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***Journal of Scientific and Engineering Research*, 2024, 11(1):126-131**

Research Article

ISSN: 2394-2630
CODEN(USA): JSERBR

Extraction and separation of paclitaxel

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Abstract To discuss the research progress of extraction, separation and detection methods of paclitaxel, and to provide relevant information for subsequent studies.

Keywords paclitaxel, extraction, separation

1. Introduction

Paclitaxel, a natural anticancer drug with the formula $C_{47}H_{51}NO_{14}$, has been widely used in the treatment of breast cancer, ovarian cancer and some head, neck and lung cancers. As a diterpenoid alkaloid with anticancer activity, paclitaxel has been greatly favored by botanists, chemists, pharmacologists and molecular biologists due to its novel and complex chemical structure, extensive and significant biological activity, new and unique mechanism of action, and scarce natural resources, making it the star and research focus of anticancer in the second half of the 20th century [1]. How to better isolate paclitaxel and its analogues is a very meaningful work both in basic research and in practical value. In recent years, the global population and the incidence of cancer have increased dramatically, and the demand for paclitaxel has also increased significantly. Paclitaxel required for clinical and scientific research is mainly extracted directly from taxus taxus. Due to the relatively low content of paclitaxel in plants (only 0.069% in the bark of short-leaf taxus taxus, the highest recognized content), about 13.6kg of bark can produce 1g of paclitaxel, and 3-12 taxus taxus trees more than 100 years old are needed to treat a patient with ovarian cancer. As a result, the deforestation of taxus chinensis has led to the extinction of this precious tree. In addition, the resources of yew itself are very poor, and the growth of taxus plants is slow, which causes great difficulties for the further development and utilization of taxol.

Although the chemical synthesis has been completed, it is not of industrial significance due to the strict conditions required, low yield and high cost [2]. Now the semi-synthetic method of paclitaxel has been relatively mature, and it is considered to be an effective way to expand the source of paclitaxel in addition to artificial cultivation. Semi-synthetic method can make greater use of plant resources, but there is no essential difference with the method of direct extraction of paclitaxel,

which needs to consume a large number of taxus trees, and still can not fundamentally solve the problem of lack of plant sources [3]. Obviously, the extraction of paclitaxel from taxus taxus plant tissue is very limited, so it is of great significance to find a new way to obtain paclitaxel. In this paper, the research progress in the extraction, separation and detection of paclitaxel is reviewed.

2. Extraction method of paclitaxel

2.1 Solvent extraction method

Solvent extraction is often used in the crude extraction stage of paclitaxel, which can be divided into primary extraction and secondary extraction. In the two-stage extraction process, the choice of solvent has an important influence on the quality of the product and the economy of the process [4].

The solvent systems used in primary extraction and secondary extraction are generally different, and the research results of the solvent systems in these two processes have been summarized in detail by researchers in various periods. Recently, Japanese scholars have carried out detailed research on the types of solvents for the extraction of paclitaxel, and the results show that: in ethyl acetate, ether, acetonitrile, acetone, methanol, hexane, isopropyl alcohol, ethyl acetate - methanol, ethyl acetate - dichloromethane, ethyl acetate - acetone, ethyl acetate - ethyl ether and other solvents, ethyl acetate - acetone (1:1) mixed solvent extraction effect is the best. The extracted extract is only 77% of the plant dry weight, and the content of paclitaxel is as high as 0.084% of the extracted extract, while the extracted extract from methanol is 20.98% of the plant dry weight, and the content of paclitaxel is 0.027% of the extracted extract [5-7]. Now it seems that using ethyl acetate-acetone (1:1) once can make the extraction amount of paclitaxel higher than that obtained by common solvents in the past, which brings great convenience to the subsequent separation and purification work. Because the price of ethyl acetate - acetone (1:1) is comparable to that of methanol, and can be recycled, so the economy of this extraction method is more reasonable. The introduction of ultrasonic technology in the primary extraction process can greatly shorten the time of the primary extraction process. For example, Chen uses methanol-dichloromethane (95.5) as the primary solvent, and the extraction time required is about 35-60min. When the solvent system remained unchanged,

ultrasonic oscillation of the mixture of raw material and solvent shortened the time to reach equilibrium to only 5min [8-9]. In contrast, Hoke et al. used pure methanol as the primary solvent without ultrasonic oscillation, and the required time was as long as 16-48h.

The introduction of ultrasonic technology, in addition to greatly shortening the extraction balance time, can also make the primary extraction at low temperature, so as to avoid the conversion of paclitaxel to other substances at high temperature caused by the reduction of yield.

The early chromatographic purification process of paclitaxel was an operation using multiple silica gel chromatography columns in series, because the irreversible adsorption of silica gel to paclitaxel caused a large loss, making the yield of paclitaxel is very low, only about 0.004% [10]. In recent years, with the advancement of chromatography technology, new chromatography technologies have been introduced into the separation and extraction process of paclitaxel, in addition to HPLC (including normal phase HPLC, reverse phase HPLC), as well as thin layer chromatography (TLC) method, micellar electrokinetic chromatography (MEKC)and high-speed counter-current chromatography (HSCCC).

The common disadvantage of various types of HPLC and TLC is that the loading capacity is small, and it is also suitable for daily processing of a large number of samples, and it can only reach the level of semi-preparative scale. For this reason, Wickremesinhe proposed to concentrate the crude methanol extract by C_{18} reversed phase column, and then purify it by C_{18} reversed phase preparation HPLC. Mattina et al. proposed to collect a large amount of concentrated taxane blends from the crude extract by C_{18} induced phase extraction (SPE) method. The results show that SPE is superior to TLC purification process, and has the advantages of saving time and using less solvent [11-12].

According to the current literature reports, HSCCC is expected to be a new method for large-scale preparation and production of paclitaxel. The main advantages of this method are: it has a high negative hub of the sample, a short separation cycle, easy to operate, because this chromatography has no solid carrier itself, to avoid the separation of the sample and the surface of the solid chemical reaction and denaturation and irreversible adsorption caused by sample loss. The disadvantage is that taxol and cephalomannine cannot be completely separated, and about half of the mixture of the two drugs can be separated by HPLC or TLC again to obtain taxol. In addition, in HSCCC, the choice of solvent system has a great influence on the separation effect, and two groups of solvent systems with short delamination time and large difference in the partition coefficient of each component of the two-phase solvent are selected.

Although MEKG has the advantages of high separation efficiency, small solvent consumption, fast speed, etc., its small processing capacity and complex operation make its application range, and it can only be used for analysis and detection.

2.2 Membrane separation method

In 1998, Pandey et al. studied the application of reverse osmosis membrane in the separation process of yew burnlike substances. The results showed that the membrane separation method could further concentrate the extract obtained from the crude extraction process, and the concentration of taxane substances could be increased by about 5 times. It is equivalent to when the extract is pretreated again, which reduces the burden of post-sequence chromatographic separation of alkali and the loss of paclitaxel [13].

When Ghasemi et al. treated the tissue culture medium of paclitaxel with 0.2μm nylon membrane and PVDF membrane, they found that the nylon membrane trapped almost all 10-deethylpapylidene paclitaxel and paclitaxel and most of cephalomarnnine, and almost no paclitaxel was trapped for other paclitaxel burning species. Most of them can be elution with 30%, 40% and 50% methanol solution, and similar results can be achieved when elution with 20% to 40% ethanol solution. When the PVDF encapsulated taxol was eluted with aqueous solvent, taxol and all taxol were eluted within the range of small changes in solvent polarity. In theory, though, the selective elution of taxol from taxane extracts can be achieved by selectively washing the taxane components from the membrane for chemical modification [14-15].

2.3 Supercritical fluid extraction method

The supercritical filter extraction (SFE) technology was introduced into the purification process of paclitaxel, which reduced the use of chlorine-containing organic solvents and was a new technology that did not pollute the environment. The most commonly used solvent for SFE is CO_2, which is itself non-toxic and has no residue in the extracted products, so from the point of view of drug safety, this technology has its unique advantages.

In 1992, Jermrings et al., using CO_2 and CO_2 with ethanol modification agent as SFE solvent, conducted a study on the extraction of taxol from the bark at 318K and 18.07-25.79MPa pressure, and found that most of the taxol in the bark could be effectively extracted. The extraction rate is as high as 0.08% (the conventional method is only 0.01%), and the selectivity of paclitaxel is better than that of traditional ethanol extraction. Nair et al. used CO_2 containing 0.001%-15% acetone or acetonitrile as solvent to extract paclitaxel by supercritical technology at 43.4MPa308K and obtained satisfactory results. Castor et al. used taxus taxus branches, leaves and buds as raw materials to extract paclitaxel with critical technology. First, pure CO_2 was used as solvent to remove the lipids in the raw materials, and then ethanol was added to adjust the polarity of the solvent, so that the yield of paclitaxel reached 0.04% [16-18].

Although supercritical technology shows the advantages of high yield and time saving in the extraction of paclitaxel, it has high requirements for equipment and strict operating conditions, and it is difficult to carry out supercritical extraction of a large number of raw materials at present.

2.4 Ion exchange resin method

Yuan Yingjin et al. used 8 kinds of resins to study the decolorization ability and adsorption of Taxus chinensis extract. The results showed that the strong resin of polyene type (Ps-A) and the weak resin of polyene polyamine epichlorohydrin condensation type (Pc-A) had good adsorption and fertilizer removal performance for dichloromethane crude extract, and were expected to be used for the purification and separation of paclitaxel [19].

Yang Xuefeng et al used PSp-6 macroporous resin to separate taxol and semi-synthetic precursors from crude extracts of Taxus yunnanensis bark and Taxus cuspidata needles by industrial reversed-phase preparative chromatography. In the test, a single column load of Yunnan taxus extract (containing taxol 1.2%) up to 5kg, by three-stage chromatography separation and recrystallization of taxol product purity is greater than 99%, the total product yield is greater than 80%, the production cycle of 196h, the production cost of about 1000 yuan/g, very promising industrialization.

3. Paclitaxel separation method

Since paclitaxel is very similar to other paclitaxel-like compounds, good separation of paclitaxel compounds is the basis for accurate quantification of paclitaxel. At present, the main separation methods of paclitaxel are as follows:

3.1 Liquid chromatography

For column chromatography, the mixture of paclitaxel and cephalomannine was obtained by atmospheric and low pressure chromatography, and then paclitaxel was isolated by conventional column chromatography. The result is incomplete separation [20].

3.2 Thin layer chromatography

Preparative TLC, HPLC and some advanced separation methods such as HPLC, HSCC (high speed countercurrent chromatography) were used to separate paclitaxel from cephalomarunine. Conclusion: the separation was incomplete.

3.3 OsO4 oxidation combined with liquid chromatography

Kingston et al. have tried to treat the mixture of paclitaxel and cephalomarnine with OsO_4 and found that OsO_4 can selectively oxidize the double bond at the end of C_{13} side chain of cephalomannine to form diols, but paclitaxel is not affected, and then column chromatography is performed with hexane-ethyl acetate as solvent. It is possible to separate paclitaxel from cephalomannine-diol (the diol of cephalomanine). Conclusion: It is highly toxic and completely isolated [21].

3.4 O_3 oxidation combined with liquid chromatography

Inspired by Kingaon's method, Murray et al. used air containing 1%~10%O_3 to effectively oxidize cephalomarnine. Some of the olefins in taxus brevifolia, which do not react to another olefins in taxus, cephalomannine, taxus, brevifolia, can be efficiently purified by silica column chromatography. Result: High cost, complete separation.

3.5 O_3 Combination with Girard's hydrazide and ACOH

Murray et al. improved the above method, specifically adding Girard's hydrazide - ACOH mixture after oxidation by O_3, so that OZO-cephalomannine (cephalomannine is oxidized by O_3, and turns into OZO-cephalomannine Girard's hydrazone. This complex) can finally be separated from taxol by selective precipitation or extraction with ethyl acetate - water, etc. Results: Low cost, complete separation [22].

3.6 Micellar electrokinetic capillary chromatography

Micellar electrokinetic capillary chromatography is a new separation and purification technology developed in recent years. It has the advantages of high separation efficiency, less reagent consumption and fast separation speed. Chan et al. used MECC to isolate paclitaxel, cephalomannine, bacatine III and their deacetylated derivatives. Hempel also reported the separation of paclitaxel from fermentation broth by MECC. In addition, Chen et al. used the same capillary electrophoresis system and sodium deoxycholic acid (SDC) as the chiral resolution agent to establish the micellar electricity of seven taxane compounds, including paclitaxel, 10-deacetylpaclitaxel, 10-deacetyl-7-epipaclitaxel, cephalomannine, bacatine III, 10-deacetyl-Barkatine III, 10-deacetyl-7-epibacatine III Dynamic capillary chromatography. However, MECC limited its application due to its small processing capacity and complex operation [23].

3.7 High-speed reflux chromatography

HSCCC technology is a relatively new liquid-liquid distribution technology in the world, which has the advantages of high separation efficiency, large preparation amount, high recovery rate, less solvent consumption, short separation cycle, simple operation, and has the inherent advantages of solid carrier free counter-current chromatography. It is expected to become a new method for large-scale preparation of paclitaxel. Chiou et al. used cyclic high speed countercurrent chromatography to separate the mixture of paclitaxel and cephalomannine. After two cycles, the separation of the two mass peaks increased from 0.7 to 1.27. However, so far, HSCCC technology is not mature enough, there are many theoretical and technical problems need to be further studied and solved, and there is no stable and reliable commercial instrument, which limits the application of HSCCC in industrialization [24].

3.8 Pharmacological action target method

Pharmacological action target method is a new separation method based on the principle of pharmacological action. In other words, anti-cancer drugs can be separated and purified by the reversible and specific interaction of anti-cancer drugs with pharmacological targets. The pharmacological research of paclitaxel shows that the target of paclitaxel's pharmacological action is microtubule, which is the polymerized state of microtubulin. The main feature of microproteins is that they are polymerized into microtubules at high temperatures and depolymerized into tubulin dimers at low temperatures. Paclitaxel can be targeted to bind to microtubules and inhibit the depolymerization of microtubules without interacting with the dimer of tubulin. Harringine, which is the most difficult to separate from paclitaxel, does not have this property. It was found that under certain conditions, the purity of paclitaxel can reach more than 95% after the mixture of paclitaxel and harringine is purified by pharmacological target method. Pharmacological targets have the following advantages: (1) The method is simple and specific. (2) Paclitaxel and its pharmacological target microtubules are representative in natural anticancer substances and pharmacological target 2, and its research results are easy to be extended to other anticancer substances and pharmacological target systems [25].

4. Conclusion

In summary, great progress has been made in the study of the extraction and initial separation technology of paclitaxel. Some new technologies and methods have been applied in this field, showing many advantages and good prospects. However, from the current situation, the series technology of liquid-liquid extraction and solid-phase extraction still plays a major role in the initial separation process. Resin chromatography is a good pre-treatment method for paclitaxel because of its simple operation and low production cost. With the continuous introduction of new technologies and new methods, the extraction and purification technology of paclitaxel has made continuous progress. These technologies will certainly promote the low-cost and high-efficiency industrialization process of paclitaxel production, change the current situation that the supply of paclitaxel is in short supply and the price is extremely high, and promote the development of the pharmaceutical industry and the great progress of human health.

Reference

[1]. Yuan H. Studies on the chemistry of paclitaxel [D]. Virginia Polytechnic Institute and State University, 1998.

[2]. Wani MC, Taylor H L, Wall M E, et al. Plant antitumour agents. VI The isolation and structure of Taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J of Am Chem Soc, 1971,93 : 2325-2327.

[3]. Expósito O, Bonfill M, Moyano E, et al. Biotechnological production of taxol and related taxoids: current state and prospects [J]. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 2009, 9(1): 109-121.

[4]. Suffness M, Wall M E. Discovery and development of taxol [M]//Taxol. CRC press, 2021: 3-26.

[5]. Kawamura F, Kikuchi Y, Ohira T, et al. Accelerated solvent extraction of paclitaxel and related compounds from the bark of Taxus cuspidate [J]. Journal of natural products, 1999, 62(2): 244-247.

[6]. Pyo S H, Park H B, Song B K, et al. A large-scale purification of paclitaxel from cell cultures of Taxus chinensis[J]. Process Biochemistry, 2004, 39(12): 1985-1991.

[7]. Kim G J, Kim J H. Enhancement of extraction efficiency of paclitaxel from biomass using ionic liquid-methanol cosolvents under acidic conditions [J]. Process Biochemistry, 2015, 50(6): 989-996.

[8]. Esclapez M D, García-Pérez J V, Mulet A, et al. Ultrasound-assisted extraction of natural products [J]. Food Engineering Reviews, 2011, 3: 108-120.

[9]. Chen Zhende, Zheng Hanchen, Zhang Hong, Wang Hongquan, Li Jinchang. A new method for preparing paclitaxel [P].1197796, 1998-11-04.

[10]. Singh I P, Ahmad F, Chatterjee D, et al. Natural products: drug discovery and development [J]. Drug Discovery and Development: From Targets and Molecules to Medicines, 2021: 11-65.

[11]. Wickremesinhe E R M, Arteea R N. Taxus callus cultures: initiation, growth optimization, characterization and taxol production[J]. Plant cell, tissue and organ culture, 1993, 35: 181-193.

[12]. Incorvia Mattina M J, Paiva A A. Taxol concentration in Taxus cultivars [J]. Journal of Environmental Horticulture, 1992, 10(4): 187-191.
[13]. Pandey R C, Yankov L K, Poulev A, et al. Synthesis and Separation of Potential Anticancer Active Dihalocephalomannine Diastereomers from Extracts of Taxus y unnanensis [J]. Journal of natural products, 1998, 61(1): 57-63.
[14]. Ghasemi S, Nematollahzadeh A. Molecularly imprinted ultrafiltration polysulfone membrane with specific nano-cavities for selective separation and enrichment of paclitaxel from plant extract [J]. Reactive and functional polymers, 2018, 126: 9-19.
[15]. Johnson M, Rivers J, Thurlow S. Paclitaxel Release System [D]. Worcester Polytechnic Institute, 2020.
[16]. Kawamura F, Kikuchi Y, Ohira T, et al. Accelerated solvent extraction of paclitaxel and related compounds from the bark of Taxus cuspidate [J]. Journal of natural products, 1999, 62(2): 244-247.
[17]. Nair U R, Sivabalan R, Gore G M, et al. Hexanitrohexaazaisowurtzitane (CL-20) and CL-20-based formulations[J]. Combustion, Explosion and Shock Waves, 2005, 41: 121-132.
[18]. Castor T P, Tyler T A. Determination of taxol in Taxus media needles in the presence of interfering component s[J]. Journal of Liquid Chromatography & Related Technologies, 1993, 16(3): 723-731.
[19]. Yuan Yingjin. Paclitaxel and polyene paclitaxel [M]. Beijing: Chemical Industry Press, 2002.1-26.
[20]. Fu Y J, Sun R, Zu Y G, et al. Simultaneous determination of main taxoids in Taxus needles extracts by solidphase extraction-high-performance liquid chromatography with pentafluorophenyl column [J]. Biomedical Chromatography, 2009, 23(1): 63-70.
[21]. Kingston D G I, Gunatilaka A A L, Ivey C A. Modified taxols, 7. A method for the separation of taxol and cephalomannine [J]. Journal of natural products, 1992, 55(2): 259-261.
[22]. Murray C W, Handy N C, Amos R D. A study of O_3, S_3, CH_2, and Be_2 using Kohn–Sham theory with accurate quadrature and large basis sets [J]. The Journal of chemical physics, 1993, 98(9): 7145-7151.
[23]. Hancu G, Simon B, Rusu A, et al. Principles of micellar electrokinetic capillary chromatography applied in pharmaceutical analysis [J]. Advanced Pharmaceutical Bulletin, 2013, 3(1): 1.
[24]. Suktham K, Daisuk P, Shotipruk A. Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia L .(Rubiaceae): Errata and review of technological development and prospects [J]. Separation and Purification Technology, 2021, 256: 117844.
[25]. Longuespée R, Theile D, Fresnais M, et al. Approaching sites of action of drugs in clinical pharmacology: New analytical options and their challenges [J]. British Journal of Clinical Pharmacology, 2021, 87(3): 858-874.