RUSSIAN FEDERATION

**FEDERAL SERVICE
FOR INTELLECTUAL PROPERTY**

(19) RU (11) 2 533 231(13) C1

(51) Int. Cl.
A61K 31/352 (2006.01)
A61K 31/715 (2006.01)

(12) **ABSTRACT OF INVENTION**

(21)(22) Application: **2013122124/15, 14.05.2013**

(24) Effective date for property rights:
14.05.2013

Priority:
(22) Date of filing: **14.05.2013**

(45) Date of publication: **20.11.2014** Bull. ÿ **32**

Mail address:
630117, g.Novosibirsk, a/ja 5, Kuchumovoj L.Ja.

(72) Inventor(s):
**Ostronkov Vladimir Sergeevich (RU),
Lashin Sergej Alekseevich (RU)**

(73) Proprietor(s):
**Ostronkov Vladimir Sergeevich (RU),
Lashin Sergej Alekseevich (RU)**

(54) **SUPRAMOLECULAR COMPLEX, POSSESSING ANTI-INFLAMMATORY AND ANGIOPROTECTIVE ACTIVITY AND METHOD OF OBTAINING THEREOF**

(57) Abstract:

FIELD: chemistry.

SUBSTANCE:supramolecular complex, possessing an anti-inflammatory and angioprotective activity, which includes dihydroquercetin, arabinogalactan and water with a specified content of components. The method of obtaining the supramolecular complex includes mixing arabinoglactan with water to complete dissolution, then the addition of dihydroquercetin, heating the solution, mixing, with the following drying of the obtained solution by a method of spraying, under specified conditions.

EFFECT: increase of dihydroquercetin water solubility. 2 cl, 1 dwg, 5 tbl, 8 ex

SUBSTANCE: group of inventions relates to the chemical-pharmaceutical industry and concerns the production of a water-soluble complex with increased pharmacological activity, consisting of dihydroquercetin, which is poorly soluble in water, and a water-soluble complexing agent. The range of application of dihydroquercetin (DHQ) is quite wide, it is used in the manufacture of various products, including the pharmaceutical industry for the production of dietary supplements and medicines, as well as in the food industry as an antioxidant. Its disadvantage is low bioavailability due to poor water solubility. In connection with the widespread use of DHQ in the food, medical, and cosmetic industries, the problem of obtaining a water-soluble complex of dihydroquercetin with high pharmacological activity and the development of improved methods for its production remains relevant.

It is known that in order to obtain water-soluble forms of drugs to various water-soluble natural oligo- and polysaccharides, such as dextran, inulin, maltodextrin, and cyclodextrin, are added to a poorly soluble drug in water, which form water-soluble complexes with the target drug and, accordingly, increase its bioavailability.

Known water-soluble composition, including a sparingly soluble antibiotic in the amount of 20-60% and a natural polysaccharide selected from the group - dextran, inulin and maltodextrin, taken in a certain ratio. A known composition is obtained by mixing solutions of antibiotics and polysaccharides, followed by drying and isolating a dry adduct, in which antibiotics are bound to polysaccharides by non-covalent and non-ionic bonds (patent US 6821959 B1, op. 23.11.2004). Known water-soluble composition,

including sparingly soluble medicinal substance and cyclodextrin, mainly beta-cyclodextrin. The composition contains a non-steroidal anti-inflammatory drug (paracetamol, ibuprofen, ketoprofen, flufenamic and mefenamic acids, etc.), a steroid, prostaglandin, prostacyclin, barbiturate, sulfonamide, cardiac glycoside as a poorly soluble drug substance. Water-soluble intermolecular complexes of lipophilic organic compounds are formed in solution due to the intercalation of their molecules into this cavity (J. Szejtli, Industrial Applications of cyclodextrins.

- In Inclusion Compounds, v.3. ed. Atwood J.L., Davies J.E., Mcnicob D.D., Academic Press, N Y., 1984, p.331-390).

Known complex based on dihydroquercetin, which is an aqueous a solution of dihydroquercetin in a molecularly encapsulated form in the form of a water-soluble associate. To obtain this complex, homogenization of dihydroquercetin is carried out in a melt of a surfactant, preferably CremophorRH 40, or in a common solvent - ethanol at a ratio of surfactant to dihydroquercetin 5:1 in the temperature range of 40-70°C, followed by mixing with water and concentration by evaporation of water (patent RU 2406496 C1, op. 20.12.2010). EFFECT: invention makes it possible to increase the solubility and bioavailability of dihydroquercetin.

The disadvantage of the known complex is the low solubility of dihydroquercetin, as well as a complex and lengthy way to obtain it. Known pharmaceutical composition, including a sparingly soluble drug and arabinogalactan (AG), taken in a ratio of 1:(5-20) by weight, which is obtained by mechanical processing of the mixture, preferably in ball mills, in particular planetary (patent RU 2337710 C2, op. 11/10/2008).

However, the method of obtaining a water-soluble composition requires the use of shock attrition and other intense mechanical effects, including pressure and shear deformation, which leads to the destruction of the crystal structure of dihydroquercetin.

The closest to the claimed complex based on dihydroquercetin and the method of its production - the prototype, is a solid nanocomposition for the delivery of biologically active substances, containing 0.1-15% of the active component, in which the nanocomposition contains taxifolin, 40-95% of the polymer, in which the nanocomposition contains arabinogalactan, or polyethylene glycols, or polyvinylpyrrolidones, or polyvinols of various molecular weights, 0-56% of a water-soluble component selected from a number of possible fillers: kollidon VA64 (copolymer of vinylpyrrolidone and vinyl acetate), kollidon 90F (high molecular weight polyvinylpyrrolidone with M.m. 1000000- 1500000), ludipress (modified lactose), powdered sugar, isomaltose, 0-6% hydrophobic or inert polymer in order to achieve controlled release of the drug substance, where the nanocomposition contains compritol 888 ATO as a hydrophobic component (composition of mono-, di- and triglycerides of behenic acid), as inert - kollidon SR. (a mixture of polymers of polyvinylpyrrolidone and polyvinyl acetate). Method for obtaining a nanocomposition containing taxifolin and arabinogalactan in

weight ratio 1:10, includes the following stages: arabinogalactan (10 g) is dissolved in 10 ml of water; separately prepare a solution of taxifolin by dissolving the latter (1 g) in 10 ml of water with stirring and heating in a thermostated vessel until taxifolin is completely dissolved; Taxifolin solution is added to a vessel with arabinogalactan solution with stirring and temperature control until a homogeneous medium is obtained, followed by lyophilization of the product (patent RU 2351352 C2, op. 10.04.2009).

The disadvantages of the known solid nanocomposite are the complexity of the composition, the use of a large number of imported hard-to-reach components, the high cost of the target product, as well as the duration and laboriousness of its production (the need to use expensive freeze-drying). The task of the group of inventions is to create a complex based on dihydroquercetin with increased solubility in water and a method for its production. EFFECT: increase in water solubility of dihydroquercetin in the complex and increase in its pharmacological activity, reduction in the duration of the method for obtaining the complex. The problem is solved by the claimed composition of the complex and the method of its production. The complex includes dihydroquercetin, arabinogalactan and water, with the following

content of components, wt.%:

dihydroquercetin	1,0-22,8
arabinogalactan	75,2-96,8
water	2,0-5,0

The method for obtaining the complex consists in mixing arabinogalactan with water until complete dissolution, then adding dihydroquercetin, heating the solution to 40-45°C, stirring for 0.5-1 hour, followed by drying the resulting solution by spraying. To do this, arabinogalactan is dissolved in a container containing deionized water until completely dissolved, then dihydroquercetin is added to the solution in a ratio of dihydroquercetin: arabinogalactan equal to 1: (3-

100), preferably 1:(50-100) by weight, respectively, the solution is heated to 40-45°C, stirred for 0.5-1 hour, then the resulting solution is dried by spraying.

Spray dryers can be used to dry the complex.
with centrifugal atomization (SRC type), equipped with a paddle (flat working surface) high-speed disc or spray dryers with nozzle spray (SRF type), equipped with pneumatic or mechanical (high pressure) nozzles. Arabinogalactan is a water-soluble polysaccharide with m.m. 9-18 kD, the main chain of which consists of galactose units, the side chains of arabinose and galactose units. This feature of the structure contributes to the formation of strong intermolecular complexes of drugs, the molecules of which are more likely to

all connected by intermolecular hydrogen bonds in the space formed by the side chains. Given the conformational mobility of arabinogalactan macromolecules, this space can vary, facilitating the formation of intermolecular complexes with a wide range of substances. In addition, the formation of complexes of different stoichiometric composition is possible, when one molecule of arabinogalactan can bind to several molecules of other organic compounds - medicinal substances, allowing you to change the ratio of these components over a wide range. This is the advantage of arabinogalactan as a complexing agent in comparison with the cyclodextrins commonly used for these purposes. The interaction of dihydroquercetin molecules with the complexing agent arabinogalactan probably occurs in the same way. Molecule

dihydroquercetin can be inserted between long polysaccharide chains
arabinogalactan, forming a supramolecular complex that has a higher water solubility compared to the sparingly soluble dihydroquercetin. As a result, while maintaining the therapeutic effect of dihydroquercetin, it is possible to increase its solubility and effectiveness tenfold. The defining differences between the proposed complex and the method of its production are
compared to the prototype are:

1. The claimed supramolecular complex after drying contains dihydroquercetin and arabinogalactan, taken in the ratio of dihydroquercetin: arabinogalactan, equal to 1: (3.3-96.8) by weight, respectively, as well as water, which makes it possible to increase the solubility of DHQ in water by 11.8 -22.5 times and increase its pharmacological activity.

2. The method for obtaining a complex based on dihydroquercetin includes mixing arabinogalactan with water until completely dissolved, then adding dihydroquercetin in a ratio of dihydroquercetin: arabinogalactan equal to 1: (3-100), preferably 1: (50-100) by weight, respectively, heating the solution to 40 -45°C, stirring for 0.5-1 hour, followed by drying the resulting solution by spraying, which reduces the duration of the method while maintaining the high quality of the target product.

The spray drying process is extremely fast (typically 15-30 seconds) and the particles of the composition in the zone of elevated temperatures have a saturated surface. Thanks to the instant drying and the low temperature of the atomized particles of the complex, the dried product is of good quality, because there is no violation of the native properties of dihydroquercetin and arabinogalactan, which is of particular importance for the chemical and pharmaceutical industry.

In addition, since the mixing of substances is carried out in the liquid phase, preferably in deionized water, then there is a uniform distribution of substances throughout the volume of the solvent, resulting in the homogeneity of the resulting complexes.

When spray drying is used, the technological cycle for obtaining the finished dry complex of dihydroquercetin with arabinogalactan is significantly reduced and completely mechanized. In this case, the processes of crystallization, precipitation, filtration, centrifugation, drying,

grinding, grinding the finished product, which significantly reduces energy costs.

The material to be dried during the drying process does not come into contact with the surfaces of the dryer until it is dry. This simplifies the solution of the problem of corrosion and the choice of material for the drying chamber. For other drying methods, damp
product comes into contact with metal surfaces.

The technical and economic performance of the spray drying method can be significantly improved by intensifying the evaporation process in spray dryers. As practice has shown, when drying highly dispersed materials, it is possible to significantly intensify the process, as a result of which the dimensions of the installation and the costs of electricity and heat are reduced. The present invention is illustrated by the following examples. Example 1. Obtaining complexes based on dihydroquercetin Arabinogalactan and dihydroquercetin were added to containers containing deionized water, taken in mass ratios equal to 1:3, 1:5, 1:10, 1:20, 1:30, 1:50 and 1:100, respectively, the solution was heated to 40-45°C, stirred for 0.5-1 hour and received aqueous solutions of DHQ/AG complexes, which were then subjected to physico-chemical methods of analysis.

The commercial product Lavitol (dihydroquercetin) was used as dihydroquercetin, batch No. 700v dated August 23, 2011, TU 9325-001-70692152-07), and as arabinogalactan, the commercial product Lavitol-arabinogalactan, batch No. 44 dated 09.11.2011, TU 9325-008-70692152-08). The results of the study of the composition of the obtained complexes, including water, in wt.%, before the drying process by spraying, are presented in table 1.

Таблица 1

Массовые соотношения ДКВ:АГ	Масса деионизированной воды, кг	Масса АГ в растворе, кг	Масса ДКВ в растворе, кг	Вес. %		
				деионизированной воды	АГ	ДКВ
1:3	48,00	9,00	3,00	80,0	15,0	5,0
1:5	49,20	9,00	1,80	82,0	15,0	3,0
1:10	50,10	9,00	0,90	83,5	15,0	1,5
1:20	50,55	9,00	0,45	84,25	15,0	0,75
1:30	50,70	9,00	0,30	84,5	15,0	0,5
1:50	50,82	9,00	0,18	84,7	15,0	0,3
1:100	50,91	9,00	0,09	84,85	15,0	0,15
Общая масса раствора перед высушиванием 60 кг						

From the data in Table 1 it can be seen that in order to obtain the complex of the desired ratio it is possible to vary the wt.% dihydroquercetin and the liquid phase at constant wt.% arabinogalactan.

Example 2. Obtaining a complex of dihydroquercetin with arabinogalactan in ratio 1:3

Complex of dihydroquercetin with arabinogalactan in a ratio of 1:3 by weight respectively received as follows. In a container containing deionized water with a volume of 48.0 l, arabinogalactan powder weighing 9.00 kg was dissolved. After complete dissolution of arabinogalactan, 3.00 kg of dihydroquercetin powder was added to the solution, the solution was heated to 40°C, stirring was carried out for 1 hour, then the resulting solution was dried by spraying. Drying of the complex was carried out by spraying in a GLP-60 dryer.
with centrifugal spray (SRC type), equipped with a paddle (flat working surface) high-speed disc. The residence time of the dried material in the dryer chamber is 1.5-2.0 sec, the temperature of the supplied air at the inlet is 180-200°C, at the outlet 70-80°C. The result was a water-soluble complex containing, wt.%:

dihydroquercetin	22,8
arabinogalactan	75,2
water	2,0

Example 3. Obtaining a complex of dihydroquercetin with arabinogalactan in ratio 1:20

Complex of dihydroquercetin with arabinogalactan in the ratio of 1:20 by weight respectively received as follows. In a container containing deionized water with a volume of 50.55 l, arabinogalactan powder weighing 9.00 kg was dissolved. After complete dissolution of arabinogalactan, dihydroquercetin powder weighing 0.45 kg was added to the solution, the solution was heated to 45°C, stirring was carried out for 0.5 hour, then the resulting solution was dried by the method

spraying as in example 2.

The result was a water-soluble complex containing, in wt.%:

dihydroquercetin	4,4
arabinogalactan	93,1
water	2,5

Example 4. Obtaining a complex of dihydroquercetin with arabinogalactan in ratio 1:100

Complex of dihydroquercetin with arabinogalactan in a ratio of 1:100 by weight respectively received as follows. In a container containing deionized water with a volume of 50.91 l, arabinogalactan powder weighing 9.00 kg was dissolved. After complete dissolution of arabinogalactan, dihydroquercetin powder weighing 0.09 kg was added to the solution, the solution was subjected to heating to 45°C, stirring was carried out for 0.5 hour, then the resulting solution was dried by spraying. The result was a water-soluble complex containing, in wt.%:

dihydroquercetin	1,0
arabinogalactan	96,8
water	2,2

Complexes of arabinogalactan with dihydroquercetin in other ratios.

Example 5. Study of the composition of the complexes

obtained Identification and quantitative content of dihydroquercetin in the complex was determined by HPLC using the Milichrome A-02 instrument (ZAO Ekonova, Novosibirsk), 290 nm, gradient mode, a mixture of acetonitrile: water was used as an eluent, caffeine was used as an internal standard , as an external standard - a standard sample of dihydroquercetin from Sigma-Aldrich. Data processing was carried out using the software "Multichrome". Chromatograms of DHQ standard sample (a) and DHQ:AG complex (1:3) (b) c

using the internal standard of caffeine are presented in the drawing. Peak dihydroquercetin included in the composition, on the chromatogram corresponds to the peak of the standard sample. Each complex obtained in the above ratios was analyzed by chromatography under similar conditions.

The content of AG in the composition was determined by the photometric method by reaction with anthrone in an acidic medium. The obtained data on the composition of the obtained water-soluble complexes are shown in Table 2.

Таблица 2

Массовые соотношения ДКВ:АГ	Количественное содержание арабиногалактана, %	Количественное содержание дигидрокверцетина, %	Суммарное содержание АГ и ДКВ в комплексе, %
1:3	75,2	22,8	98,0
1:5	80,1	14,9	95,0
1:10	86,6	8,7	95,3
1:20	93,1	4,4	97,5
1:30	94,6	3,2	97,8
1:50	95,5	1,9	97,4
1:100	96,8	1,0	97,8

It follows from Table 2 that when the complex is formed by spray drying, no new chemical bonds are formed, the chemical substances do not change in composition, but only intermolecular bonds are formed between the DHQ molecule and AG, which increase the DHQ solubility in water.

Example 6. Study of the water solubility of dihydroquercetin complexes with arabinogalactanoma

An experiment was carried out to study the water solubility of the resulting complexes. To do this, the samples were dissolved in 100 ml of distilled water at a temperature of 20°C on a magnetic stirrer (400 rpm). The dissolution was carried out until the added weights ceased to dissolve. The concentration of DHQ in the solution was determined by the photometric method.

Таблица 3

Массовые соотношения ДКВ:АГ	Способ получения	Растворимость ДКВ в воде, г/л	Увеличение растворимости ДКВ, (~ количество раз)
1:3	метод распылительной сушки	7,1	11,8
1:5		8,0	13,3
1:10		9,5	15,8
1:20		11,3	18,3
1:30		11,7	19,5
1:50		12,0	20,0
1:100		13,5	22,5
Растворимость ДКВ в воде – 0,6 г/л			

Table 3 shows that with an increase in the content of AG in the complex, the solubility DKV increases.

Example 7. Study of anti-inflammatory activity of complexes of dihydroquercetin with arabinogalactan

Anti-inflammatory activity was studied on the model of acute exudative inflammation in mice caused by sublantal injection of 0.04 ml of 2% formalin solution into the left hind paw. The studied complexes were administered intragastrically in water purified 1 hour before the administration of formalin. The group of control animals received an equivalent amount of purified water. 3 hours after the injection, the weight of the left and right hind legs was measured, and the difference between them was used to judge the severity edema.

The data obtained on the effect of a single intragastric administration of the studied complexes on the increase in the mass of the left paw relative to the right paw (ÿ, %) are presented in Table 4.

Таблица 4

Группа (n=7)	Δ, %
Контроль	57,7±6,2
ДКВ, 100 мг/кг	54,5±5,4
(1:3), 400 мг/кг	39,1±3,3
(1:5), 600 мг/кг	39,7±3,1
(1:10), 1100 мг/кг	40,0±2,7
АГ, 100 мг/кг	46,9±4,5

Table 4 shows that the complex of dihydroquercetin with arabinogalactan in the ratio (1:3), (1:5) and (1:10) showed greater anti-inflammatory activity than pure dihydroquercetin.

Example 8. Study of the angioprotective activity of complexes of dihydroquercetin with arabinogalactan

The study of angioprotective activity was carried out by reaction to xylene, which was administered to rats in an amount of 0.02 ml intradermally into the depilated abdominal area 10 minutes after an intravenous injection of 2 ml/kg of 1% Evans blue solution. The criterion for vascular permeability was the time (s) between the introduction of xylene and the appearance of the first signs of skin coloration. The test substances were administered intragastrically for 7 days in purified water. The group of control animals received an equivalent amount of purified water. The last administration of substances was carried out 1 hour before the injection of Evans blue solution. Data on the effect of course intragastric administration of complexes on capillary permeability in rats are shown in Table 5.

Таблица 5.

Группа (n=5)	Время выхода красителя, с
Контроль	105±2
ДКВ, 50 мг/кг	113±4
(1:3), 200 мг/кг	128±10
(1:5), 300 мг/кг	124±8
(1:10), 550 мг/кг	119±8
АГ, 250 мг/кг	95±2

From the data in Table 5 it follows that the studied complexes of dihydroquercetin with arabinogalactan have pronounced angioprotective properties.

EFFECT: invention makes it possible to increase the solubility of dihydroquercetin in water up to 11.8-22.5 times, to improve its bioavailability by increasing the absorption of the complex, as a result of which it becomes possible to reduce the dose of the dihydroquercetin drug while maintaining its pharmacological activity.

The invention also makes it possible to significantly reduce the duration of the method obtaining the complex while maintaining the high quality of the target product.

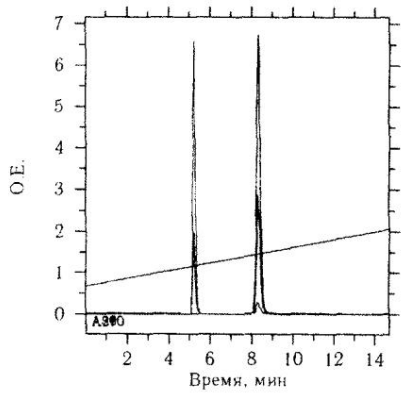
Claim

1. Supramolecular complex with anti-inflammatory and angioprotective activity, including dihydroquercetin, arabinogalactan and water, with the following content of components, wt.%:

dihydroquercetin	1,0-22,8
arabinogalactan	75,2-96,8
water	2,0-5,0

2. The method of obtaining a supramolecular complex according to claim 1, including mixing arabinogalactan with water until complete dissolution, then adding dihydroquercetin, heating the solution to 40-45°C, stirring for 0.5-1 hour, followed by drying the resulting solution by spraying.

а



б