TLC analysis as a method for assessing the authenticity of medicinal plants fees

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TLC as a method for herbal mixtures identification

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RESUME

According to the modern requirements for the standard documentation for the herbal medicinal products, the national quality standards must contain issue Identification. There is no entry "Qualitative analysis" in the actual documentation on some herbal mixtures. It was shown the possibility to identify up to 100% of components in the herbal mixtures by TLC.

Keywords: herbal mixtures, TLC.

SUMMARY

Modern requirements for regulatory documents for herbal medicinal products imply the mandatory inclusion of the section "Qualitative reactions" in national quality standards. In the current documentation for some medicinal plant fees, the section "Qualitative reactions" is missing. This article shows the possibility of identifying up to 100% of the components of medicinal plant collections using TLC analysis.

Key words: medicinal herbs, TLC analysis.

INTRODUCTION

Multicomponent medicinal vegetable drugs for a long time are used in medical practice, due to their effectiveness, mild action, the absence in most cases of undesirable side effects with prolonged use, availability.

Modern requirements for regulatory documents for herbal medicinal products imply the mandatory inclusion of the section "Qualitative reactions" in national quality standards. And in this section, modern methods of analysis should be used, for example, chromatography in a thin layer of sorbent (TLC). In the current documentation for some medicinal plant fees, there is no section "Qualitative reactions" at all.

The purpose of our work was to consider the possibility of detecting components of some medicinal herbal collections.

MATERIALS AND METHODS

The object of study was model mixtures and industrial series of Antihemorrhoidal collection and Breast collection No. 4; the determination of the presence of certain components of the collection was carried out by determining the chromatographic profile of biologically active compounds by TLC.

RESULTS AND DISCUSSION

When developing methods for determining the authenticity of collections by TLC in order to select the optimal conditions for the extraction of substances from herbal medicinal preparations, the following extractants were analyzed: ethanol 96% - toluene - water (50: 100: 1); ethanol 96% - toluene (1: 1); ethanol 96% - toluene (4: 1); ethanol 96%.

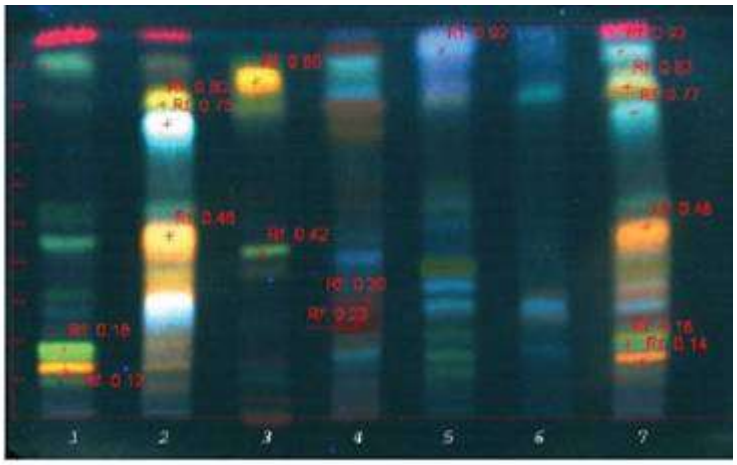
Also, to select the optimal conditions for the separation of biologically active substances in a thin layer of sorbent, the mobile phases used in the analysis of raw materials containing phenolic compounds were analyzed:

hexane - acetone - toluene (60:20:10); toluene - ethyl acetate (95: 5); 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 - glacial acetic acid (30: 10: 6: 3: 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 - glacial acetic acid - anhydrous formic acid - water (60: 20: 5: 5: 4); ethyl acetate - ethanol - water - anhydrous formic acid (77: 13: 10: 2); ethyl acetate - ethanol - water - toluene (50: 8: 2: 5); ethyl acetate - ethanol - water (77:13:10) [1-3].

The studies carried out made it possible to establish that the optimal extractant for the extraction of substances from the antihemorrhoidal collection is ethanol 96% and the best separation of phenolic compounds is achieved in the system ethyl acetate - ethanol 96% - water - anhydrous formic acid (77: 13: 10: 2) on chromatographic plates " TLC Silica gel 60 F254 »Aluminum sheets (MERCK, Germany). As reference solutions, we used solutions of standard samples of barbaloin and quercetin in ethanol, the zones of which served as markers on the chromatogram. A 1% solution of diphenylboryloxyethylamine in ethanol 96% and a 5% solution of polyethylene glycol in ethanol 96% were used as a detecting reagent.

After treatment successively with solutions of diphenylboryloxyethylamine and polyethylene glycol in UV light at 365 nm, two orange zones with R_s (for barbaloin) about 0.3 (senna leaves) and 1.1 (yarrow herb); green zone with R_s about 0.4 (senna leaves); brown-red zone with R_s about 0.5 (buckthorn bark); blue zone with R_s about 1.75 (yarrow herb); yellow-green zone with R_s about 1.8 (yarrow herb); zone blue or blue-cyan with R_s about 2.1 (licorice roots).

A photograph of the chromatogram of phenolic compounds of the model collection mixture and individual collection components is shown in Fig. 1.



Rice. 1. Photo of the chromatogram of phenolic compounds of the model collection mixture and collection components Antihemorrhoidal in UV light at a wavelength of 365 nm after treatment with solutions of diphenylboryloxyethylamine and polyethylene glycol

1. Senna leaves;
2. Yarrow herb;
3. Barbaloin and quercetin;
4. Buckthorn bark;
5. Licorice roots;
6. Fruits of coriander;
7. Model Antihemorrhoidal Collection Blend.

The antihemorrhoidal collection contains 5 components (Senna leaves, Yarrow herb, Licorice roots, Buckthorn bark and Coriander fruits), and the developed method allows identifying the presence of only fourcomponents (Senna leaves, Yarrow herb, Buckthorn bark, Licoriceroots). The developed technique does not allow identifying the presence of the fifth component - Coriander fruit, because phenolic compounds are practically absent in coriander fruits.

We made an attempt to determine the authenticity of the coriander fruit in the collection Antihemorrhoidal TLC method for lipophilic substances. The studies carried out to determine the lipophilic substances in this collection were unsuccessful: neither the selection of the extractant, nor the selection of the chromatographic system made it possible to establish the authenticity of coriander fruits by TLC in the presence of senna leaves, yarrow herb, buckthorn bark and licorice roots in the Antihemorrhoidal collection.

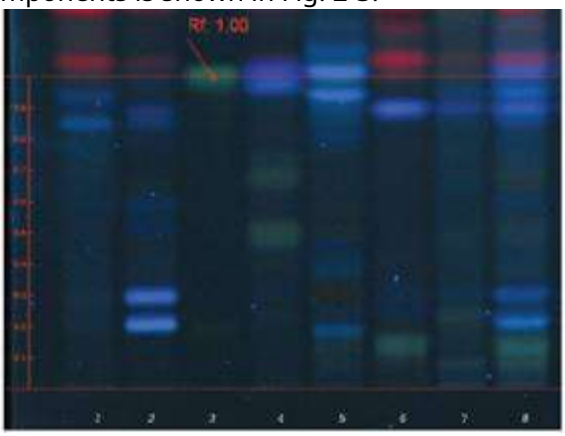
The conducted researches deigned to establish that the optimal mixture for the extraction of substances from the collection of Breast No. 4 is a mixture of ethanol 96% - toluene - water (50: 100: 1) and the best separation of phenolic compounds is achieved in the system ethyl acetate - toluene - anhydrous formic acid - water - glacial acetic acid (30: 10: 6: 3: 1) on chromatographic plates on chromatographic plates “TLC Silica gel 60 F254 »Aluminum sheets (MERCK, Germany), allowing to determine all components of the collection of the Breast

No. 4. We used as reference solutions0.5% solution of rutin in ethanol 96% and 0.5% solution of quercetin in ethanol 96%, the zones that served as a marker on the chromatogram. We proposed to use a 1% solution of diphenylboryloxyethylamine in ethanol 96% and a 5% solution of polyethylene glycol in ethanol 96% as a detecting reagent.

When viewing the chromatogram of the test solution in UV light at 365 nm, a violet zone with R_s (quercetin) about 1.0 (chamomile flowers).

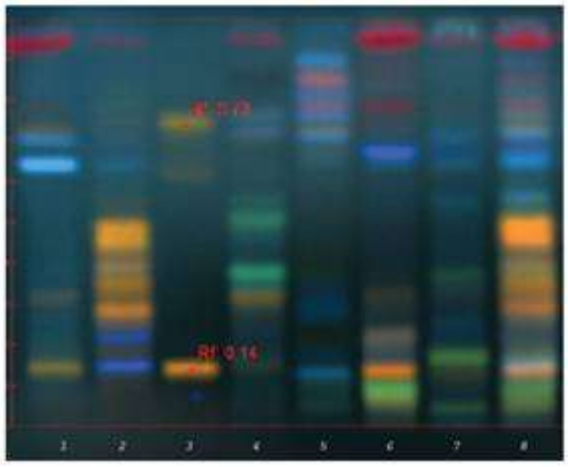
After treatment with a solution of diphenylboryloxyethylamine in UV light at 365 nm, green zones with R_s (for quercetin) about 0.05 (calendula flowers) and 0.1 (violet herb); orange colored zones with R_s about 0.3, and 0.6 (wild rosemary shoots); blue zones with R_s about 0.85 (peppermint leaves) and 1.2 (licorice roots); purple zone with R_s about 0.9 (violet herb).

A photograph of the chromatogram of phenolic compounds of the model collection mixture and individual collection components is shown in Fig. 2-3.



Rice. 2. Photo of the chromatogram of phenolic compounds of the model mixture of collection and components collection Pectoral No. 4 in UV light at a wavelength of 365 nm

1. Peppermint leaves;
2. Shoots of wild rosemary;
3. Rutin and quercetin;
4. Chamomile flowers;
5. Licorice roots;
6. Violet herb;
7. Calendula flowers;
8. Model mixture of collection of the Breast №4.

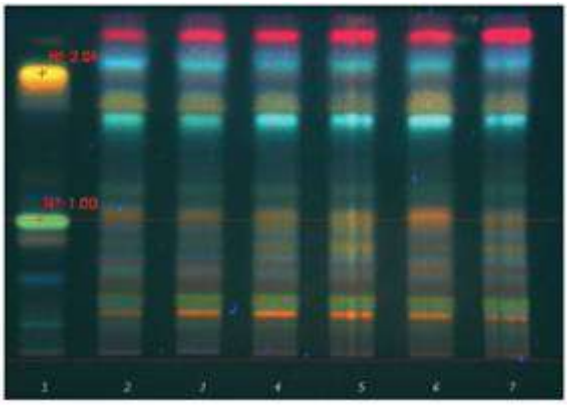


Rice. 3. Photo of the chromatogram of phenolic compounds of the model mixture of collection and components collection of Breast No. 4 in UV light at a wavelength of 365 nm after treatment with solutions diphenylboryloxyethylamine and polyethylene glycol

1. Peppermint leaves;
2. Shoots of wild rosemary;
3. Rutin and quercetin;
4. Chamomile flowers;
5. Licorice roots;
6. Violet herb;
7. Calendula flowers;
8. Model mixture of collection of the Breast №4.

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 series of samples, according to the main zones with each other, and their correspondence to the description of the method. The number of subjects in the collection series was at least three. Industrial collection batches were analyzed. Chromatographic profiles of different series coincided in the main zones with each other and correspond to the description of the methods.

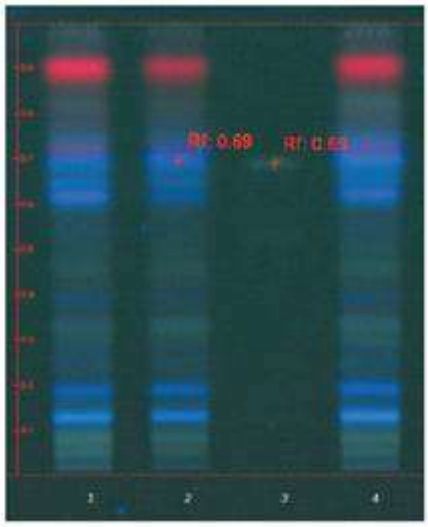
Photographs of chromatograms of industrial series of collections are shown in Fig. 4-6.



Rice. 4. Photo of the chromatogram of phenolic compounds of industrial collection series Proctophytol® (Antihemorrhoidal Collection) in UV light at a wavelength of 365 nm after treatment with solutions of diphenylboryloxyethylamine and polyethylene glycol

1. Barbaloin and quercetin;

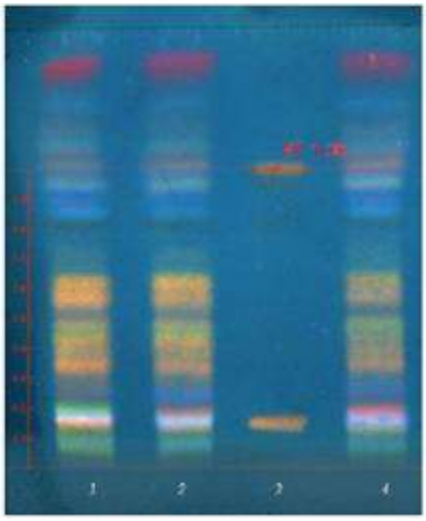
2. Series 50706;
3. Series 20807;
4. Series 51107;
5. Series 20308;
6. Series 70908;
7. Series 10209.



Rice. 5. Photo of the chromatogram of phenolic compounds of extracts from the industrial batch collection Pectoral No. 4 in UV light at 365 nm

1. Series 251108;
2. Series 271108;
3. Rutin and quercetin;
4. Series 121108.

The resolution between the characteristic zones of standard samples was chosen as an indicator of the suitability of chromatographic systems. The value of the resolution between the indicated zones was not less than 5.0 for the Hemorrhoidal collection, and not less than 15.0 for the Breast collection No. 4.



Rice. 6. Photo of the chromatogram of phenolic compounds of extracts from industrial seriescollection of Breast No. 4 in UV light at 365 nm after treatment with solutions of diphenylboryloxyethylamine and polyethylene glycol

1. Series 251108;
2. Series 271108;
3. Rutin and quercetin;
4. Series 121108.

Conclusions:

1. Methods for determining the authenticity of two fees, allowing to detect up to 100% of the components of the collection.

2. The obtained results are included in the section "Qualitative reactions" Projects of FSP OJSC Krasnogorskleksredstva.

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