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STUDY ON IDENTIFICATION OF ACTIVE INGREDIENTS OF COMBINED GEL WITH SALVIA AND WILLOW PLANT EXTRACTS

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Abstract

Over recent years, there has been increased attention of scientists around the world to the problem of rheumatic diseases. Rheumatic diseases affect the national economy, lead to significant costs of treatment and reduce the quality of life of patients.

The aim of our study was newly developed combined gel with herbal and synthetic ingredients that intended to be used for rheumatic diseases treatment. In the composition of the combined gel as active ingredients dry extracts of leaves of sage and willow bark and methyl salicylate were used. The physicochemical properties of the gel is a homogeneous mass of brown colour with a characteristic smell of methyl salicylate. This emulgel, was made on the basis of hydroxyethyl cellulose, mixes well with water. Chemical reactions and TLC were used to identify the gel components.

For the identification of methyl salicylate in the investigated gel, it is recommended to use a reaction with a solution of ferrum (III) chloride, which resulted in a violet coloration. Substances of polyphenolic structure of dry extract of willow leaves and leaves of sage should be confirmed by reactions of interaction with solutions of potassium hydroxide and concentrated sulfate acid. The method of thin-layer chromatography in comparison with reference standards has proved the presence of both in dry extracts and in gel derivatives of salicylic acid, mainly similar in structure with salicin and substances of a flavonoid structure, similar in structure to luteolin. Specificity of the test was confirmed by the ability of the proposed solvent system to divide the components of the dosage form. Thus, this solvent system can identify not only substances of the flavonoid structure of plant extracts, but also confirm the presence of other components of the gel - methyl salicylate and methyl parahydroxybenzoate.

Key words: *gel, identification, thin-layer chromatography, dry extract, common sage, white willow*

Introduction

Over recent years, there has been increased attention of scientists around the world to the problem of rheumatic diseases. Rheumatic diseases (RD) – is a group of multifactorial fever, represented by a large number of nosological forms, differing in origin and combined, mainly on the basis of localization of the basic pathological process in the connective tissue and for such a clinical manifestation as articular syndrome. Rheumatic diseases affect the national economy, lead to significant costs of treatment and reduce the quality of life of patients [1, 2].

The object of our study was newly developed combined gel with herbal and synthetic ingredients that intended to be used for rheumatic diseases treatment. In the composition of the combined gel as active ingredients dry extracts of leaves of sage and willow bark and methyl salicylate were used. The physicochemical properties of the gel is a homogeneous mass of brown colour with a characteristic smell of methyl salicylate. This emulgel, was made on the basis of hydroxyethyl cellulose, mixes well with water.

The aim of this study was development of identification methods to include them in specifications for the medicine.

Methods

The quality control of medicinal combinations containing herbal ingredients is a complex analytical task. For the identification of plant components in combination agents, chemical reactions confirming the presence of a particular group of compounds, as well as more specific methods, such as thin-layer chromatography or high-performance thin-layer chromatography (TLC / HPTLC), high performance liquid chromatography (HPLC) or gas chromatography (GC) are used. In the development of techniques the methods of analysis used in pharmacopoeial monographs on the ingredients that are part of the combined gel were taken into account [3, 4, 5].

To obtain dry extracts of leaves of sage and willow bark, the pharmacopoeial raw material of salvia leaves – Salviae officinalis folium (SPHU 2.0, vol. 3) and willow bark – Salicis cortex (SPHU 2.0, vol. 3) were used [6]. The chemical composition of the willow bark includes phenolic glycosides (salicin, salicortin, tremulacin, salireposide, picein and triandri), salicylates, flavonoids [7, 8, 9]. The chemical composition of the leaves of the sage includes phenolic acids (chlorogenic, coffee, ferulic, rosmarinic), flavonoids, di- and triterpenes, tannins, essential oils [7, 10, 11].

According to State Pharmacopoeia of Ukraine (SPHU) 2.0, vol. 3 for the identification of the willow bark, the TLC method is used, as the reference solutions salicin and chlorogenic acid are used; the TLC method is also used to identify the sage leaves, as the solution for comparison cineol and thujone are used.

Thus, chemical reactions and the method of thin-layer chromatography were used to identify the components of the gel.

Results

For chemical reactions, 0.15 g of willow dry extract, 0.15 g of sage leaves dry extract, 1.0 g of gel in 10 ml of water, 0.10 g of methyl salicylate were shaken with 10 ml of water. The obtained solutions were used to conduct chemical reactions with alcoholic solution of potassium hydroxide, acid sulfate concentrated and ferrum (III) chloride solution to substances of phenolic nature, and in particular, flavonoids (**Tab. 1**).

As can be seen from the data of **Table 1**, after adding to 1 ml of the resulting model solutions 1 ml of alcohol solution of potassium hydroxide, there is a bright yellow color; after adding drops to the walls of the tube 1 ml of sulphuric acid concentrated on the border of two layers, a coloration (substances of the flavonoid structure) is formed; after adding 0.5 ml of 3% solution of ferrum (III) chloride, on the border of two layers, a coloration characteristic of the substances of the polyphenolic structure is formed.

In the development of TLC techniques—the scheme described in that work was used [5]. As a stationary phase, a TLC plate with a F254 silica gel layer (25 μm) was used. As a mobile phase, a universal mobile phase was used on substances of phenolic nature and flavonoid glycosides. Detection of bioactive substances was carried out in several detection modes using a combination of reagents

for derivatization according to the method, which allows detection of different classes of compounds.

Determination of willow bark BAS.

Test solution a. To 1.0 g of the test gel is added 10 ml of methanol, heated in a water bath at a temperature of about 50 °C for 10 minutes, constantly shaking, cooling and filtering.

To 5.0 ml of the resulting solution, add 1.0 ml of a solution of 50 g / l of anhydrous sodium carbonate, heat in a water bath at a temperature of about 60 °C for 10 minutes, cool and filter if necessary.

Test solution b. 0.015 g of Salix alba L. dry Extract dissolved in 5.0 ml of water, add 5.0 ml of methanol and stir. To 5.0 ml of the obtained solution add 1.0 ml of a solution of 50 g / l of sodium carbonate anhydrous, heat in a water bath at a temperature of about 60 °C for 10 minutes and cool.

Comparison solution a. 2 mg of salicin and 2 mg of chlorogenic acid is dissolved in 1 ml of methanol.

Plate: TLC plate with F254 silica gel (25 μm).

Mobile phase: water P–methanol P–ethyl acetate P (8:15:77).

Volume of applied sample: 10 μl, bands.

The distance that must pass the mobile phase: 15 cm from the starting line.

Drying: in a stream of warm air.

Detection: treated with a mixture of sulfuric acid – methanol (5:95), heated at a temperature (100-105) °C for 5 minutes and revised in daylight.

Specificity of the test can be confirmed by the fact that in the case of chromatography under the same conditions of solutions of the extract of leaves of sage, methyl salicylate and methyl parahydroxybenzoate, no staining zones were detected.

The next stage of the research was the selection of conditions for the chromatographic determination of other biologically active substances and components of the dosage form. The tests were carried out on thin-layer plates with a layer of silica gel GF254 from the Merk company in the mobile phase n-butanol-acetic acid-water (4: 1: 2). After drying, the plates were treated with a boric citrate reagent and detected UV light at wavelengths of 254 nm and 366 nm.

Test solution a. 1.00 g of the test gel is dissolved in 5.0 ml of water, 5.0 ml of methanol is added and stirred.

Test solution b. 15 mg of dry extracts of Salvia officinalis L. and Salix officinalis L. salts are dissolved in 5.0 ml of water, 5.0 ml of methanol is added and mixed.

Comparison solution a. 5 mg of luteolin P and 5 mg of rutine P was dissolved in 5 ml of 70% ethanol R.

Comparative solution b. 10 mg of methyl salicylate is dissolved in 10 ml of methanol and stirred.

Comparison solution c. 2 mg of methyl parahydroxybenzoate is dissolved in 10.0 ml of methanol.

Plate: TLC plate with F254 silica gel (25 μm).

Mobile phase: n-butanol P – glacial acetic acid P - water P (4: 1: 2).

Volume of applied sample: 10 μl, with bands10 mm.

The distance that must pass the mobile phase: 13 cm from the start line.

Drying: in the air.

Detection: the plate is sprayed with a boron citrate reagent, heated at a temperature from 100 °C to 105 °C for 3 minutes and immediately examined in UV light at wavelengths of 254 nm and 365 nm.

Preparation of boron citrate reagent. 0.5 g of boric acid and 0.5 g of citric acid are dissolved in 20 ml of methanol.

Determination of BAS of willow bark.

Test solution a. To 1.0 g of the test gel is added 10 ml of methanol, heated in a water bath at a temperature of about 50 °C for 10 min, constantly shaking, cooled and filtered.

To 5.0 ml of the resulting solution add 1.0 ml of a solution of 50 g / l of sodium anhydrous carbonate, heat in a water bath at a temperature of about 60 °C for 10 min, cool and filter if necessary.

Test solution b. 0.015 g of dry bark extract of Salix alba L. is dissolved in 5.0 ml of water, 5.0 ml of methanol are added and mixed. To 5.0 ml of the resulting solution is added 1.0 ml of a solution of 50 g / l of sodium carbonate anhydrous, heated in a water bath at a temperature of about 60 °C for 10 min and cooled.

Comparison solution a. 2 mg of salicin and 2 mg of chlorogenic acid are dissolved in 1 ml of methanol.

Plate: TLC plate with a layer of silica gel F_{254} (25 μm).

Mobile phase: water P - methanol P - ethyl acetate P (8: 15: 77).

The volume of the sample applied: 10 µl, stripes.

The distance that the mobile phase must pass: 15 cm from the starting line.

Drying: in a stream of warm air.

Detection: treated with a mixture of sulfuric acid - methanol (5:95), heated at a temperature of (100-105) ℃ for 5 min and viewed in daylight.

The specificity of the test can be confirmed by the fact that in the case of chromatography under the same conditions, solutions of sage leaf extract, methyl salicylate and methyl parahydroxybenzoate stained areas were not detected.

The chromatograms of the solutions a and b to be tested should show, at the level of zones on the chromatogram of the comparison solutions a, b, and c, corresponding to the color of the main spots (**Tab. 2**). Other fluorescent zones may also be detected.

According to the results of the research, when detecting in UV light at a wavelength of 254 nm, chromatograms of the test solutions observed zones (yellowish-brown) at the level of the spots on the chromatogram of the solution of luteolin, corresponding to a color with Rf of about 0.50. Zones of yellow color on the chromatograms of the investigated solutions with Rf 0.27 were located at the level of the rutine zone. Gray-blue zones with Rf of about 0.70 on the chromatogram of hydroalcoholic extract from the gel coincided with the color and position of the methyl salicylate zone, and browned with Rf 0.77 – the zone of nipagin. After viewing the chromatograms of the investigated solutions in UV light at 366 nm, the location of the zones remained the same, but they slightly changed the color and there were additional blue fluorescence zones with Rf of about 0.20.

Specificity of the test was confirmed by the ability of the proposed solvent system to divide the components of the dosage form. Thus, this solvent system can identify not only substances of the flavonoid structure of plant extracts, but also confirm the presence of other components of the gel – methyl salicylate and methyl parahydroxybenzoate.

Discussion

Consequently, for the identification of methyl salicylate in the investigated gel, it is recommended to use a reaction with a solution of ferrum (III) chloride, which resulted in a violet coloration. Substances of polyphenolic structure of dry extract of willow leaves and leaves of sage should be confirmed by reactions of interaction with solutions of potassium hydroxide and concentrated sulfate acid. The method of thin-layer chromatography in comparison with reference standards has proved the presence of both in dry extracts and in gel derivatives of salicylic acid, mainly similar in structure with salicin and substances of a flavonoid structure, similar in structure to luteolin.

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Table 1

Results of research of gel ingredient and investigated gel with chemical reactions

Ingredient	With an alcoholic solution of potassium hydroxide	With sulphuric acid concentrated	With a solution of ferrum (III) chloride
Willow bark dry extract	Bright yellow color	On the border of two layers there is a pink color	Green color
Leaves of sage dry extract	Bright yellow color	On the border of two layers there is a yellow-brown color, which after shaking becomes bright yellow	Green color
Methyl salicylate	No reaction occurs	There is an increase in odor	Violet color
Investigated gel	Bright yellow color	On the border of two layers there is a pinkish-yellow color	Pale purple color

Table 2

Results of determination of active and auxiliary substances of the developed gel by thin-layer chromatography

Model sample	Comparison solution a	Comparison solution b	Comparison solution c	Test solution a	Test solution b
Dry extract of white willow bark	Reddish-purple zone (salicin)	–	–	Reddish-purple zone (salicin)	Reddish-purple zone (salicin)
Dry extract of common sage leaves	Yellowish brown zone (luteolin); yellow (rutine)	–	–	Yellowish brown zone (luteolin); yellow (rutine)	Yellowish brown zone (luteolin); yellow (rutine)
Methyl salicylate	–	Gray-blue zone	–	Gray-blue zone	–
Methyl parahydroxybenzoate	–	–	Brown zone	Brown zone	–