Development and validation of a methodology for assessing the authenticity of the Collection of choleretic No. 3

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SUMMARY

Nowadays, the standardization of herbal products is especially important. Modern requirements for regulatory documents for herbal medicinal products imply the mandatory inclusion of the section "Qualitative reactions" in the national quality standards for a medicinal product. This article proposes a new method for determining the authenticity of the collection of choleretic No. 3 by chromatography in a thin layer of sorbent, which makes it possible to identify 80% of the collection components by this method.

Key words: medicinal herbal collection, definitionauthenticity, chromatography in a thin layer of sorbent.

INTRODUCTION

Diseases of the gallbladder and biliary tract (primarily chronic cholecystitis and primary dysfunction of the biliary tract) are among the most common and severe diseases of the digestive system. For these diseases, choleretic agents are prescribed and, first of all, complex herbal remedies, the most interesting of which are herbal preparations. These drugs have undeniable advantages - they are effective and safe, do not cause addiction, their market prices are lower than for drugs of synthetic origin. Therefore, choleretic herbal preparations are of interest to all segments of the population, including the needy.

Nowadays, the standardization of herbal products is especially important. Modern requirements for regulatory documents for herbal medicinal products imply the mandatory inclusion of the section "Qualitative reactions" in the national quality standards for a medicinal product. In the current documentation for Collecting Choleretic No. 3 [1], the section "Qualitative reactions" includes a method for determining rutin, hyperoside and quercetin by TLC. However, this technique does not allow to establish the authenticity (the presence of certain components) of the collection, but allows to establish the presence of only three flavonoids widespread in nature.

The purpose of our work was to develop a method for the detection of components in the Choleretic Collection No. 3 and its validation.

MATERIALS AND METHODS

The objects of the study were model and industrial samples of the Collection of Choleretic No. 3. Thin-layer chromatography was performed on plates

"TLC Silica gel 60 F254" Aluminum sheets (MERCK, Germany) 100 x 100 mm in size.

During the development of the method, the optimal mixtures for the extraction of substances from the collection were investigated and selected: 1. Ethyl alcohol 96%; 2. Ethyl alcohol 96% - toluene (4: 1); 3. Ethyl alcohol 96% - toluene (1: 1).

The optimal separation conditions were also selected, including the analysis of the mobile phases used in the analysis of raw materials containing phenolic compounds [2–4]: ethyl acetate - anhydrous formic acid - water (65:15:20); ethyl acetate - glacial acetic acid - water (5: 1: 1); ethyl acetate - toluene - anhydrous formic acid - water (60: 20: 10: 4); ethyl acetate - toluene - glacial acetic acid - water (60: 20: 10: 4); ethyl acetate - toluene - anhydrous formic acid - water (60: 10: 10: 4); ethyl acetate - toluene - anhydrous formic acid - water (35: 5: 6: 4).

A 0.5% rutin solution and a 0.5% quercetin solution in ethyl alcohol 96% were used as reference solutions, the zones of which served as a marker on the chromatogram. A 1% solution of diphenylboryloxyethylamine in ethyl alcohol 96% and a 5% solution of polyethylene glycol were used as a detecting reagent.

In addition, we analyzed the mobile phases used in the analysis of raw materials containing lipophilic compounds [2–4]: 1. Toluene - ethyl acetate (95: 5); 2. Toluene - ethyl acetate - glacial acetic acid - anhydrous formic acid (38: 2: 3: 2); 3. Toluene - ethyl acetate - glacial acetic acid - anhydrous formic acid (38: 1: 3: 2); 4. Toluene - ethyl acetate - glacial acetic acid - anhydrous formic acid (38: 0.5: 3: 2).

A 0.02% solution of Sudan III in toluene and a 0.1% solution of menthol in ethyl alcohol 96% were used as reference solutions, the zones of which served as a marker on the chromatogram. A 0.5% solution of anisic aldehyde was used as a detecting reagent.

RESULTS AND DISCUSSIONThe studies carried out made it possible to establish that the best separation of phenolic compounds of the Choleretic collection No. 3 is achieved in the system ethyl acetate - toluene - anhydrous formic acid - water (35: 5: 6: 4) in the case of extraction of biologically active substances from the collection with a mixture of ethyl alcohol 96% - toluene (4: 1).

After the solvent front passed a distance of 8 cm from the start line, the plate was removed from the chamber, dried under a draft (at room temperature) until traces of solvents were removed, and viewed in UV light at a wavelength of 365 nm. On the chromatogram of a solution of standard samples (CO) of rutin and quercetin, a zone of yellow, green-yellow or yellow-green color with R_f about 0.85 (quercetin) taken as $R_s = 1.0$.

The collection chromatogram showed a yellow-green or green zone with R_s about 0.5 (quercetin), blue zones with R_s about 0.8 and 0.9, purple zone with R_s about 1.0 (the zone corresponds to chamomile flowers). Then the plate was heated at 100–105-C for 5–10 minutes and the warm plate was treated

successively 1% solution of diphenylboryloxyethylamine in ethanol and 5% solution of polyethylene glycol. After 15 minutes. after treatment, the plate was viewed under UV light at a wavelength of 365 nm.

A yellow zone with R_f about 0.85 (quercetin) taken as R_s = 1.0 and a yellow zone with R_s for quercetin about 0.2 (rutin).

The collection chromatogram showed a yellow-green or green zone with R_s (for quercetin) about 0.05 (zone corresponds to calendula flowers), zones of greenish-yellow, yellow-orange or orange color with R_s about 0.45 and 1.0; blue zones with R_s about 0.35, 0.6, 0.8 and 0.9 (zone corresponds to peppermint leaves). The presence of a yellow-orange or orange zone is allowed R_s (quercetin) about 0.2 and the red zone with R_s about 0.25; as well as the presence of other zones. A photograph of the chromatogram of phenolic compounds of the model collection mixture and individual collection components is shown in Fig. 1. Due to the fact that the composition of the Choleretic Collection No. 3 includes 5 components (Chamomile flowers, Peppermint leaves, Marigold flowers, Yarrow herb and Tansy flowers), and the above technique allows identifying the presence of only three components (Chamomile flowers, Mint pepper leaves, Marigold flowers), we proposed to determine the authenticity of the components of the collection for lipophilic substances by TLC. The studies carried out made it possible to establish that the best separation of lipophilic compounds of the Choleretic Collection No. 3 is achieved in the system toluene - ethyl acetate - glacial acetic acid - anhydrous formic acid (38: 0.5: 3:

After the solvent front passed a distance of 8 cm from the starting line, the plate was taken out of the chamber and dried under a draft (at room temperature) until traces of solvents were removed.

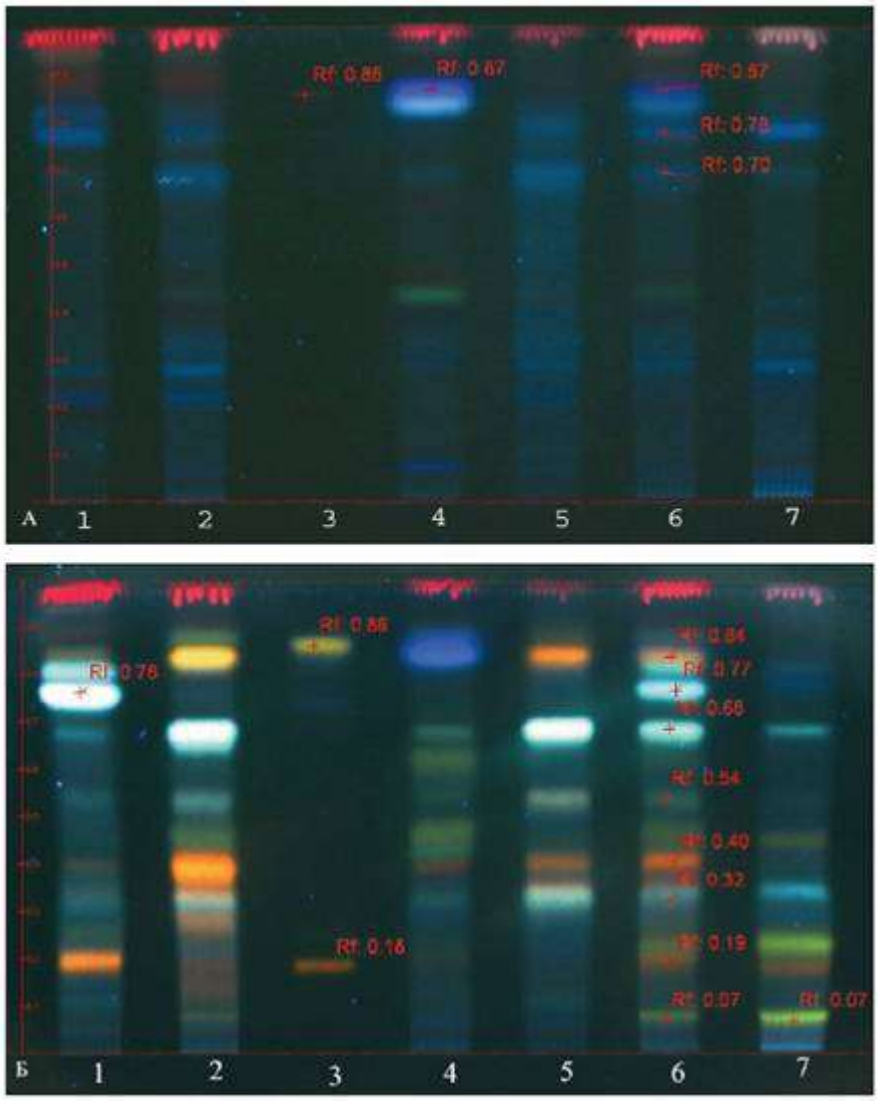
Then the chromatogram was treated with a solution of anisaldehyde and heated in an oven for 2–3 min. at 100-105-C. The chromatogram was viewed immediately in daylight.

On the chromatogram of a solution of CO of Sudan III and menthol, a zone of violet, violet-blue, blue or light blue color with R_f about 0.6 (Sudan III), taken as R_s = 1.0 and the zone is purple with an Rs of about 0.65 (menthol). On the collection chromatogram, zones of violet color with R_s about 0.65; 0.75; 0.80 and 1.40, pale purple zone with R_s about 1.20 (zone corresponds to yarrow grass).

A photograph of the chromatogram of lipophilic compounds of the model collection mixture and individual collection components is shown in Fig. 2. The proposed method allows to identify in the Choleretic Collection No. 3 the herb of yarrow among the 5 components of the collection. The developed methods were validated according to the specificity and suitability of the chromatographic system. The specificity of the methods was assessed by the coincidence of the chromatographic profiles of different collection series, by the main zones with each other, and their correspondence to the description of the method. The number of test collection series was at least three. Chromatographic profiles of different series coincided in the main zones between

themselves and correspond to the description of the method. As an indicator of the suitability of the chromatographic system in the case of identification of phenolic compounds, we chose the resolution between the characteristic zones of standard samples of rutin and quercetin with Rs about 0.2 and Rs about 1.0, respectively. The resolution value between the indicated zones was at least 10.0.

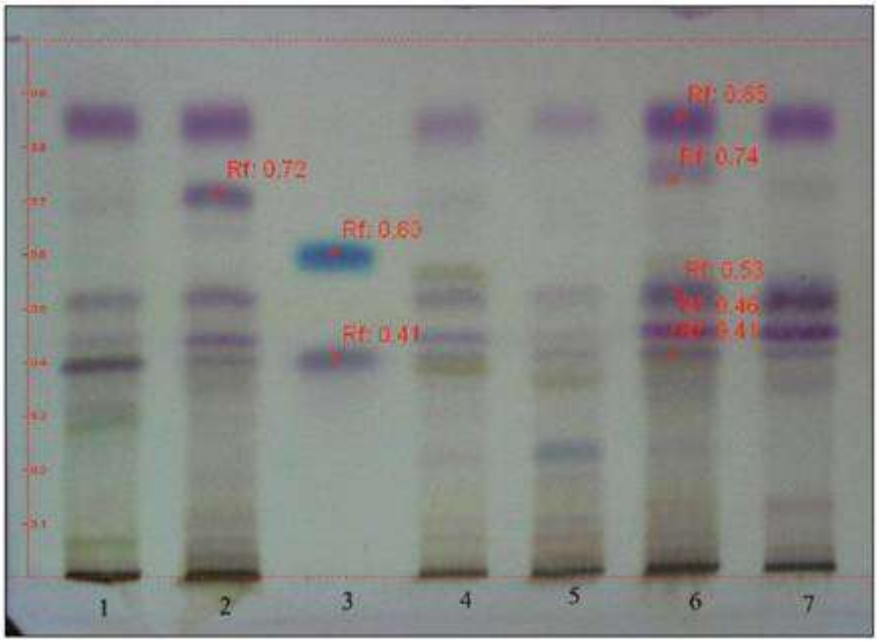
As an indicator of the suitability of the chromatographic system in the case of identification of lipophilic substances, we chose the resolution between the characteristic zones of standard samples of Sudan III and menthol with Rs about 1.0 and Rs about 0.65; respectively. The resolution value between the indicated zones was not less than 2.0.



Rice. 1. Photographs of the chromatogram of phenolic compounds of the model collection mixture and collection components.

A - chromatogram in UV light before treatment with 1% diphenylboryloxyethylamine solution in ethanol and 5% polyethylene glycol solution.
B - chromatogram in UV light after treatment with 1% diphenylboryloxyethylamine solution in ethanol and 5% polyethylene glycol solution:
1. Peppermint leaves;
2. Yarrow herb;
3. Rutin and quercetin;
4. Chamomile flowers;
5. Flowers of tansy;
6. Model mixture of collection of Cholagogue No. 3;
7. Calendula flowers.

Solvent system: ethyl acetate - toluene - anhydrous formic acid - water (35: 5: 6: 4); plate "TLC Silica gel 60 F254" Aluminum sheets (MERCK, Germany); solvent travel distance 8 cm.



Rice. 2. Photo of the chromatogram of lipophilic compounds of the model mixture collection and collection components in daylight:
1. Peppermint leaves;
2. Yarrow herb;
3. Rutin and quercetin;
4. Chamomile flowers;
5. Flowers of tansy;
6. Model mixture of collection of Cholagogue No. 3;
7. Calendula flowers.

Solvent system: toluene - ethyl acetate - glacial acetic acid - anhydrous formic acid (38: 0.5: 3: 2); plate "TLC Silica gel 60 F254" Aluminum sheets (MERCK, Germany); solvent travel distance 8 cm

CONCLUSIONS

1. A method for the determination of phenolic compounds has been developed and validated. Collected Choleretic TLC, which allows the detection of 3 components of the collection - Chamomile flowers, Peppermint leaves, Marigold flowers. The results obtained were included in the draft FSP Fitohepatol® No. 3 of Krasnogorskleksredstva OJSC.

2. A method for the determination of lipophilic substances in Collecting by the choleretic method TLC, which allows detecting 1 component of the collection - Yarrow herb. The results obtained were included in the draft FSP Fitohepatol® No. 3 of Krasnogorskleksredstva OJSC.

3. Tansy flowers were not identified by either of the two methods. their content in the collection is 8% and their biologically active substances are masked by the components of other components of the collection.

LITERATURE

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