : Acetone/Water ratio (1:4, v/v) | 98.2±0.4 | 95.9±0.9 | |
| | Step 1 : Acetone/Pentane ratio (1:10, v/v) | 85.3±1.1 | 98.2±1.0 | |
| Method 24 | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 96.7±0.5 | 97.0±2.1 | 91.6±3.5 |
| | Step 3 : Acetone/Water ratio (1:1, v/v) | 98.2±1.1 | 96.2±1.5 | |
| | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | |
| Method 25 | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 96.5±1.5 | 96.1±2.1 | 90.8±0.2 |
| | Step 3 : Acetone/Water ratio (1:4, v/v) | 98.1±0.7 | 96.0±0.1 | |
| | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | |
| Method 26 | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 96.6±0.8 | 98.0±1.5 | 92.1±1.2 |
| | Step 3 : Acetone/Water ratio (1:5, v/v) | 98.1±1.2 | 95.4±1.0 | |
| | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | |
| Method 27 | Step 2 : Methanol/Water ratio (55.0:45.0, v/v) | 96.0±0.4 | 97.5±1.0 | 92.8±0.7 |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.1±1.1 | 97.0±0.6 | |
| | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | |
| Method 28 | Step 2 : Methanol/Water ratio (60.0:40.0, v/v) | 96.7±0.2 | 97.9±0.5 | 93.4±0.4 |
| | Step 3 : Acetone/Water ratio (1:3, v/v) | 98.1±0.4 | 97.5±0.8 | |
| | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | |
| Method 29 | Step 2 : Methanol/Water ratio (60.0:40.0, v/v) | 96.0±1.1 | 97.0±0.9 | 91.1±0.9 |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.0±0.2 | 95.4±1.2 | |
| | Step 1 : Acetone/Pentane ratio (1:8, v/v) | 85.6±0.7 | 98.5±0.8 | |
| Method 30 | Step 2 : Methanol/Water ratio (70.0:30.0, v/v) | 95.0±2.0 | 97.0±0.5 | 93.2±0.2 |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.0±0.8 | 97.5±0.9 | |
| | Step 1 : Acetone/Pentane ratio (1:9, v/v) | 88.5±0.5 | 98.1±1.1 | |
| Method 31 | Step 2 : Methanol/Water ratio (61.5:38.5, v/v) | 97.2±0.7 | 97.7±0.6 | 92.5±0.1 |
| | Step 3 : Acetone/Water ratio (1:5, v/v) | 98.0±0.4 | 96.5±0.1 | |
| | Step 1 : Acetone/Pentane ratio (1:10, v/v) | 85.3±1.1 | 98.2±1.0 | |
| Method 32 | Step 2 : Methanol/Water ratio (65.0:35.0, v/v) | 96.6±0.8 | 97.4±0.2 | 91.2±2.7 |
| | Step 3 : Acetone/Water ratio (1:2, v/v) | 98.0±0.6 | 95.7±1.3 | |

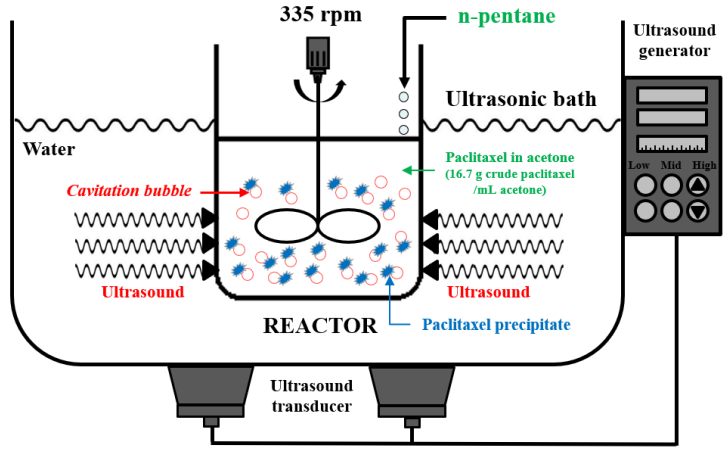
**(Table 3)**

| Assignments | Compounds IR, ν ($cm^{-1}$) | |
|---|---|---|
| | **Standard paclitaxel** | **Purified paclitaxel by three-step tandem cavitation fractional precipitation** |
| N-H/O-H stretching | 3485 | 3485 |
| $CH_3$/C-H stretching | 2945 | 2933 |
| C=O stretching | 1735 | 1734 |
| C-C stretching | 1657 | 1645 |
| $CH_3$ deformation | 1371 | 1374 |
| C-N stretching | 1250 | 1252 |
| C-O stretching | 1073 | 1066 |
| C-H in-plane deformation | 979 | 977 |
| C-H out-of-plane/C-C=O deformation | 706 | 711 |

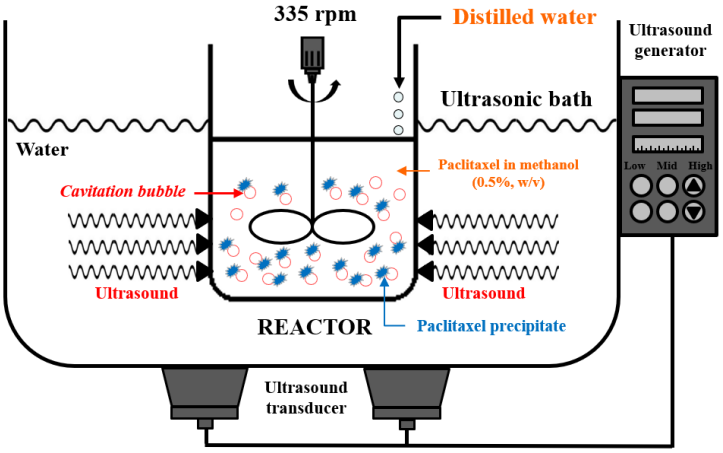



**(Fig. 1)**

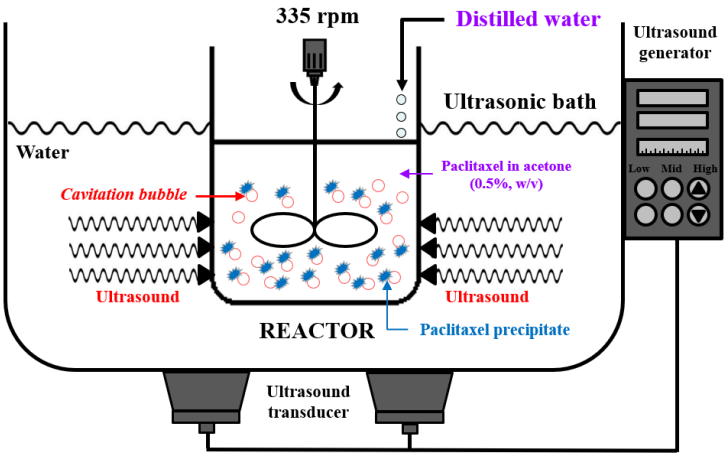
(A)


335 rpm
n-pentane
Ultrasound generator
Ultrasonic bath
Water
Paclitaxel in acetone (16.7 g crude paclitaxel /mL acetone)
Cavitation bubble
Low Mid High
Ultrasound
Ultrasound
REACTOR
Paclitaxel precipitate
Ultrasound transducer

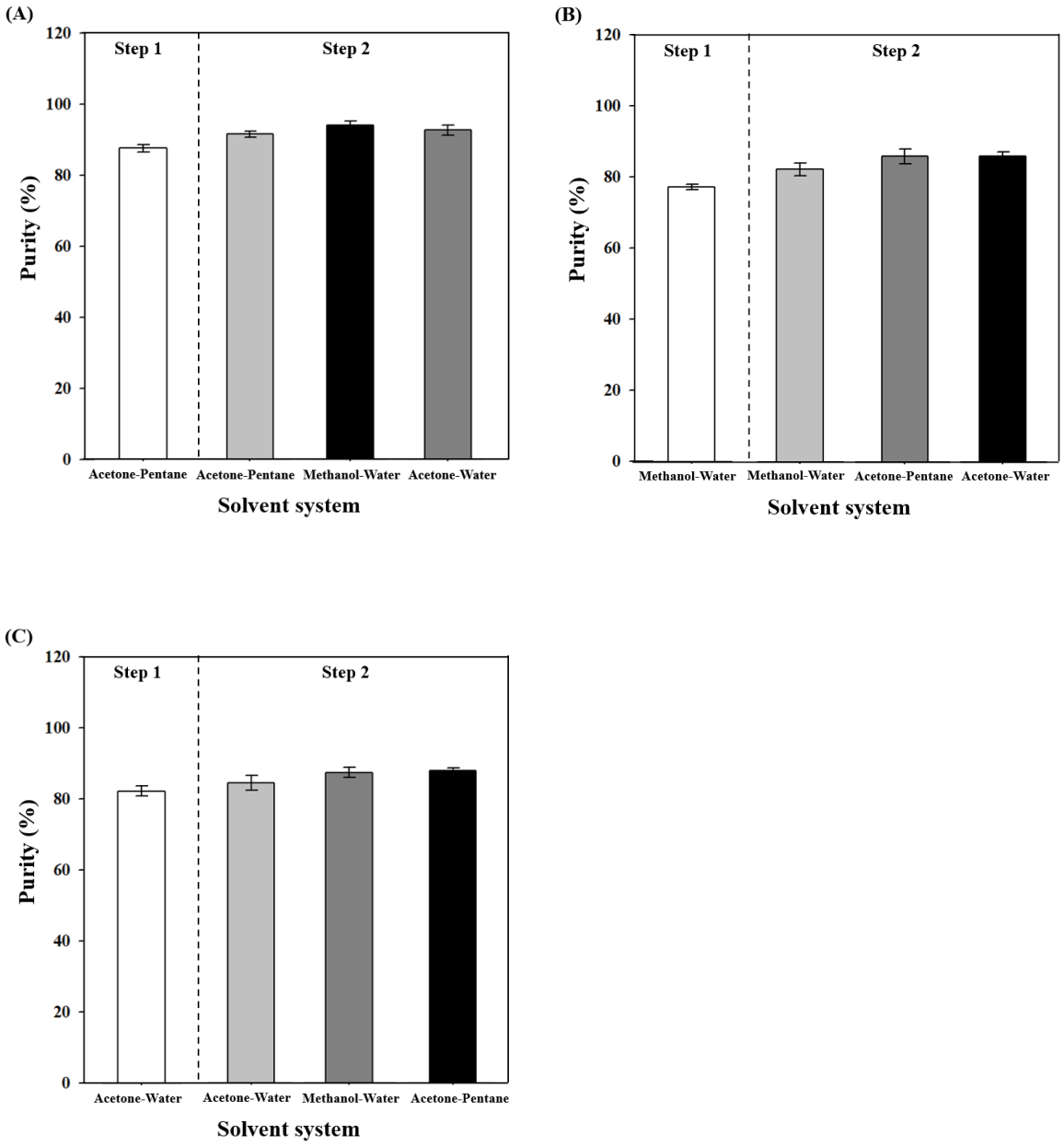
(B)


335 rpm
Distilled water
Ultrasound generator
Ultrasonic bath
Water
Paclitaxel in methanol (0.5%, w/v)
Cavitation bubble
Low Mid High
Ultrasound
Ultrasound
REACTOR
Paclitaxel precipitate
Ultrasound transducer

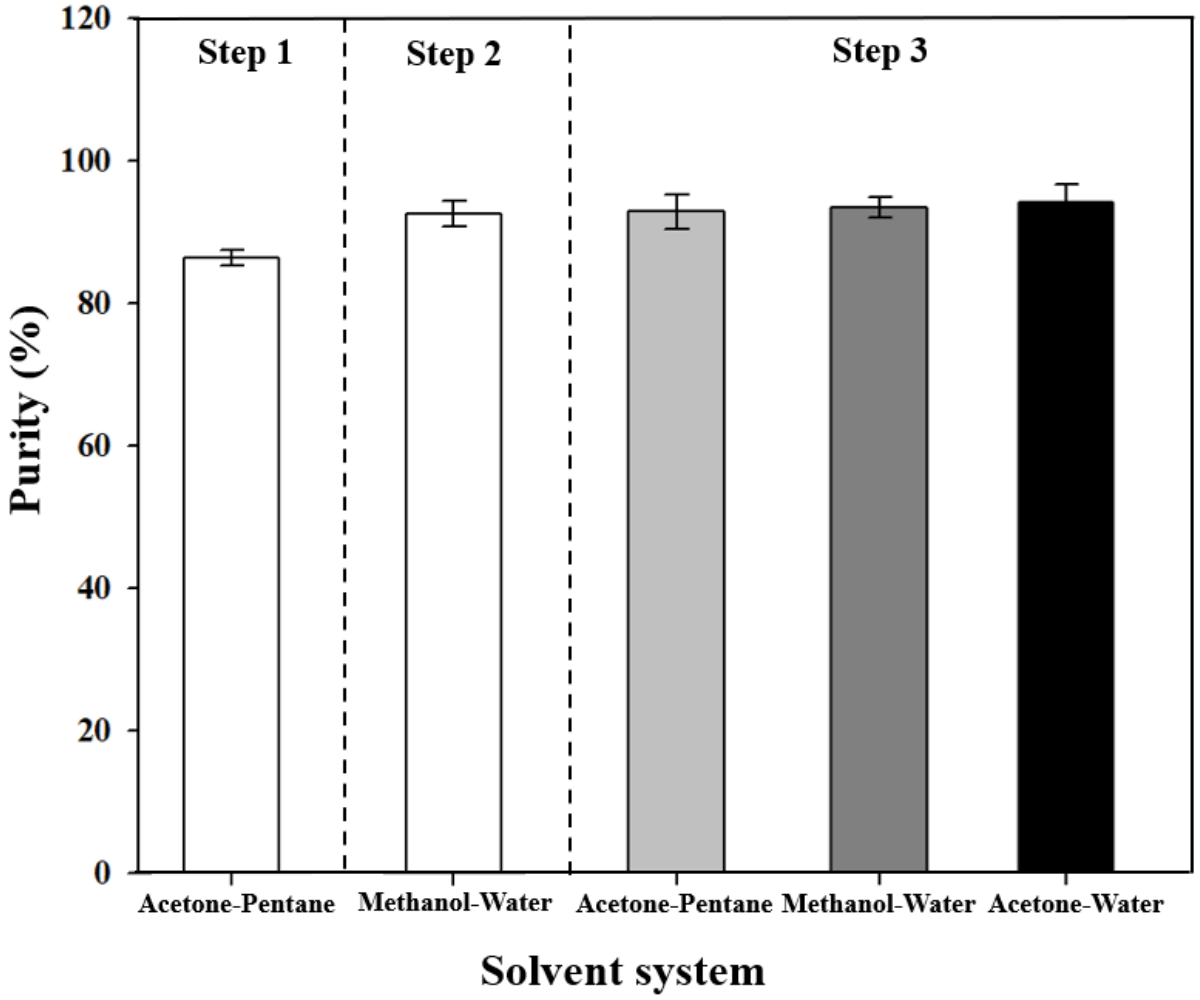
(C)


335 rpm
Distilled water
Ultrasound generator
Ultrasonic bath
Water
Paclitaxel in acetone (0.5%, w/v)
Cavitation bubble
Low Mid High
Ultrasound
Ultrasound
REACTOR
Paclitaxel precipitate
Ultrasound transducer

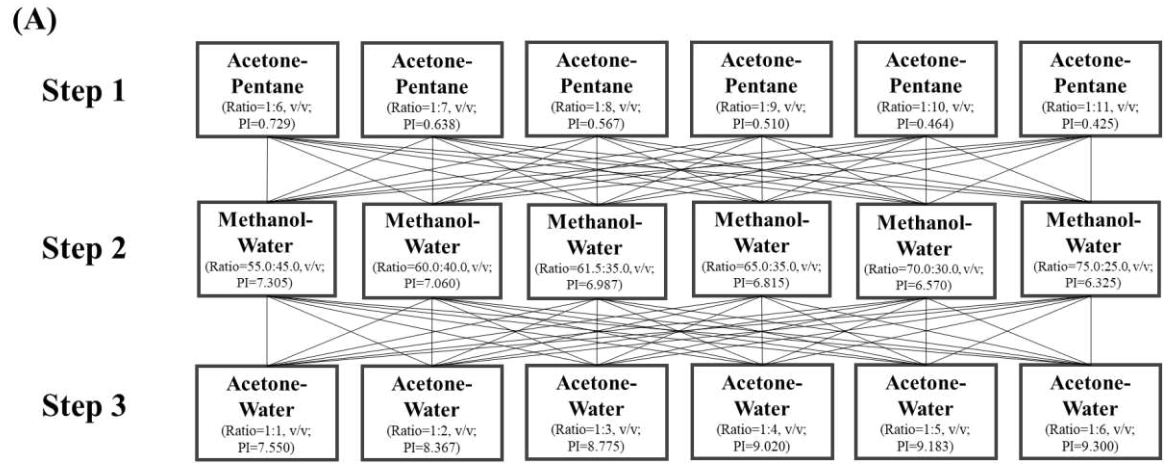
(Fig. 2)

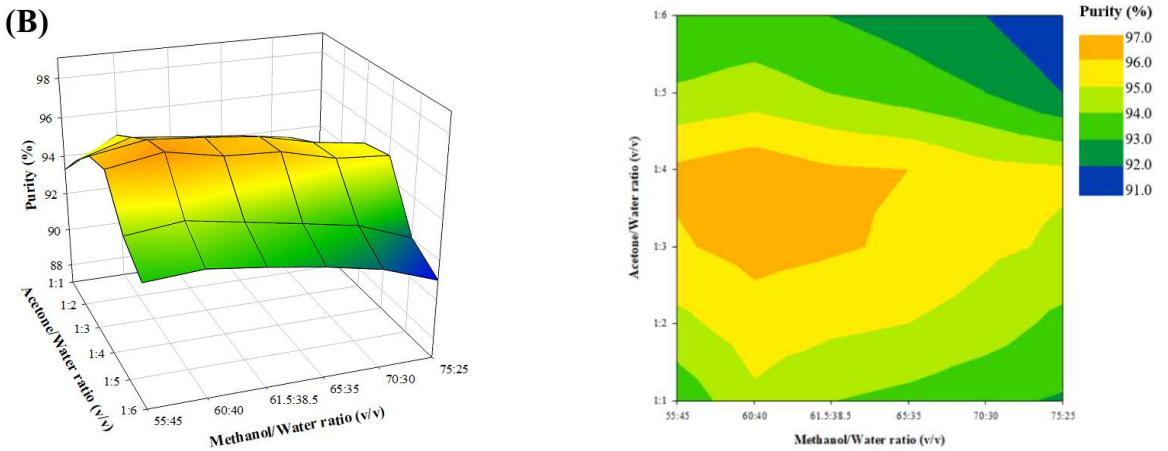

(A)
Step 1
Step 2
Purity (%)
120
100
80
60
40
20
0
Acetone-Pentane
Acetone-Pentane
Methanol-Water
Acetone-Water
Solvent system
(B)
Step 1
Step 2
Purity (%)
120
100
80
60
40
20
0
Methanol-Water
Methanol-Water
Acetone-Pentane
Acetone-Water
Solvent system
(C)
Step 1
Step 2
Purity (%)
120
100
80
60
40
20
0
Acetone-Water
Acetone-Water
Methanol-Water
Acetone-Pentane
Solvent system

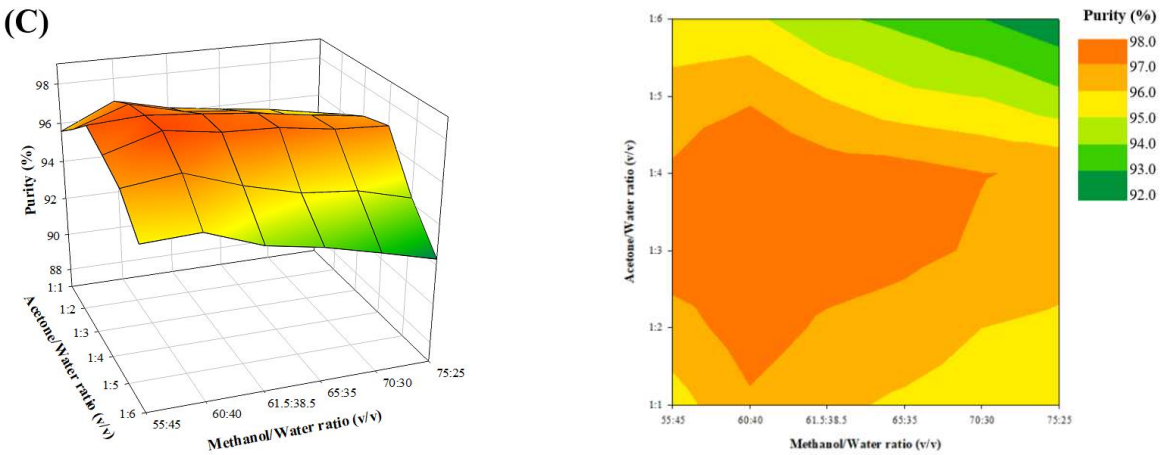
**(Fig. 3)**


Step 1
Step 2
Step 3
120
100
80
60
40
20
0
Purity (%)
Acetone-Pentane
Methanol-Water
Acetone-Pentane
Methanol-Water
Acetone-Water
Solvent system

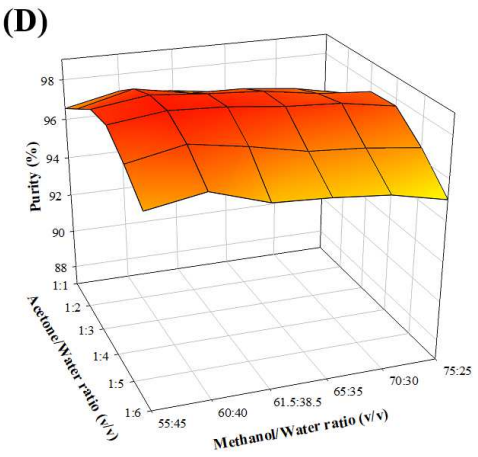
**(Fig. 4)**

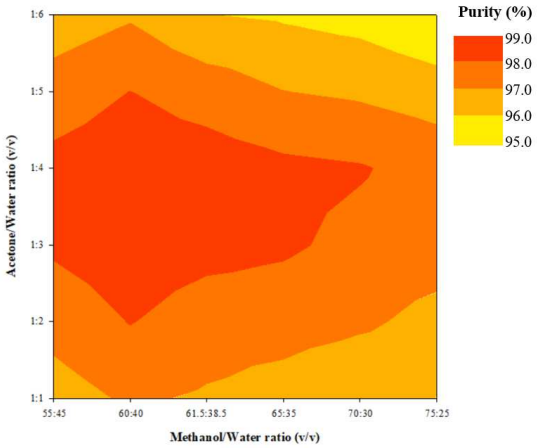

(A)
Step 1
Step 2
Step 3
Acetone-Pentane (Ratio=1:6, v/v; PI=0.729)
Acetone-Pentane (Ratio=1:7, v/v; PI=0.638)
Acetone-Pentane (Ratio=1:8, v/v; PI=0.567)
Acetone-Pentane (Ratio=1:9, v/v; PI=0.510)
Acetone-Pentane (Ratio=1:10, v/v; PI=0.464)
Acetone-Pentane (Ratio=1:11, v/v; PI=0.425)
Methanol-Water (Ratio=55.0:45.0, v/v; PI=7.305)
Methanol-Water (Ratio=60.0:40.0, v/v; PI=7.060)
Methanol-Water (Ratio=61.5:35.0, v/v; PI=6.987)
Methanol-Water (Ratio=65.0:35.0, v/v; PI=6.815)
Methanol-Water (Ratio=70.0:30.0, v/v; PI=6.570)
Methanol-Water (Ratio=75.0:25.0, v/v; PI=6.325)
Acetone-Water (Ratio=1:1, v/v; PI=7.550)
Acetone-Water (Ratio=1:2, v/v; PI=8.367)
Acetone-Water (Ratio=1:3, v/v; PI=8.775)
Acetone-Water (Ratio=1:4, v/v; PI=9.020)
Acetone-Water (Ratio=1:5, v/v; PI=9.183)
Acetone-Water (Ratio=1:6, v/v; PI=9.300)


(B)
Purity (%)
Acetone/Water ratio (v/v)
Methanol/Water ratio (v/v)
97.0
96.0
95.0
94.0
93.0
92.0
91.0

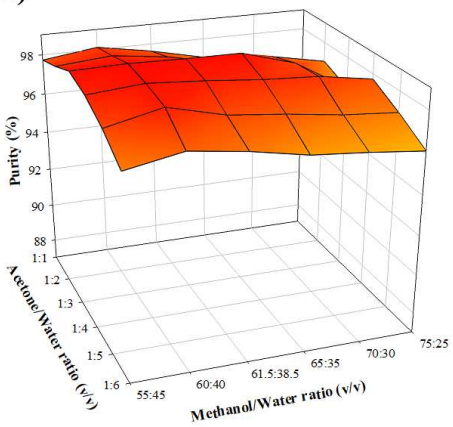

(C)
Purity (%)
Acetone/Water ratio (v/v)
Methanol/Water ratio (v/v)
98.0
97.0
96.0
95.0
94.0
93.0
92.0

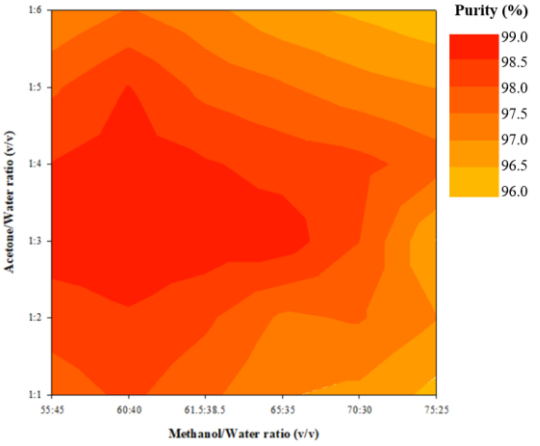
(D)


Purity (%)
98
96
94
92
90
88
Acetone/Water ratio (v/v)
1:1
1:2
1:3
1:4
1:5
1:6
55:45
60:40
61.5:38.5
65:35
70:30
75:25
Methanol/Water ratio (v/v)

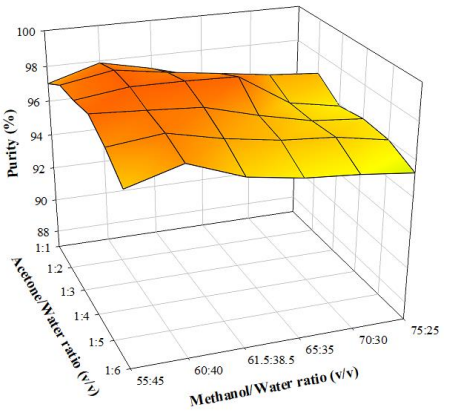

Purity (%)
99.0
98.0
97.0
96.0
95.0
Acetone/Water ratio (v/v)
1:6
1:5
1:4
1:3
1:2
1:1
55:45
60:40
61.5:38.5
65:35
70:30
75:25
Methanol/Water ratio (v/v)

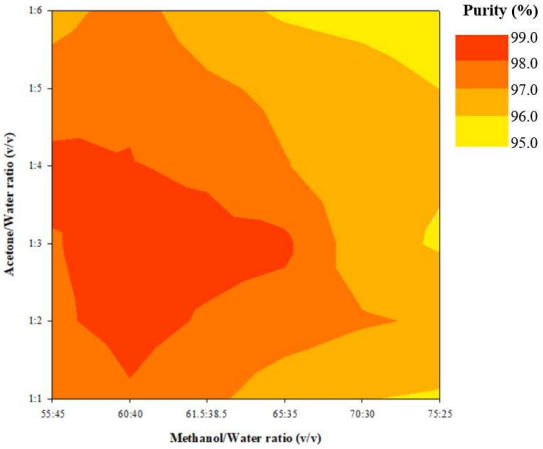
(E)


Purity (%)
98
96
94
92
90
88
Acetone/Water ratio (v/v)
1:1
1:2
1:3
1:4
1:5
1:6
55:45
60:40
61.5:38.5
65:35
70:30
75:25
Methanol/Water ratio (v/v)

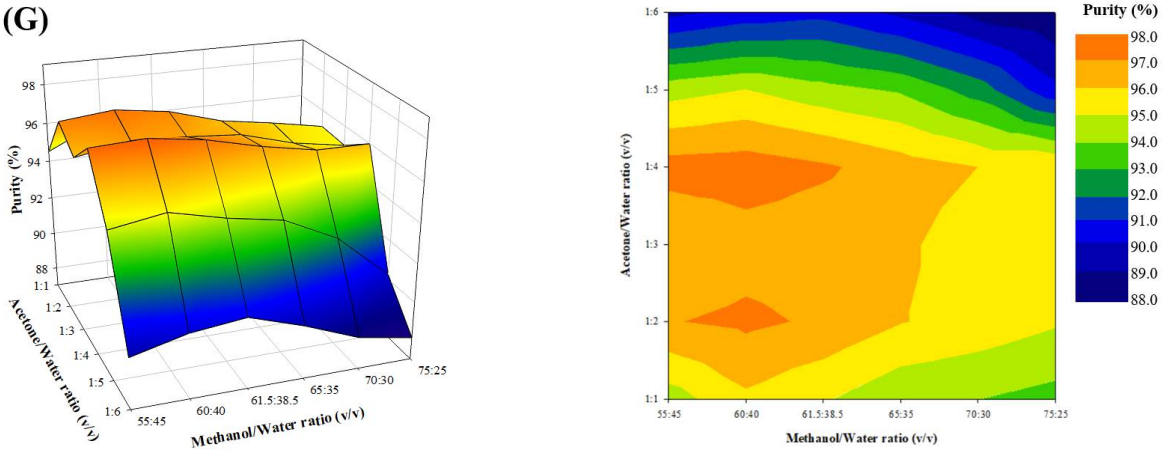

Purity (%)
99.0
98.5
98.0
97.5
97.0
96.5
96.0
Acetone/Water ratio (v/v)
1:6
1:5
1:4
1:3
1:2
1:1
55:45
60:40
61.5:38.5
65:35
70:30
75:25
Methanol/Water ratio (v/v)

(F)

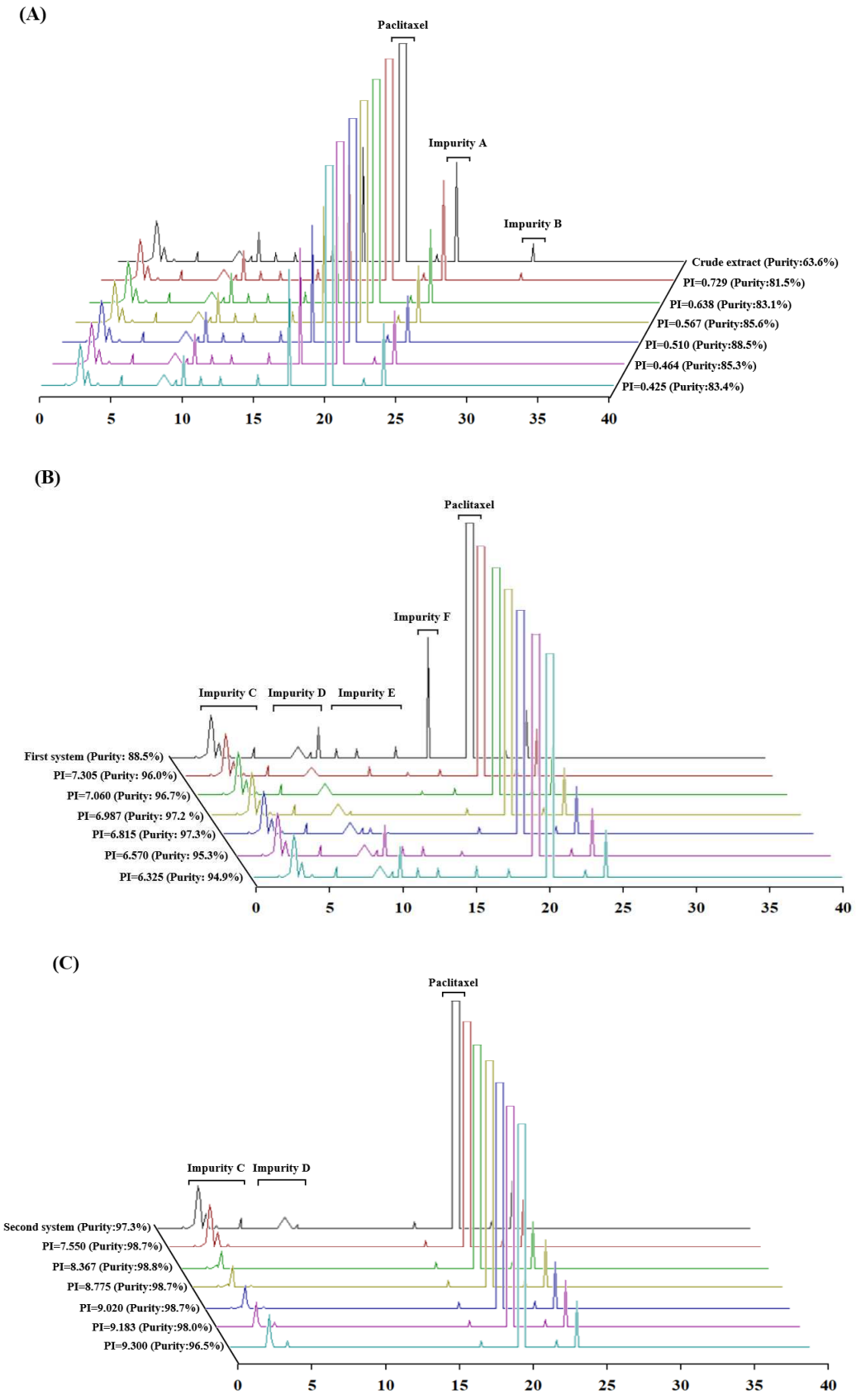

Purity (%)
100
98
96
94
92
90
88
Acetone/Water ratio (v/v)
1:1
1:2
1:3
1:4
1:5
1:6
55:45
60:40
61.5:38.5
65:35
70:30
75:25
Methanol/Water ratio (v/v)


Purity (%)
99.0
98.0
97.0
96.0
95.0
Acetone/Water ratio (v/v)
1:6
1:5
1:4
1:3
1:2
1:1
55:45
60:40
61.5:38.5
65:35
70:30
75:25
Methanol/Water ratio (v/v)

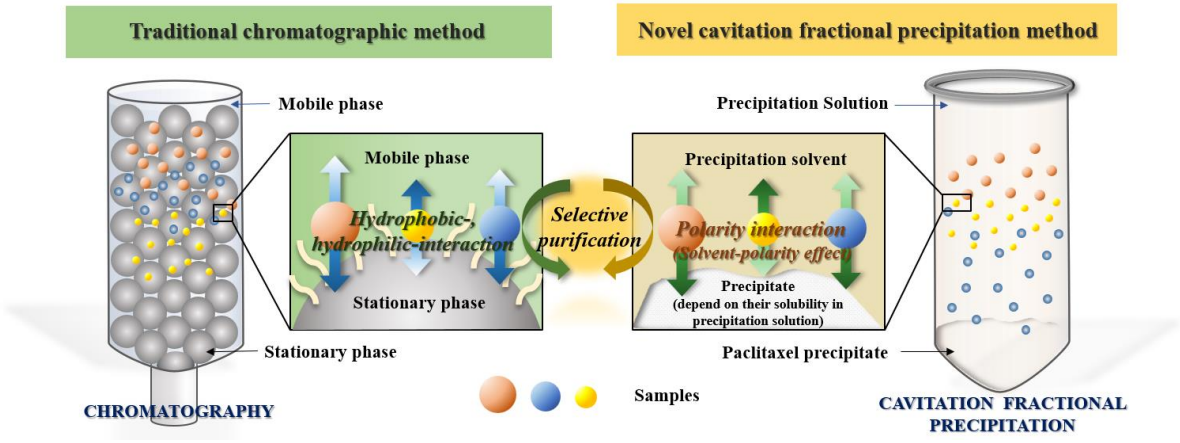
**(G)**


Purity (%)
98
96
94
92
90
88
1:1
1:2
1:3
1:4
1:5
1:6
Acetone/Water ratio (v/v)
55:45
60:40
61.5:38.5
65:35
70:30
75:25
Methanol/Water ratio (v/v)
Purity (%)
98.0
97.0
96.0
95.0
94.0
93.0
92.0
91.0
90.0
89.0
88.0

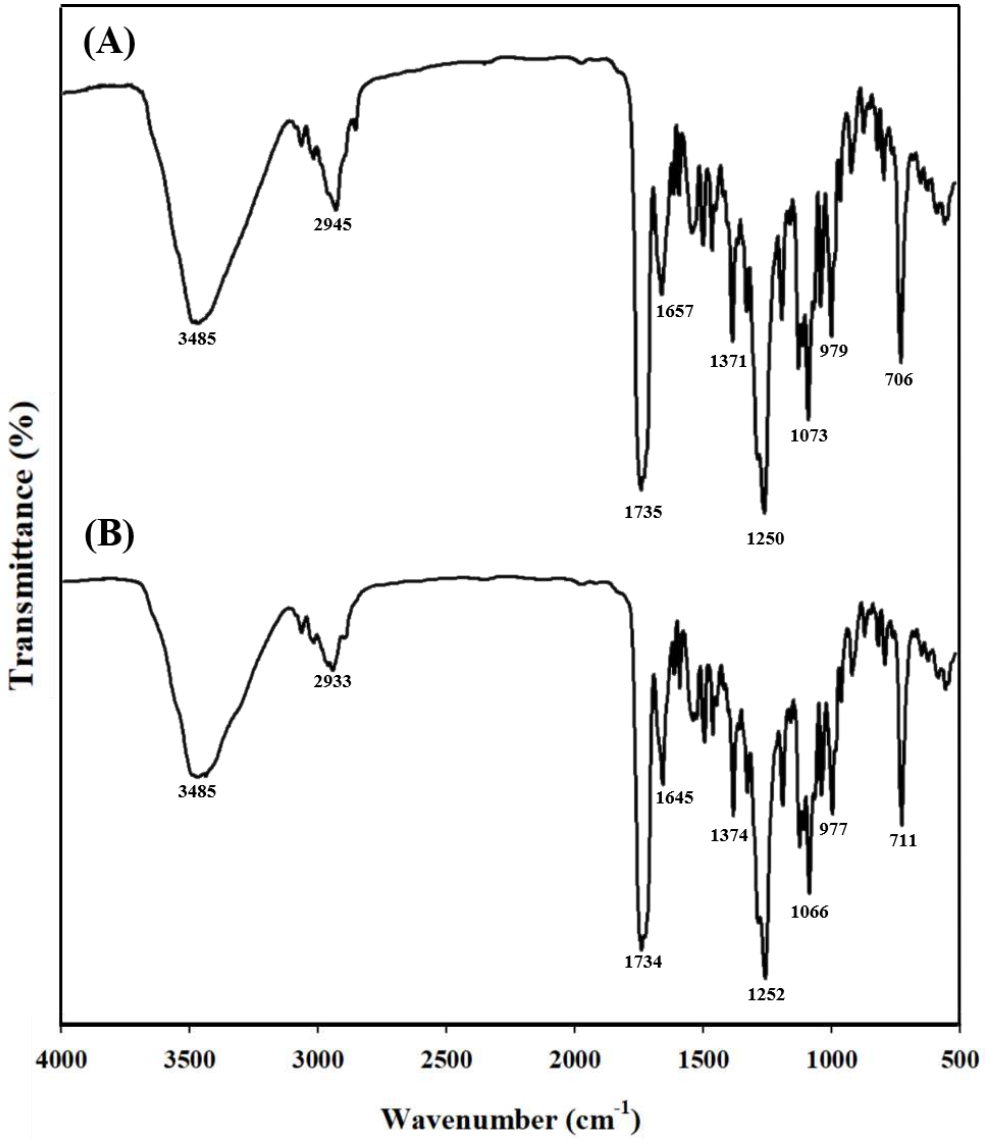
**(Fig. 5)**


(A)
Paclitaxel
Impurity A
Impurity B
Crude extract (Purity:63.6%)
PI=0.729 (Purity:81.5%)
PI=0.638 (Purity:83.1%)
PI=0.567 (Purity:85.6%)
PI=0.510 (Purity:88.5%)
PI=0.464 (Purity:85.3%)
PI=0.425 (Purity:83.4%)
0 5 10 15 20 25 30 35 40
(B)
Paclitaxel
Impurity F
Impurity C
Impurity D
Impurity E
First system (Purity: 88.5%)
PI=7.305 (Purity: 96.0%)
PI=7.060 (Purity: 96.7%)
PI=6.987 (Purity: 97.2 %)
PI=6.815 (Purity: 97.3%)
PI=6.570 (Purity: 95.3%)
PI=6.325 (Purity: 94.9%)
0 5 10 15 20 25 30 35 40
(C)
Paclitaxel
Impurity C
Impurity D
Second system (Purity:97.3%)
PI=7.550 (Purity:98.7%)
PI=8.367 (Purity:98.8%)
PI=8.775 (Purity:98.7%)
PI=9.020 (Purity:98.7%)
PI=9.183 (Purity:98.0%)
PI=9.300 (Purity:96.5%)
0 5 10 15 20 25 30 35 40

**(Fig. 6)**


Traditional chromatographic method
Novel cavitation fractional precipitation method
Mobile phase
Mobile phase
Hydrophobic-, hydrophilic-interaction
Stationary phase
Selective purification
Stationary phase
CHROMATOGRAPHY
Precipitation Solution
Precipitation solvent
Polarity interaction (Solvent-polarity effect)
Precipitate
(depend on their solubility in precipitation solution)
Paclitaxel precipitate
Samples
CAVITATION FRACTIONAL PRECIPITATION

**(Fig. 7)**


(A)
3485
2945
1735
1657
1371
1250
1073
979
706
(B)
3485
2933
1734
1645
1374
1252
1066
977
711
Transmittance (%)
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)

**(Fig. 8)**