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Short communication

Microwave-assisted drying of paclitaxel for removal of residual solvents

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ABSTRACT

In this study, the residual solvent in final purified paclitaxel was effectively removed using microwave-assisted drying. When the sample final purified by silica-HPLC was concentrated using a rotary evaporator, the residual methanol easily met the ICH-specified value (3000 ppm), but methylene chloride did not meet the ICH-specified value (600 ppm). Thus, the efficiency of microwave-assisted drying according to microwave power (100, 200, and 300 W) and drying time was investigated using the sample (methylene chloride conc.: 26,000 ppm, methanol conc.: 50 ppm) concentrated by rotary evaporation. A higher microwave power was effective in removing methylene chloride, and the ICH requirements were met by drying at 300 W for 21 h. In addition, when the sample concentrated by rotary evaporation was vacuum dried (35 °C, 24 h), the concentration of methylene chloride could be reduced to 8500 ppm. When the vacuum-dried sample was subjected to microwave-assisted drying, the ICH requirements could be met by drying for 10 h at 200 W and 8 h at 300 W. The lower the initial concentration of the solvent and the higher the microwave power, the greater the improvement in the efficiency of microwave-assisted drying.

1. Introduction

Paclitaxel is a diterpenoid anticancer substance that was discovered in the bark of the yew tree. It has been approved by the U. S. Food and Drug Administration as a treatment for ovarian cancer, breast cancer, Kaposi's sarcoma, and non-small cell lung cancer. Paclitaxel is currently the most widely used anticancer drug [1]. The demand for this drug is expected to increase steadily; its indications, which include acute rheumatoid arthritis and Alzheimer's disease, are expanding continuously, and clinical tests for combined prescription with other therapies are being conducted. The main paclitaxel production methods are direct extraction from yew trees, semi-synthesis and plant cell culture. The latter method has the advantage of being able to mass produce paclitaxel of a certain quality because it can be produced reliably within the bioreactor without being affected by external factors such as climate and environment [2].

For active pharmaceutical ingredients (APIs), the specifications of the final purified product are very important. The quality requirements for paclitaxel are well-defined by the U. S. Pharmacopeial Convention (USP 27-NF22). The purity of APIs as well as the residual solvent concentration, impurity content and endotoxin content should be met thoroughly. In particular, the concentration of residual solvent has been strictly limited by the International Conference on Harmonisation (ICH) Q3C guidance [3]. For example, the concentration of residual methylene chloride and methanol is limited to less than 600 ppm and 3000 ppm, respectively [4–6]. To meet the requirements for these residual solvents, the drying method is very important. In the case of rotary evaporation and vacuum drying, it is very difficult to manufacture a product with less than the ICH-specified concentration of residual solvent even if samples are dried for a long time because of the phenomenon of case hardening, in which the sample surface is hardened before the residual solvent is fully removed in the process of drying paclitaxel [1,5,6]. In order to solve these problems, a drying method using supercritical carbon dioxide [5] and a spray drying method [6] have been proposed. However, when using supercritical carbon dioxide, a high-pressure equipment is required, and paclitaxel can decompose due to high operating pressure [5,7]. In addition, in the case of spray drying, there are the problems because the operation takes a long time and a large installation space is required [6].

In general, the heating rate of drying using microwaves is quite high because the heated material itself becomes a heating element and is heated by heat transfer in the entire interior. In addition, the equipment space can be reduced and equal drying of the product can improve its quality [8–10]. Drying using microwaves is widely used for wood, vegetables, fruit, and ore because energy conservation and shortening of the process are possible [11–13]. Therefore, this study attempted to effectively remove the methylene chloride and methanol that are the residual solvents in the final purified paclitaxel using microwaves. We also investigated the effect of the microwave power and the initial solvent concentration according

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to drying time and compared the drying efficiency with that of the conventional vacuum drying method. These results should prove useful for the removal of residual solvent in the mass production of APIs by providing an efficient drying method using microwaves.

2. Materials and methods

2.1. Plant materials and culture conditions

A suspension of cells originating from *Taxus chinensis* was maintained in darkness at 24 °C with shaking at 150 rpm. The cells were cultured in modified Gamborg's B5 medium supplemented with 30 g/L sucrose, 10 mM naphthalene acetic acid, 0.2 mM 6-benzylaminopurine, 1 g/L casein hydrolysate, and 1 g/L 2-(N-morpholino) ethanesulfonic acid. Cell cultures were transferred to fresh medium every 2 weeks. During prolonged culture for production purposes, 4 mM $AgNO_3$ was added at the initiation of the culture as an elicitor, and 1% and 2% (w/v) maltose were added to the medium on days 7 and 21, respectively [14]. Following cultivation, biomass was recovered using a decanter (CA150 Clarifying Decanter; Westfalia, Germany) and a high-speed centrifuge (BTPX 205GD-35CDEFP; Alfa Laval, Sweden). The biomass was provided by Samyang Genex Company, South Korea.

2.2. Paclitaxel analysis

Dried residue was redissolved in methanol for quantitative analysis using an HPLC system (SCL-10AVP; Shimadzu, Japan) with a Capcell Pak C_{18} column (250 mm × 4.6 mm; Shiseido, Japan). Elution was performed in a gradient using a distilled water–acetonitrile mixture varying from 65:35 to 35:65 within 40 min (flow rate = 1.0 mL/min). The injection volume was 20 μL, and the effluent was monitored at 227 nm with a UV detector. Authentic paclitaxel (purity: 97%) was purchased from Sigma–Aldrich and used as a standard [15]. Each sample was analyzed in triplicate.

2.3. Analysis of residual solvents

The concentration of residual methylene chloride and methanol was analyzed using gas chromatography (GC-2014; Shimadzu, Japan). An HP-5 column (0.20 mm ID × 25 m, 0.33-μm film) and a flame ionization detector were employed, and a separation temperature within the column from 40 °C to 100 °C was used by programming at a rate of 10 °C/min. The carrier gas used was helium and was analyzed at a flow rate of 1 mL/min.

2.4. Preparation of sample for microwave-assisted drying

After the sample was extracted four times at room temperature using biomass recovered from plant cell culture and methanol at a ratio of 1:1 (w/v), liquid–liquid extraction was performed by concentration (30% of original) using a rotary evaporator (CCA-1100; EYELA, Japan). Methylene chloride was added to the concentrated methanol solution (25% of concentrated methanol extract) and then suspended after stirring for 30 min to induce phase separation. The liquid–liquid extraction was repeated three times. Paclitaxel was collected into the lower methylene chloride layer for concentration, followed by vacuum filtration with filter paper (150 mm; Whatman) and drying. To remove tar/waxy compounds derived from plant cells, dried crude extract was dissolved in methylene chloride at a ratio of 20% (v/w) and the adsorbent Sylopute (Fuji Silysia Chemical Ltd., Japan) was added at a ratio of 50% and stirred for 30 min in a 40 °C water bath (PS-1000; EYELA), then filtered. The filtrate was dried at 30 °C under vacuum and then subjected to hexane precipitation. Dried crude extract was dissolved in methylene chloride, which was dropped into hexane to induce precipitation and remove non-polar impurities (methylene chloride/hexane = 1:10, v/v). After hexane precipitation, the paclitaxel precipitate was obtained through filtration, then dried in a 35 °C vacuum oven (UP-2000; EYELA) for 24 h. After dissolving the crude extract obtained through hexane precipitation (pure paclitaxel basis: 0.5%, w/v) in methanol, distilled water was slowly added one drop at a time until the methanol concentration reached 61.5%, then the solution was kept at 4 °C to obtain paclitaxel precipitate. The precipitate was obtained through filtration with filter paper (Whatman Grade 4, 20–25 μm particle retention, 150 mm diameter) and was vacuum dried for 24 h at 35 °C. The HPLC purification system was composed of an ODS C_{18} column (50 mm × 500 mm; Shiseido) and a silica column (50 mm × 500 mm; Shiseido). First, for ODS column HPLC, injection of crude extract was done at 50–150 mg/mL, the flow rate was 3–5 cm/min, the wavelength of the UV detector was 227 nm, and elution was done with methanol/water (65:35, v/v). Also, using silica column HPLC to remove trace impurities, injection of crude extract was done at 50–150 mg/mL, elution was performed with methylene chloride/methanol (98:2, v/v), and the UV detector wavelength was set at 227 nm [16]. The purity of paclitaxel after final purification was 98.7%. The sample preparation process for the removal of residual solvent is shown in Fig. 1.

2.5. Microwave-assisted drying method

For microwave-assisted drying, the sample obtained from silica-HPLC (mobile phase: methylene chloride/methanol (98:2, v/v)), which is the final purification step in the production process, was used [16]. The sample, 1 g of paclitaxel (purity: 98.7%), was dissolved in the elution solution of 20 mL methylene chloride/methanol (98:2, v/v) and was concentrated at 35 °C under reduced pressure using a rotary evaporator (CCA-1100) [5]. The solution was concentrated and then the samples were dried using microwave equipment (2450 MHz, Model 1501; Korea Microwave Instrument Co., Korea). The microwave cavity was W420 mm × D380 mm × H420 mm in size and consisted of cooling fan and thermocouple. A thermocouple was used to measure temperature changes continuously during drying. The power supplied to the microwave generator (capacity: 1.5 kW) was heated by changing to 100, 200 and 300 W. This experiment was carried out only up to 300 W because a problem with the operation (i.e. microwave overheating) occurred when the microwave power was 400 W or more. The concentration of residual methylene chloride and methanol in the sample to be dried was analyzed by GC after being dissolved in dimethylacetamide. In order to compare the efficiency of the vacuum and microwave-assisted drying methods, experiments were carried out under the same conditions (drying time and temperature) using a vacuum drying oven (UP-2000).

3. Results and discussion

3.1. Effect of microwave power on the removal of residual solvents

In general, the final purification step in the production of paclitaxel derived from biomass utilizes silica-HPLC (mobile phase: methylene chloride/methanol (98:2, v/v)) [16]. In order to identify the change in residual solvent concentration with concentration time, 1 g of paclitaxel was dissolved in 20 mL methylene chloride/methanol (98:2, v/v) and was then was concentrated (35 °C, reduced pressure) using a rotary evaporator. The residual concentration of methylene chloride rapidly decreased up to 4 h of concentration to 26,000 ppm and after that there was little change. The residual concentration of methanol also rapidly decreased up to 3 h of concentration to 50 ppm and after that there was little change (data not shown). This result seems to be due to the case hardening phenomenon, by which the sample surface is hardened before the residual solvent is fully removed in the concentration process [1,5,6]. According to the ICH Q3C guidance [3], the maximum residual concentration of methylene chloride and methanol is strictly limited to 600 ppm and 3000 ppm, respectively. When the sample final purified by silica-HPLC was concentrated by rotary evaporation, the residual methanol met the ICH-specified value (3000 ppm) but methylene chloride did not meet the ICH-specified value (600 ppm). Therefore, in order to reduce the concentration (~26,000 ppm) of residual methylene chloride in the sample concentrated by rotary evaporation to the ICH-specified value (600 ppm or less), the microwave-assisted drying method was introduced. The efficiency of microwave-assisted drying according to microwave power (100, 200, 300 W) and drying time was investigated using the sample (methylene chloride conc.: 26,000 ppm, methanol: 50 ppm) concentrated (35 °C, 5 h, reduced pressure) by rotary evaporation. In the case of a microwave power of 100 W, as shown in Fig. 2, the methylene chloride concentration remained high (>10,000 ppm) despite drying for a long time (~25 h). The reason is that, at low microwave power, the paclitaxel heating rate is low, and thermal equilibrium is easily reached [8]. At a microwave power of 200 W and 300 W, the concentration of methylene chloride rapidly decreased early during drying, but slowly decreased after a drying time of 5 h. The methylene chloride content of paclitaxel was very high during the initial phase of drying, resulting in a higher absorption of microwave power and higher drying rates due to higher methylene chloride diffusion. As drying progressed, the loss of methylene chloride in the paclitaxel caused a decrease in the absorption of microwave power and resulted in a fall in the drying rate [17,18]. At 200 W, the concentration of methylene chloride was reduced to approximately 1200 ppm by drying for a long time (~25 h) but did not decrease below that. In the case of 300 W, the concentration of methylene chloride met the ICH-specified value (600 ppm or less) after drying for 21 h. From these results, it was found that increased microwave power