Technological methods of sesquiterpene lactones extraction from raw materials

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Abstract

Methods of sesquiterpene lactones extraction used in pharmaceutical manufacture have been summarized. The alternative method of extraction and purification of sesquiterpene lactones of arglabin and leucomisin has been offered.

Terpenoids are the wide class of natural compounds basically widespread in plants and being a potential source of new medical preparations.

At present the different pharmaceutical enterprises produces about 100 types of medications on the basis of terpenoids which are widely used in medicine.

The sesquiterpene γ-lactones takes a special place among terpenoids that is due to its polyfunctional structure and presence of molecule of pharmacophore groups with a wide spectrum of biological activities: antibacterial, fungicidal, antifeedant, insecticidal, antihelmintic, cardiotonic, antitumor [1-4].

The first sesquiterpene γ-lactone which obtained a medical application is α-santonin – a typical representative of bicyclic sesquiterpenoids of eudesmane group.

α-Santonin has been firstly extracted in 1830 in Germany from flower anthodiums of Artemisia cina Berg and has been used actively for treatment during the three years. In the middle of XVIII century in Germany and England there were a number of the factories producing flower anthodiums with various containing of santonin of Artemisia maritima Bess. population which grows in Northern Africa, India and South America.

In 1892 in Shymkent the santonin factory was established and for a long time it was a unique industrial pharmaceutical enterprise in Kazakhstan. The organization of pharmaceutical factory in Southern Kazakhstan has been connected with raw-material source of santonin, namely, presence of industrial stocks of endemic species of Artemisia cina Berg.

Technological process of obtaining of α-santonin consists of 5 stages [5].

At the first stage the flower anthodiums of Artemisia cina Berg. is steeped in water and mixed with lime containing calcium oxide no less than 60 %. α-Santonin is dissolved in alkalis with the disclosing of lactone ring, forming salt of santonin acid.

At the second stage the sevenfold leaching of calcium salt of α-santonin acid is mixed with water. The content of α-santonin in an extract is varying about 0,7-1,4 %.

At the third stage an extract in an extractor is evaporated and essential oil with water vapor is distilled. The obtained oil is settled and dried under sodium sulfate as external antirheumatic drug. The extractor is unloaded at the end of distillation of essential oil. The yield of α-santonin at a stage of extraction is 95 %.

At the fourth stage the concentrated extract containing calcium salt of santonin acid, gum and others extractive substances is acidified with nitric acid. Thus calcium nitrate and santonin acid is formed which slowly turned in α-santonin. α-Santonin - raw product is tenfoldly washed with water before reaction of neutralization, then squashed in centrifuge, transfered in drying chamber and dried at temperature 66-68°C. The yield is about 80 % at this stage.

The fifth stage of α-santonin production is purification of technical α-santonin with repeated

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crystallization from ethanol and the subsequent filtration on specially designed filter. After crystallization the α-santonin is transferred on centrifuge where it's squashed and washed with distilled water. The yield of pure α-santonin is 80-84 % at this stage. The total yield of α-santonin depends on the content of target substance in initial raw material and on number of recrystallizations.

The second sesquiterpene lactone which is used in medical practice is endemic number – tauremisin extracted from aerial part of Artemisia taurica Willd. Preparation of "Tauremisin" was developed and introduced in manufacture on its basis [1, 6]. It has been recommended for application as drug in medicine which is toning up the central nervous and cardiovascular system. It is also recommended for application at intellectual and physical fatigue, condition of lassitude at present.

The industrial method of the tauremisin obtaining consists of the air-dry aerial stage of Artemisia taurica Willd. which is processed by principle of back-flow of hot water for 30 minutes at temperature 75-80 °C [6].

Further the obtained water extractions is chloroformed three times at the ratio of 10:1, 20:1, 20:1; chloroform extractions is united, chloroform is distilled off without vacuum up to residual volume 1,5 l and then the remained chloroform is evaporated at vacuum 15 mm of mercury. Ethyl ether (10:1) is added to the obtained tight dark-brown crystallizing mass to up the termination of loss of residue. Residue was dissolved in chloroform, washed with 5 % solution of sodium carbonate for separation of pitch substances of acid character and watered twice. Chloroform was distilled off. Residue was diluted in ethanol, added activated carbon, boiled for several minutes and filtered. After cooling crystals were drafted and recrystallizated from ethanol. Yield of tauremisin is about 0,2 % in re-calculation on air-dry raw material.

Alanton is a galenical new antiulcer preparation, containing pure sum of sesquiterpene lactones of Inula helenium L. [7]. The basic lactones in content of preparation: alantolactone and isoalantolactone can be not less than 95%.

Manufacture of substance "Alanton" from aerial part of Inula helenium L. has been carried out at a few stages.

At the first extraction of crushed raw material of 85% ethanol (1:3) in battery of percolators has been carried out. Infusion has been made for 12 h.

The next stage the obtained extract has been evaporated in vacuum to up 2/5 initial volume. Then water has been added and continued to evaporate to up 1/5 of loaded extract.

The third stage of manufacture has been an extraction of terpenoid fraction from water residue of methylene chloride. Chlormethylene extracts have been poured out, united, deaquated with calcinated sodium sulfate for 2 h, filtered and evaporated up to 1/10 from initial volume.

At the fourth stage purification of obtained solution has been carried out with a method of column chromatography on aluminium oxide. Elution has been made with methylene chloride. The obtained eluate has been evaporated to up full distillation of solvent. Residue has been hard mass with dark-yellow color.

At a final stage of the obtaining of alanton residue has been added to 30-fold quantity of ethanol and at mixing added step-by-step equal quantity of purified water. Mixture has been clouded. Yellow flocks of residue have been formed. At the subsequent addition of water, some clots have been showed. Suspension has been poured out in crystallizer where cooled up to 0-5 °C. Crystallization of alanton has ben made for 2 day. Alanton has been filtered and washed out with 4-fold quantity of the petrol cooled up to 0-5 °C. Residue has been dried for 10-12 h. Dried alanton has been crushed in spherical mill, sieved and packed. The yield of alanton has been made 1 % in recalculation on air-dry raw material.

Authors [8, 9] have developed an alternative method of manufacture of substance "Alanton". Technology of the obtaining of sesquiterpene lactones alantolactone and isoalantolactone from plant raw material on this method has been carried out 2-fold extraction of roots and rootstocks of Inula helenium L. with mixture of ethanol:hexane (1:4) at boiling, mass ratio of roots and rootstocks with extractan (1:5) for 3 h. The obtained extracts have been united, filtered from mechanical impurity and evaporated up to ¼ volume. Thus, residue has been determined. With the purpose of purification of attendant substances, mixtures of ethanol:hexane (1:4) have been dissolved in the minimal volume (50 ml) and sesquiterpene lactones have been upset with addition of pure hexane to up the steady cloud. The maximal loss of crystals of sesquiterpene lactones has been reached at mixtures for 24 h at room temperature. Yellowish crystals of the purified sum of sesquiterpene lactones alantolactone and isoalantolactone, being a quantitative ratio of 2:1 with melting point 86-88°C, have been obtained

with application of column chromatography. Silica gel and eluent of mixture of petrol ether and ethyl acetate (9:1) have been used as a sorbent. At application of this method, time on isolation of lactone has been reduced from 192 h to 58 h and yield has been increased from 1,3-1,5 to 1,7-1,9 %.

Preparation of "Alanton" is produced in the form of tablets at Borshagovsky factory at present time and applies as antiulcer agent.

Isolated sesquiterpene lactone artemisinin from Artemisia annua L. has high antimalarial action. Artemisinin has been isolated with extraction of Artemisia annua L. by subsequent column chromatography. Nonpolar solvents have been used as an extractant. The method of obtaining of artemisinin without application of column chromatography has been known. For this purpose an aerial part of Artemisia annua L. has been extracted with ethanol, then extract has been evaporated, the obtained residue re-extracted with hexane. Extract has been evaporated. Residue has been dissolved in ethyl acetate, added activated carbon for bonding of chlorophylls, filtered and crystallized artemisinin from the obtained filtrate [10].

On other method [11] of manufacture of artemisinin has been carried out on the following scheme: the air-dry and crushed raw material of Artemisia annua L. has been 6-fold extracted for 8 h in the Soxhlet with hexane at temperature 60-80 °C. Liquid extract has been evaporated up to 5 % from initial volume. The obtained residue of hexane extract has been mixed with water acetonitrile in the ratio 1:5 for 3 h. Then the mixture has been settled and divided after full separation of phase. Acetonitrile solution has been dried up fresh calcined sodium sulfate and evaporated up vacuum.

The obtained residue has been chromatographed on column with silica gel by mixture of ethylacetate:hexane in various ratios. At elution of column with 8 % mixture of ethylacetate:hexane has been obtained artemisinin. Artemisinin has been recrystallizated from mixture of ethylacetate:hexane (1:4), the dropped out crystals have been filtered and dried. The yield has been made 0,045 % in recalculation on air-dry raw materials.

The highly effective antimalarial drug "Qinghaosu" has been developed on the basis of artemisinin in China [12].

The component lactone fraction of aerial part of Tanacetum parthenium L. - sesquiterpene lactone parthenolide is a basis of medical drugs for prophylaxis of migraine [13, 14]. Parthenolide has been an active substance of total biologically active admixture "Feverfew" [15], produced by firm MH Pharma Ltd (Great Britain) in the form of capsules, used for headache and arthritis. Parthenolide has been isolated with the following scheme: air-dry, crushed raw material of aerial part Tanacetum parthenium L. has been exhaustively extracted with 90 % water ethanol at room temperature. Extract has been filtered and evaporated up vacuum. Residue has been chromatographed on column with silica gel, as eluent has been used dichloride methane. The obtained fractions have been united, evaporated and rechromatographed on column with silica gel by mixture gexane:dichloride methane.

As it has been showed from the above described technologies, a basis of the obtaining of sesquiterpene lactone has been made extraction of raw materials with various organic solvents with the subsequent chemical or chromatographic purification. Thus, there is an actual problem in development of effective and ecologically safe methods of isolation of lactones from plant raw materials, in conformity with standards of the international rules of manufacture of medical drugs - GMP.

At JSC "International research and production holding "Phytochemistry" some phytopreparations have been developed and produced in manufacture, their basis has been sesquiterpene lactones.

The first and second turns of Karaganda pharmaceutical complex have been constructed and introduced in exploitation on the basis of holding "Phytochemistry", there has been section of extraction and synthesis of substance of phytopreparations, section of sublimation ampulling of medical preparations and laboratory of quality control.

Antitumor preparation "Arglabin" has been developed on the basis of sesquiterpene lactone arglabin, isolated from aerial part of Artemisia glabella Kar. et Kir. Preparation has been recommended by Ministry of Health of Republic of Kazakhstan for treatment of cancers of breast, lungs and liver.

Method of obtaining "Arglabin" has been patented in 11 countries of the world (the USA, Great Britain, Germany, Switzerland, France, Austria, Italy, the Netherlands, Sweden, Japan, China), protected by European, the USA, China and Euroasian patents [16-20]. Arglabin has been registered as antitumor preparation in Republic of Kazakhstan, the Russian Federation, the Kirghiz Republic, Republic of Tajikistan, Georgia.

Sesquiterpene lactone leucomisin isolated from

Artemisia leucodes Schrenk. is a substance of hypolipidemic and anti-atherosclerotic preparation "Aterolid" [21].

At present, the preparation is a final stage of clinical tests on the basis of scientific research institute of cardiology and internal diseases of Ministry of Health of Republic of Kazakhstan. Preparation is effective for treatment of the diseases connected with dislipoproteinemia and atherosclerosis.

The basis of technology of these preparations has been made extraction with organic solvents with the subsequent partition chromatography of the obtained extracts.

The researches on optimization of technology of the obtaining of substances of antitumor preparation "Arglabin" and hypolipidemic preparation "Aterolid" in the plan of manufacture to the international standards and increases of competitiveness of preparations have been carried out.

Thus, the supercritical fluid extraction of plant raw materials with carbon dioxide has been used for the isolation of sesquiterpene lactones [22, 23].

The centrifugal partition chromatography (CPC) has been used for isolation and produce of arglabin and leucomisin from CO_2-extractions of Artemisia glabella Kar. et Kir. and Artemisia leucodes Schrenk.

The basic advantage of this method is to not use solid sorbents. The division of the sum of substances has been made between two unmixed phases. Also this method has a number of advantages before high performance liquid chromatography (HPLC) (table 1).

Table 1

Comparison of chromatography of normal pressure (CNP), high performance liquid chromatography (HPLC) and centrifugal partition chromatography (CPC) dates

Basic characteristics	CNP	HPLC	CPC
Sorbent	yes	yes	not
Percent of return of test	< 100 %	< 100 %	100 %
Quality of solvent	medium	high	low
Consumption of solvent	large	large	low
Preparation of test	filtration	overhead	filtration
Production costs	medium	very high	not large

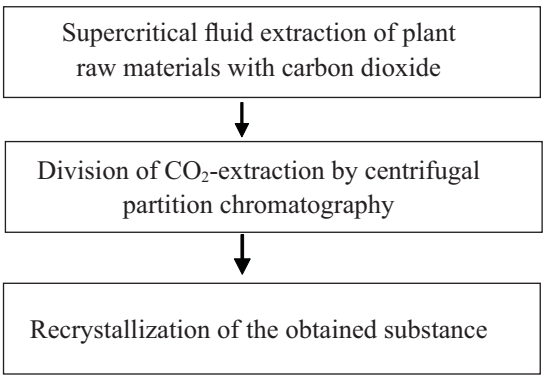

Fig. 1. The basic technological scheme of "Arglabin" and "Aterolid" substances production

The basic technological scheme of the pilot manufacture of pharmacologically active substances of preparations of "Arglabin" and "Aterolid", including only three stages (figure 1) has been developed on the basis of the results.

The developed technology of isolation and purification of sesquiterpene lactones of arglabin and leucomisin has been introduced in manufacture of phytopreparations of "Arglabin" and "Aterolid" on the basis of LLC "Karaganda pharmaceutical complex". The pilot orders on manufacture of substance leucomisin (PO 40653870-04-09) and substance arglabin native (PO 40653870-01-09) have been approved by Committee of pharmaceutical control MH RK.

This technology has been characterized by high speed of division. It has been not demanded application of sorbent and application of high-purified solvents. The consumption of solvents has been less in 10 times. Productivity has been made above in 10 times. The industrial costs have been decreased in 5-10 times. Thus, the cost price of substances has been reduced.

Thus, the economic, ecologic safe technology of extracting and purification of sesquiterpene lactones from plant raw materials has been developed and introduced in manufacture. It showed the great perspectives of introduction of phytopreparations on the base of biologically active compounds in medicine.

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