Research to develop quality control methods for collection for treatment pyelonephritis

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SUMMARY

As a result of the studies, a method was developed using TLC analysis to establish the authenticity of the collection and a method for the quantitative determination of the amount of phenolic compounds in the collection using spectrophotometry and RSO of luteolin-7-glucoside. A nomenclature of numerical indicators has been developed to control the quality of the collection for the treatment of pyelonephritis.

Key words: quality control, collection.

One of the directions of pharmaceutical science is the expansion of the range and the search for new drugs of natural origin.

The solution to this problem is possible by studying the raw materials of promising traditional medicine plants and developing effective and low-toxic collections [6]. Urinary tract infections are among the most common diseases in both outpatient and nosocomial practice.

Acute pyelonephritis is the most common kidney disease in all age groups.

For the treatment of diseases of the urinary tract, antibiotics are widely used, the use of which is always associated with a certain risk associated not only with the main, but also with concomitant diseases of the patient.

The use of fees for the treatment of diseases of the urinary tract is not only justified, but also highly safe, because water extracts from them can be used for a long time and at the same time do not cause complications associated with their use.

All of the above was the basis for the development of an effective and low-toxic multicomponent collection, the infusion of which is recommended for the treatment of pyelonephritis [1, 3, 4].

Studies of the collection infusion for the treatment of pyelonephritis on various experimental models have shown that it has a pronounced diuretic effect, has an antimicrobial, anti-inflammatory and capillary-protective effect, and has some analgesic effect.

The method of using the water extract from the collection was tested in the urology clinic of the I.M. Sechenov.

The composition of the collection and its purpose are protected by a patent of the Russian Federation.Objective of the study: determination of criteria for quality control and development of regulatory documents for collection for the treatment of pyelonephritis.

To solve this problem, we carried out:

- development of a methodology for the qualitative determination of the main groups biologically active substances contained in the components of the collection;

- development of a method for the quantitative determination of the amount of phenolic connections;

- the establishment of merchandising indicators ("numerical indicators"), characterizing the quality of the collection.

To establish the presence in the collection of the main groups of biologically active substances (BAS), specific for each component of the collection, a method of chromatography in a thin layer of sorbent (TLC) is proposed. Determination of natural compounds in medicinal plant materials by TLC has a number of advantages, which include selectivity and high sensitivity.

In the course of the development of the technique, studies were carried out to select the optimal chromatographic conditions, which made it possible to clearly separate and identify biologically active substances specific for each of the types of medicinal plant materials included in the collection [5, 7, 8, 9].

Technique for qualitative TLC analysis of the collection

An analytical collection sample is ground to a particle size passing through a sieve with holes 2 mm in diameter, thoroughly mixed and a sample (about 1.00 g) is placed in a 50 ml conical flask, 20 ml of 70% ethanol is added and boiled on an electric stove with an asbestos mesh within 30 minutes. with a reflux condenser. After cooling the extract to room temperature, it is filtered through a paper filter.

To the start line of the plate "Silufol UV_{254}"Or" Sorbfil - PTSKh - PA - UF "(Russia) 10 x 10 cm in size with a micropipette apply 0.02 ml of the obtained filtrate in the form of a strip 3 cm long.

The plate with the applied sample is dried in air for 15 minutes, then placed in a chamber, which is pre-saturated for 2 hours with a mixture of chloroform: methanol: water (71: 3: 7) and chromatographed in an ascending manner. When the front of the mobile phase reaches the end of the plate, the plate is removed from the chamber and dried in a fume hood for 15 minutes. Then the chromatogram is sprayed (treated) with a 3% alcoholic solution of aluminum chloride, after which it is heated in an oven for 10 min at a temperature of 100–105 ° C and viewed in UV light at a wavelength of 366 nm.

The chromatogram should show at least 14 bands of substances with different colors of fluorescence: with Rf about 0.18, 0.76, 0.86 - yellow, blue, violet, respectively, which are the predominant BAS in the rhizomes and roots of Eleutherococcus spiny; with an Rf of about 0.36; 0.45; 0.54; 0.57 - gray, yellow, blue, yellow, respectively, which are the main biologically active substances contained in bearberry leaves; with Rf about 0.20 - yellow - a substance specific to sweet clover grass; with an Rf of about 0.31; 0.73; 0.81 - yellow, purple, brown, respectively, which are the main biologically active substances contained in the raw material of licorice; with an Rf of about 0.1 - a bright blue color - a compound characteristic of peppermint leaves.

The basis for determining the content of the sum of phenolic compounds in terms of luteolin-7-glucoside in the collection is based on the spectrophotometric method using a bathochromic shift reagent [2].

Spectrophotometric method for quantifying the amount
complete phenolic compounds for the treatment of pyelonephritisAn analytical sample of the collection is ground to a particle size passing through a sieve with holes of 2 mm in diameter. Then about 2.00 g (accurately weighed) of the collection is placed in a flask with a thin section with a capacity of 250 ml, add 100 ml of 70% ethyl alcohol and weighed. The flask is connected to a reflux condenser, heated in a boiling water bath for 2.5 hours. Then the flask with its contents is cooled to room temperature, weighed and brought to the initial weight with 70% ethyl alcohol.

The extract is filtered through a filter paper into a 250 ml flask, discarding the first 20 ml of the filtrate (solution A). In a volumetric flask with a capacity of 25 ml, place 1 ml of solution A, 0.5 ml of acetic acid diluted with 2 ml of a 3% alcohol solution of aluminum chloride and bring the volume of the solution to the mark with the same alcohol, mix (solution B).

After 40 minutes, the absorption of solution B is measured on a spectrophotometer at a wavelength of 410 nm in a cuvette with a layer thickness of 10 mm against the background of a reference solution. As a reference solution, a solution consisting of 1 ml of extraction (solution A), 0.5 ml of diluted acetic acid and brought to the mark with 70% ethyl alcohol in a 25 ml volumetric flask is used.

At the same time, the absorption of 0.0025% Luteolin-7-glucoside PCO solution prepared similarly to the test solution is measured. The content of the sum of phenolic compounds (X) in terms of luteolin-7-glucoside and absolutely dry raw materials is calculated by the formula:

$$\frac{Д_1 \times m_0 \times 100 \times 25 \times 10 \times 100 \times 100}{Д_0 \times m_1 \times 1 \times 100 \times 25 \times (100 - W)} = \frac{Д_1 \times m_0 \times 100 \times 100}{Д_0 \times m_1 \times (100 - W)}$$

where D_1 Is the optical density of the test solution;

D_0 - the optical density of the Luteolin-7-glucoside PCO solution; m_1 - the mass of the collection, g;

m_0 Is the mass of the RSO of luteolin-7-glucoside, g; W is the loss in mass during drying of the collection,%.

Note. Preparation0.0025% solution of RSO luteolin-7-glucoside. 0.0025 g (accurately weighed) of the PCO luteolin-7-glucoside, dried to constant weight, is dissolved in 40 ml of 95% ethanol in a 100 ml volumetric flask when heated to 60 ° C, cooled to room temperature and the volume of the solution is brought to with the same alcohol up to the mark and mix.

The shelf life of the solution is 3 months. In addition to the content of the sum of phenolic compounds, two more indicators were proposed to control the quality of the collection, determined according to well-known pharmacopoeial methods: the content of extractives extracted by water (GF XI edition, issue 1, p. 295 with the following addition - the weight of the sample of collection 5 g (with with an error of ± 0.01 g) and the content of tannins in terms of tannin (GF XI edition, issue 1, p. 286 with

by the following addition: the collection is removed from the sample for 1 h).

When developing regulatory documents for collection for the treatment of pyelonephritis, the following nomenclature and norms of numerical indicators characterizing the quality of collection were proposed: the sum of phenolic compounds in terms of luteolin-7-glucoside - not less than 0.8%; extractives extracted by water - not less than 35%, tannins - not less than 9%, moisture - not more than 14%, total ash - not more than 7%, ash insoluble in 10% hydrochloric acid solution - not more than 3%, particles that do not pass through a system with holes with a diameter of 5 mm - no more than 8; particles passing through a sieve with holes of 0.25 mm - no more than 5%, organic impurities - no more than 1%, mineral impurities - no more than 1%.

conclusions

1. A technique has been developed using TLC analysis to establish the authenticity of the collection for the treatment of pyelonephritis.

2. A method has been developed for the quantitative determination of the amount of phenolic compounds assembled using spectrophotometry and PCO luteolin-7-glucoside.

3. A nomenclature of numerical indicators has been developed and their norms have been proposed to control the quality of the collection.

Literature

1. Belikov V.V., Tochkova T.V. Phenolic compounds and their physiological properties. - Alma-Ata, 1973. - P. 168–172

2. Belikov V.V., Schreiber M.S. Methods for the analysis of flavonoid compounds // Pharmacy. - 1970. - No. 1. - S. 66–72.

3. Blazhiy A .; Shutyy L. Phenolic compounds of plant origin. - M., 1970 .-- 240 p.

4. Georgievsky V.P., Komisarenko N.F., Dmitruk S.E. Biologically active substances of medicinal plants. - Novosibirsk: "Science". Siberian branch, 1990 .-- 333 p.

5. State Pharmacopoeia of the USSR. Issue 1. - General methods of analysis (MoH USSR) - 11th ed. add. - M .: Medicine, 1987 - 336 p.

6. State register of medicines. Official edition (by as of April 1, 2008) - M., 2008. - T. I. - 1398 p., T. II. - 1208 p.

7. Zaprometov M.N. Fundamentals of the biochemistry of phenolic compounds. - M .: Higher school, 1981 - 268 p.

8. Harborne J. Biochemistry of phenolic compounds. - M .: Mir, 1968 .-- 572 p.

9. Wagner H., Bladt S. Plant Drug Analysis. A Thin Layer Chromatography Atlas. Berlin; Heidelberg; New York; Springer - Verlag, 1995 .-- 384 s.

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