Development of a TLC method for the detection of flavonoids and coumarins in matrix tinctures from raw materials of various types of wormwood

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Due to the species diversity of wormwood, some species of the genus Artemisia are of great interest as sources of homeopathic medicines. In particular, wormwood, wormwood, and wormwood are divine tree. Currently, there is no regulatory documentation for matrix tinctures and freshly harvested raw materials of all the listed types of wormwood.

Due to significant differences in the pathogenesis of monopreparations Artemisia absinthium (ABSINTHIUM), Artemisia vulgaris (ARTEMISIA VULGARIS), and Artemisia abrotanum (ABROTANUM) [6], it is of practical interest to develop criteria for assessing the authenticity of matrix tinctures from raw materials of different types of wormwood. diagnostics of objects.

Thin layer chromatography (TLC) remains the generally accepted pharmacopoeial method in the study and analysis of the chemical composition of medicinal plants (MP) and drugs (MP) [3, 4, 10, 11, 12].

The aim of this study was to develop a TLC technique for detecting the main groups of biologically active substances (BAS) in homeopathic matrix tinctures from freshly harvested raw materials Artemisia absinthium, Artemisia vulgaris and Artemisia abrotanum.

The initial raw material for the preparation of matrix tinctures from raw materials of different types of wormwood in homeopathy is the aerial part of plants. In accordance with the modern pharmacognostic terminology adopted in the Russian Federation, the aerial part is understood as freshly harvested grass, leaves, flowers, stems, or their mixture. Matrix tinctures from freshly harvested raw materials of all studied types of wormwood were prepared according to the method described in the general FS "Matrix homeopathic tinctures" [2], in accordance with method 3a. The stocking of raw materials was carried out in the phase of budding and the beginning of flowering on the territory of Moscow and the Moscow region in 2006. According to the literature, the main groups of biologically active substances contained in all three studied species of wormwood are flavonoids and coumarins [1, 7, 8, 9].

1. Development of a TLC method for the determination of flavonoids

The following systems are most often used for TLC of flavonoids with various sorbents: 1) chloroform: methanol - 8: 3; 2) chloroform: methanol: water - 60: 35: 7; 3) ethyl acetate: acetic acid: water - 5: 1: 1; 4) ethyl acetate: methanol: water - 100: 17: 13; 5) butanol-1: acetic acid: water - 4: 1: 2; 6) benzene: acetic acid: water - 115: 72: 3; 7) benzene: methanol - 8: 2; 8) benzene: acetic acid - 5: 2; 9) 15% acetic acid; 10) chloroform: toluene: 95% alcohol - 15: 2: 3 [7, 8, 9].

Our research made it possible to establish that the best separation is achieved in the following systems: benzene: ethyl acetate - 2: 1; chloroform: methanol: water - 60: 35: 7; benzene: hexane: ethyl acetate - 3: 4: 2.

During chromatography in each of the three systems, adsorption zones of compounds were found in all the samples under study, which we presumably referred to as flavonoids (according to their characteristic absorption in visible light in UV light), which, according to the literature, are quantitatively dominant group of biologically active substances.

In this case, the largest number of adsorption zones was found on chromatographic plates during analysis in the chloroform: methanol: water - 60: 35: 7.

Chromatography was carried out in pre-saturated sandwich chambers (saturation time 30 min.) On Sorbfil plates (sorbent type STX-1A silica gel, ZAO Sorbpolymer, Russia) with dimensions of 100x150 mm.

At the next stage of the research, an attempt was made to identify the detected compounds of a flavonoid nature using rutin and quercetin as "witnesses" of GSO, since the literature contains information about the presence of these flavonoids in the raw materials of the studied species of wormwood [1, 7, 8, 9]. To detect the compounds under study, we used freshly prepared alcoholic solutions of GSO rutin and quercetin.

Methodology

On the start line of the pre-washed chromatographic plate Sorbfil is applied in the form of a strip 1 cm long or pointwise 10 µl of a sample of tincture of the studied species of wormwood. When chromatography in the presence of "witnesses", solutions of witnesses are applied to the start line at a distance of 1 cm from each other and from the applied sample. The plate is allowed to dry at room temperature.

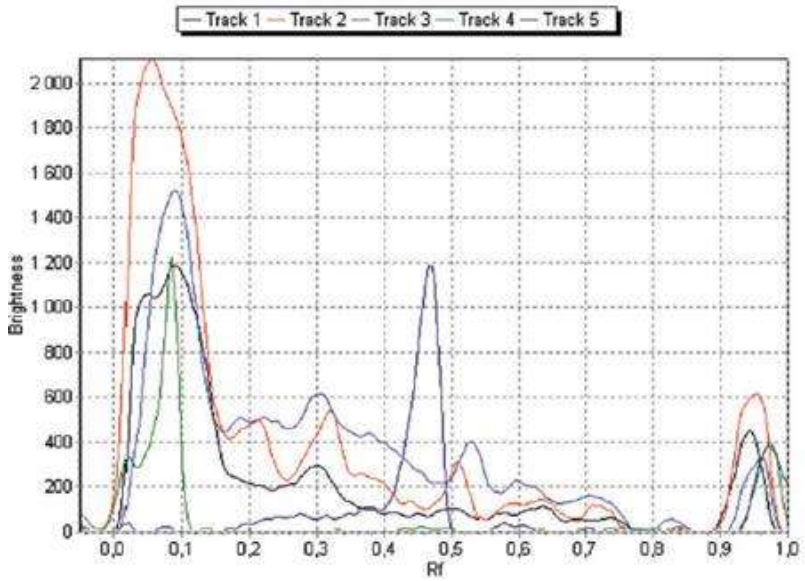
The dried plate is carefully placed in a pre-saturated chamber. After the front of the solvents has passed at least 8 cm, the plate is removed, the position of the front is noted and left under traction until dry. The chromatogram is then viewed in visible and UV light at 365 nm. After that, the chromatogram is treated with a 2% solution of aluminum chloride and dried in air (about 15 minutes). The chromatogram is viewed again after UV light treatment at 365 nm.

results

When viewing the obtained chromatogram before and after treatment with AlCl3 in UV light at 365 nm, up to 5 adsorption zones with a color characteristic of flavonoids are detected. In total, we found from 12 to 16 zones of adsorption from bright blue and yellow-green to bright red and dark brown shades belonging to substances of various chemical nature.

We processed the obtained chromatograms using a Sorbfil videodensitometer, version 1.7 (Russia, b. 23965-02). Densitometry is considered the most convenient modern method of processing and analyzing chromatograms. Densitometers scan a TLC plate with a narrow beam of light of a specific wavelength. They allow you to process any TLC, with zones of adsorption of various biologically active substances, visible in daylight or UV light with a wavelength of 254 or 365 nm. Video densitometers make automatic calculations based on video images of chromatograms. Their advantages include high speed of data processing and ease of documentation [5].

The results of chromatography followed by densitometric processing are shown in Fig. 1. The densitograms show that matrix tinctures from raw wormwood and wormwood have a peak with Rf 0.09 (close in value to the peak of rutin with Rf 0.08). On chromatograms of matrix tinctures from raw wormwood and wormwood, a similar peak with quercetin - Rf 0.97 was found. At the same time, in both cases, the nature of the UV light glow of the "bystanders" adsorption zones and the corresponding matrix tincture zones is the same. Based on the results obtained, TLC in the chloroform: methanol: water - 60: 35: 7 system can be used for differential diagnostics of the three studied types of wormwood.



Rice. 1. Densitogram of matrix tinctures: track1 - wormwood; track 2 - wormwood; track 3 - the wormwood tree of god; track 4 - GSO routine; track 5 - GSO quercetin; chloroform system: methanol: water - 60: 35: 7; the analyzed image was recorded from video source videodensitometer "Sorbfil" Russia, version 1.7

Table 1

Results of calculating densitograms

Значения Rf	Зона адсорбции							
	1	2	3	4	5	6	7	8
Матричная настойка полыни горькой	0,05	0,09	0,30	0,94				
Матричная настойка полыни обыкновенной	0,05	0,21	0,32	0,97				
Матричная настойка полыни божье дерево	0,09	0,19	0,22	0,24	0,31	0,38	0,53	0,97
ГСО рутина	0,02	0,08	0,97					
ГСО кверцетина	0,47	0,97						

2. Development of a TLC method for the determination of coumarins

Chromatographic methods, and in particular TLC, are widely used for the analysis of coumarins. The literature describes several systems of solvents for TLC coumarins [1, 12]: 1) cyclohexane: ethyl acetate - 3: 1; 2) toluene: ethyl formate: formic acid - 5: 4: 1; 3) chloroform: acetic acid: water - 4: 1: 1; 4) cyclohexane: ethyl acetate: methanol - 12: 14: 1; 5) cyclohexane: ethyl acetate: methanol - 3: 2: 1; 6) chloroform: petroleum ether - 1: 2; 7) ethyl acetate: benzene - 1: 2.

In the German homeopathic pharmacopoeia, TLC in the n-butanol system is used as one of the criteria for the authenticity of wormwood in the system of n-butanol: glacial acetic acid: water - 68:16:16, witness scopoletin dissolved in methanol; stationary phase - silica gel HF 254 [12].

We tested all of the above systems using Sorbfil plates (type of sorbent silica gel CTX-1A, Sorbpolymer CJSC, Russia) 100x150 mm in size and initial matrix tinctures. The best separation of coumarins was achieved using the ethyl acetate: benzene solvent system - 1: 2. However, due to the presence of a large number of adsorption zones with a blue glow in UV light, which is characteristic of both coumarins and a number of phenolic compounds, including phenol carboxylic acids, we considered it expedient to carry out preliminary fractionation of the initial matrix tinctures in order to separate

coumarins from other phenolic compounds. Preliminary fractionation was carried out according to the generally accepted technique using solvents with different polarities.

Methodology

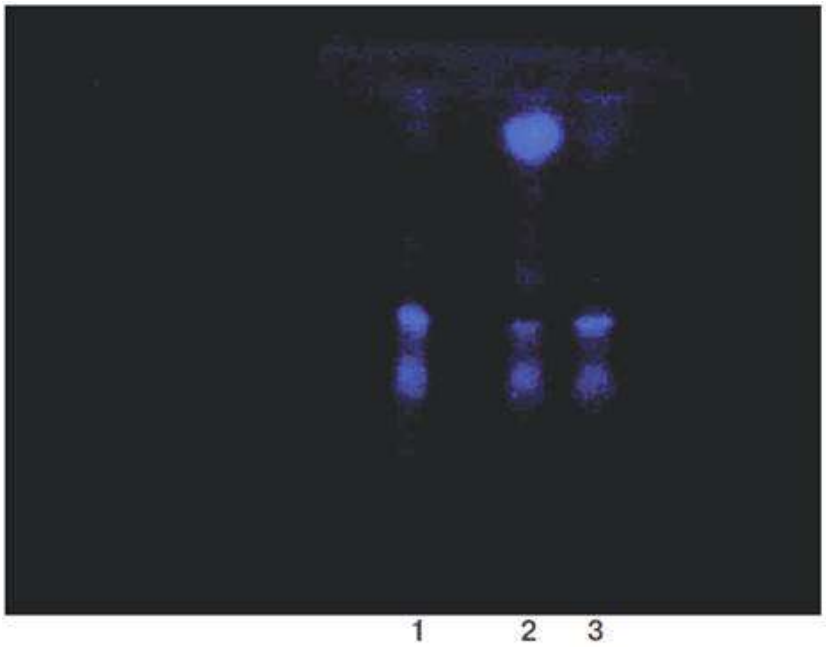
2 ml of each of the studied matrix tinctures are evaporated to 0.5 ml in a water bath. Then, 1 ml of distilled water is added to each sample and treated in a separating funnel 3 times successively with petroleum ether (2 ml). Each time, the homogeneous phases are combined and then separated from each other. Thus, organic and aqueous extracts are obtained from the studied matrix tincture. In this case, substances of the coumarin nature are extracted into the organic phase.

10 µl of the organic fraction obtained from each of the studied tinctures is applied to the start line of the previously washed chromatographic plate with a 1 cm strip or pointwise. After the solvent front has passed at least 8 cm in the chromatographic chamber, the plate is removed, the position of the front is noted and left under traction until dry. The chromatogram is then viewed in visible and UV light at 365 nm. After that, the chromatogram is treated with a 10% alcohol solution of NaOH and dried in air (about 15 minutes). Then viewed again under UV light at 365 nm.

results

When viewing the chromatogram in UV light at 365 nm before treatment with 10% alcohol solution of NaOH, up to three zones of adsorption with a characteristic glow were found in each of the studied samples, and after processing, one more zone appeared in samples from the raw material of wormwood and wormwood. adsorption.

A photograph of the chromatogram of all studied samples from raw materials of three types of wormwood is shown in Fig. 2. As a result of the study, it was found that matrix tinctures from raw wormwood and wormwood are similar in coumarin composition, and wormwood has a characteristic adsorption zone of bright purple color. The intensity and color of the remaining adsorption zones were similar (Table 2).



Rice. 2. Photo of the chromatogram of the studied matrix tinctures:1 - bitter wormwood; 2 - wormwood divine tree; 3 - common wormwood. Conditions

chromatography: Solvent system: ethyl acetate: benzene (1: 2); Sorbfil records, sorbent - silica gel CTX-1A; solvent travel distance 80 mm

table 2

Characteristics of adsorption zones during TLC of the organic fraction from matrix homeopathic tinctures of various types of wormwood in the ethyl acetate: benzene - 1: 2 system

Матричные настойки из сырья (органическая фракция)	Rf	Цвет зоны при 365 нм	
		до обработки	после обработки 10% спиртовым раствором NaOH
Полынь божье дерево			
1-я зона	0,15	ярко-голубой	зеленый
2-я зона	0,23	голубой	бледно-голубой
3-я зона	0,52	ярко-фиолетовый	зелено- голубой
4-я зона		---	----
Полынь обыкновенная			
1-я зона	0,15	слабо-голубой	бледно-зеленый
2-я зона	0,23	голубой	голубой
3-я зона		----	----
4-я зона	0,33	----	светло-голубой
Полынь горькая			
1-я зона	0,15	ярко-голубой	зеленый
2-я зона	0,23	ярко-голубой	голубой
3-я зона		----	----
4-я зона	0,33	----	светло-голубой

At the next stage of research, we made an attempt to check the homogeneity of the adsorption zones that we assigned to coumarins. This study acquired particular relevance, since the brightness and intensity of the luminescence of the adsorption zone on the chromatogram of the sample obtained from the raw material of the wormwood tree differed significantly from the samples from the raw material of other types of wormwood.

To establish the homogeneity of the adsorption zones, two-dimensional chromatography was carried out in the following systems: a) ethyl acetate: benzene - 1: 2; b) chloroform: petroleum ether - 1: 2.

As a result of the study, no additional adsorption zones were found. Therefore, we made a conclusion about the homogeneity of the studied zones. The results of chromatographic analysis of matrix tinctures of various types of wormwood for the detection of coumarins are summarized in Table 2.

From the data in Table 2, it can be seen that the technique developed by us can have a diagnostic value and makes it possible to distinguish matrix tinctures from raw materials of various types of wormwood.

conclusions

1. Developed methods of chromatography in a thin layer of sorbent matrix homeopathic tinctures from raw materials Artemisia absinthium, Artemisia vulgaris and Artemisia abrotanum in order to detect flavonoids and coumarins in them.

2. It has been shown that matrix homeopathic tinctures of wormwood, wormwood wood and wormwood differ in flavonoid composition. Wormwood and common wormwood have a similar flavonoid composition. The wormwood tree can be differentiated using this technique.

3. Chromatographic and densitometric study of the test samples matrix tinctures using GSO quercetin and rutin made it possible to establish that in

the studied samples from the raw material of wormwood and wormwood are substances that, in their chromatographic behavior, coincide with the adsorption zone of GSO rutin; and in the samples from the raw material of common wormwood and wormwood, the substances coinciding in chromatographic behavior with the GSO of quercetin.

A method for chromatographic detection in a thin layer of coumarins in matrix homeopathic tinctures from raw materials of various types of wormwood has been developed. It has been established that tinctures of wormwood and wormwood have a similar coumarin composition; wormwood tincture can be differentiated from them using this technique.

The developed methods of TLC analysis of flavonoid and coumarin composition can be used to determine the authenticity of matrix homeopathic tinctures from raw wormwood, wormwood, and wormwood.

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