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RESEARCH ARTICLE

The Development of Definition Methodic of The Nasal Gel's "Phytorin-Plus" Active Substances

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ABSTRACT:
Objectives: the development of definition methodic of the nasal gel's "Phytorin-plus" active substances. **Materials and Methods:** the development of identification and quantitative definition methodic of licorice root dry extract in the experimental series of the nasal gel "Phytorin-plus" was carried out by HPLC method. To determine the glycyrrhizin acid (GA) was used the modification of the procedure described in the State Pharmacopoeia, validation was carried out only for proof of specificity. To confirm the presence of essential oils in the medicine, their identification and quantitative determination using the gas chromatography method were performed in accordance with the requirements of the State Pharmacopoeia. **Results and Discussions:** based on the conducted research we have developed identification and quantitative definition methodic of active substances of the nasal gel "Phytorin-plus", description of which is given in the article. **Conclusion:** using the HPLC method, an identification was made and the content of GA in the medicine was determined. The content of GA was 13.03 mg per gram of the nasal gel. The methodic of identification and quantitative determination of essential oils of pine and eucalyptus in the nasal gel by the gas chromatography method was proposed. Their contents ranged from 8.5 mg to 11.5 mg and 17.0 mg to 23.0 mg respectively.

KEYWORDS: Nasal gel, Development, Identification, Quantitative definition, Methodic, Active substances.

INTRODUCTION:
Today, viral rhinitis is a common pathology, often complicated by otitis media, sinusitis and bronchial asthma. Modern methods of treatment of this disease are relatively ineffective and are aimed at reducing the symptoms, and not at inhibiting the provoking factor of its development, in particular, adenovirus and other infectious agents[1-3].

In local therapy of viral rhinitis, along with liquid dosage forms and aerosols, soft medicinal forms, in particular nasal gels, have not lost their significance. It is known that among modern soft dosage forms, it is nasal gels that provide the optimal content of active ingredients on the nasal mucosa, prolonged therapeutic effect, do not violate the movement of the ciliary epithelium, and maintain the natural moisture of the mucous membrane[4,5].

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Nasal gels on the modern pharmaceutical market are mainly represented by medicines of foreign production based on synthetic compounds. Thus, the urgent task is to develop a new combined nasal gel based on natural raw materials for the treatment of viral rhinitis, followed by the study of its pharmacological, physical and chemical parameters[6,7].

At the National University of Pharmacy, the nasal gel under the conventional name "Phytorin-plus" with licorice root dry extract and essential oils of pine and eucalyptus is created[8-10].

The **objective** of this work is the development of definition methodic of the nasal gel's "Phytorin-plus" active substances.

MATERIALS AND METHODS:
The development of identification and quantitative definition methodic of GA of licorice root dry extract in the medicine was carried out on the Varian ProStar analytical chromatograph (ProStar 210 pump; ProStar 330 spectrophotometric detector; ProStar 410 autosampler with 20µl dosing loop volume), and

Columns Nucleosil 100-3C18100*4.6 with pre-colon.

The following solvents and reagents were used: acetonitrile "gradient grade" (Sigma-Aldrich), water for chromatography (Millipore Direct-Q5), acetic acid (chemically pure), ammonia water (chemically pure).

The standard samples that were used: the Pharmacopoeia Standard Sample of monoammonium glycyrrhizate and the working standard sample of the licorice root dry extract.

The development of identification and quantitative definition methodic was carried out using the experimental series of the nasal gel "Phytorin-plus" and placebo by HPLC method.

The first stage in the development of methodic for the determination of GA was to determine the requirements for the selection of active component from the matrix consisting of a gel base (carbopol, triethanolamine, propylene glycol and ethanol) and essential oils of pine and eucalyptus. It was necessary to propose a methodic for sample preparation, which would ensure the complete release of active substance and prevent the matrix components from being sampled. As a solvent for the determination of GA was chosen an aqueous ammonia solution, capable of forming an easily soluble monoammonium glycyrrhizate with it.

To prevent the components of the matrix entering the sample, after dispersing the suspension, the solution was centrifuged for 5 minutes at 5000 min^{-1} and filtered through a membrane filter with a pore size of no more than 0.45 microns. To determine the GA was used the modification of the procedure described in the State Pharmacopoeia; validation was carried out only for proof of specificity.

To confirm the presence of essential oils of pine and eucalyptus in the medicine, their identification and quantitative determination using the gas chromatography method was performed in accordance with the requirements of the State Pharmacopoeia.

Studies were conducted based on the State scientific-research laboratory of National University of Pharmacy for medicinal substances quality control.

RESULTS:

After making changes in the pharmacopoeial methodic for sample preparation in determining the GA in the nasal gel "Phytorin-plus", the methodic is proposed as follows.

Sample mixture:
Mix 25ml of ammonia concentrated solution and 975ml of water.

Mobile phase:
Mix 55ml of acetic acid, 335ml of acetonitrile and 610 ml of water.

Test solution:
1.0g of the medicine place in a 100ml volumetric flask, add 50ml of the sample mixture, sonicate until complete dispersion of the sample, bring to the mark with the same solvent and centrifuge. Filter the supernatant liquid through a nylon membrane filter with a pore size of 0.45 microns, rejecting the first portions of the filtrate.

Comparison solution:
Approximately 50mg of Pharmacopoeia Standard Sample of monoammonium glycyrrhizate place in a 50 ml volumetric flask, dissolve in a sample mixture; bring to the mark with the same solvent. 5.0ml of the resulting solution adjust to a volume of 50ml and filter through nylon membrane filter with a pore size of 0.45 microns.

Chromatography is carried out on a liquid chromatograph with UV-detector under the following conditions: a column 0.10m × 4.6cm filled with silica gel for chromatography, with a particle size of 3.0 microns, with a pre-column (Nucleosil 100-3C18100*4.6); speed of the mobile phase: 1.5ml/min; column temperature: 25°C; detection at wavelength: 254nm; injection volume: 20µl.

The chromatographic system is considered suitable if the following requirements are met:

- The coefficient of symmetry, calculated at the peak of monoammonium glycyrrhizate on the chromatogram of the comparison solution must be at least 0.8 and not more than 2.0;
- The efficiency of the chromatographic column calculated on the basis of the peak of monoammonium glycyrrhizate on the chromatogram of the comparison solution is not less than 2000.

In order to fulfill the requirements of the suitability test of the chromatographic system, chromatographic conditions may be adjusted.

Serially obtain n_0 = 2, 3, ... 8 parallel chromatograms of the comparison solution and calculate the relative standard deviation of RSD. The value n_0 is sufficient if the RSD value calculated for the peak area of the monoammonium glycyrrhizate glyceride does not exceed the RSD_{max} shown below.

Number of parallel injections, n_0						
2	3	4	5	6	7	8
RSD_{max}						
0.51	1.34	1.92	2.37	3.75	3.08	3.38

If the RSD values obtained do not exceed the value of RSD_{max}, the same amount of comparison solution and the test solution are alternately chromatographed n≥n0 times.

The content of GA (X), in mg per gram of the nasal gel, is calculated by the formula:

$$X = \frac{S_1 * m_0 * 5 * 50 * P * 823}{S_1 * m_0 * 50 * 50 * 100 * 840} = \frac{S_1 * m_0 * P * 823}{S_1 * m_1 * 1000 * 840}$$

where:
S_1 – average value of peak area of the test substance, calculated from the chromatogram of the test solution;
S_0 – average value of peak area of the test substance, calculated from the chromatogram of the comparison solution;
m_1 – weight of the sample of the medicine, g;
m_0 – weight of Pharmacopoeia Standard Sample of monoammonium glycyrrhizate, mg;
P – content of the active substance in Pharmacopoeia Standard Sample of monoammonium glycyrrhizate, %;
823 – molecular weight of GA;
840 – molecular weight of monoammonium glycyrrhizate (without taking into account crystallization water).

The content of GA should be at least 7.50mg per gram of the nasal gel.

Identification:
The maintenance time of the main peaks on the chromatograms of the test solution obtained with the quantitative determination of GA coincides with the maintenance periods of peaks on the chromatograms of the solution of Pharmacopoeia Standard Sample of monoammonium glycyrrhizate (Fig. 1).

The methodic of determining essential oils of pine and eucalyptus in the nasal gel “Phytorin-plus” is proposed in the following form.

Test solution:
Approximately 10.0g (precise weight) of the medicine place in a 500ml round-bottomed flask, add 300ml of purified water and attach to the apparatus for determining the essential oils.

Into the receiver place 2.0ml of the internal standard solution, heat the sample flask to boil and continue distillation for 60 minutes. The resulting detachment is then passed through 0.5g of anhydrous sodium sulfate, collecting the resulting filtrate in a 5ml volumetric flask, washing the filter with 2ml of toluene, combining the wash solution with the filtrate in the flask, adjusting the solution to the toluene, and mixing.

Pine essential oil comparison solution:
Approximately 100mg (precise weight) of pine essential oil place in a 5ml volumetric flask, add 2.0ml of internal standard solution, adjust the volume of the solution with toluene to the mark and mix.

Eucalyptus essential oil comparison solution:
Approximately 300mg (precise weight) of eucalyptus essential oil place in a 5ml volumetric flask, add 2.0ml of internal standard solution, adjust the volume of the solution with toluene to the mark and mix.

Internal standard solution:
0.50ml of heptanol-1 place in a 25ml volumetric flask, add 20ml of toluene, mix, adjust the volume of the solution with toluene to the mark and mix.

Comparison internal standard solution:
2.0ml of the internal standard solution place in a 5ml volumetric flask, adjust the volume of the solution with toluene to the mark and mix.

For 1µl of the test solution, comparison solution and internal standard solution is chromatographed on a gas chromatograph with a flame-ionization detector under the following conditions: a quartz capillary column, 50 m x 0.2mm in diameter with a stationary phase of FFAP; a layer thickness of 0,33 microns; the temperature of the column is programmed: 70°C is maintained for 5 minutes, then the temperature is raised at a rate of 3°C/min to a temperature of 220°C and maintain for 10 minutes; unit temperature – sample injection – 230°C; temperature of the detector – 240°C; speed of carrier gas (nitrogen) – 0.9 ml/min; flow separation – 1:50.

The content of essential oils of pine and eucalyptus (X), in mg per gram of the nasal gel, is calculated by the formula:

$$X = \frac{B_i \times m_0 \times 5}{B_0 \times m \times 5} = \frac{B_i \times m_0}{B_0 \times m}$$

where: B_i – the average value of the ratio of the sum of the areas of components of the essential oils of pine and eucalyptus to the peak area of the internal standard, calculated from the chromatograms of the test solution; B_o – the average value of the ratio of the sum of the areas of components of the essential oils of pine and eucalyptus to the peak area of the internal standard, calculated from the chromatograms of the comparison solution of essential oils of pine and eucalyptus; m_o – weight of essential oil, in mg; m – weight of the sample of the medicine, in g.

The content of pine essential oil should range from 8.5 mg to 11.5mg.
The content of eucalyptus essential oil should range from 17.0mg to 23.0mg.

Results are considered reliable if the following conditions are true:

- The separation coefficient of the peaks of limonene and 1.8-cyneol, calculated from the chromatograms of the comparison solution of the essential oil of eucalyptus and the test solution must be at least 1.5;
- The relative standard deviation of the ratio of the sum of the areas of the components of the essential oil of pine to the peak area of the internal standard calculated from the chromatograms of the test solution and the pine essential oil comparison solution and the chromatograms of the eucalyptus essential oil comparison solution must meet the requirements of the State Pharmacopoeia 1.1 2.2.24N.

Identification of essential oils pine and eucalyptus in the developed medicine was carried out at the main peaks of limonene, 1.8-cyneol and α-pinen, the retention times of which should coincide with the maintenance times of the peaks of limonene, 1.8-cyneol and α-pinen on the chromatograms of the comparison solution (Fig. 2).

DISCUSSION:

Using the developed methodic, the content of GA was controlled in the experimental series of the nasal gel "Phytorin-plus". The content of GA was 13.03mg per gram of the nasal gel.

Typical chromatograms obtained in the determination of GA are given in Fig. 1.

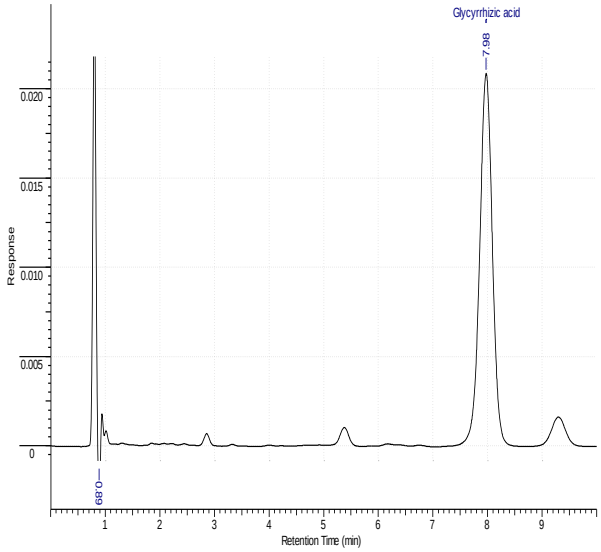
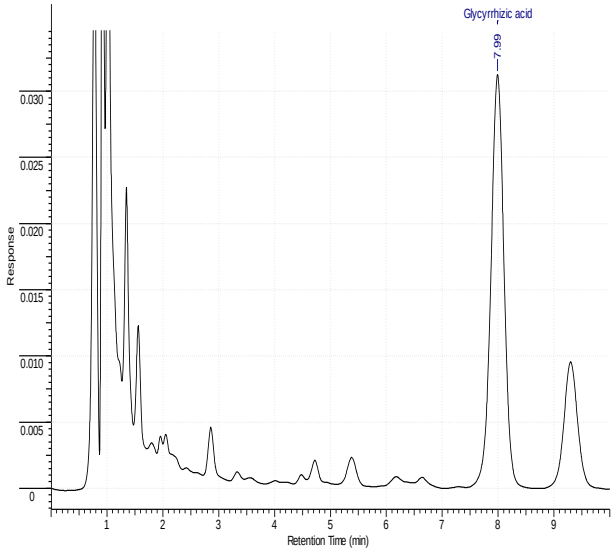


Fig. 1. Chromatogram of the comparison solution (GA)



Chromatogram of the test solution (GA)

To demonstrate the specificity of the methodic for determining GA, solution of placebo was prepared and analyzed. Based on the conducted researches it was established that the determination of this active substance in the nasal gel according to the proposed methodic does not interfere with the placebo components.

Using the developed methodic for determining the essential oils of pine and eucalyptus in the nasal gel "Phytorin-plus", their contents in the experimental series of the medicine was monitored. They ranged from 8.5 mg to 11.5mg and 17.0mg to 23.0mg respectively.

Chromatograms of test solution, comparison solution and comparison internal standard solution are given in Fig. 2.

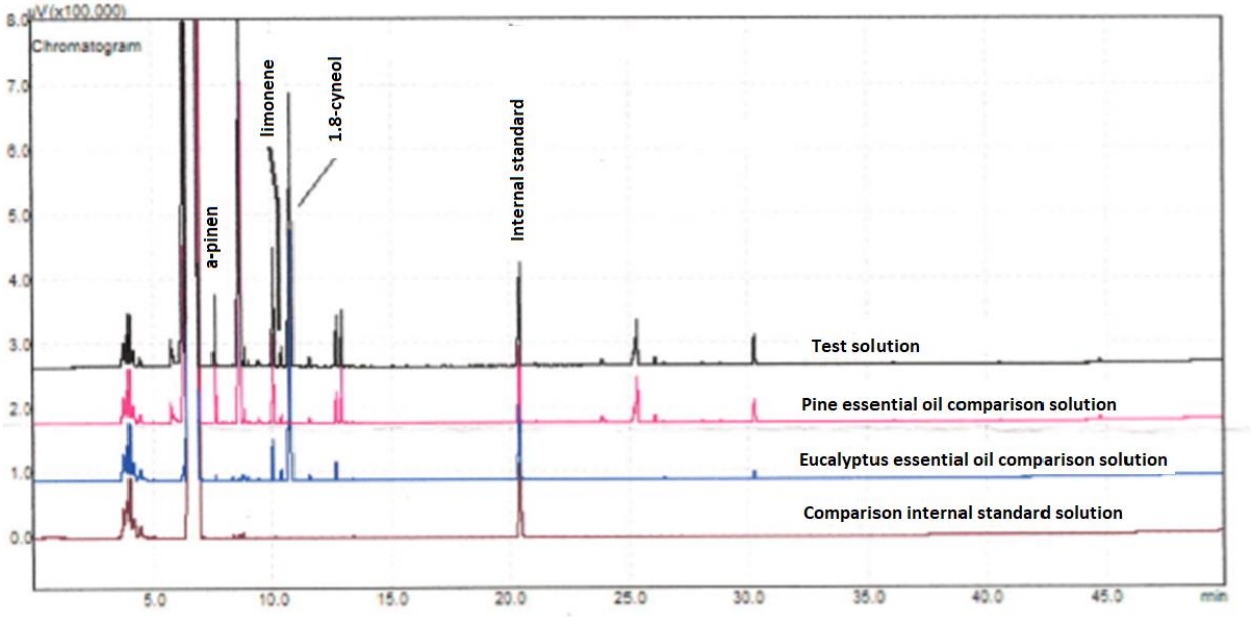


Fig. 2. Chromatograms of test solution, comparison solution and comparison internal standard solution

CONCLUSIONS:

1. Methodic of identification and quantitative determination of active substances of the nasal gel "Phytorin-plus" are developed.
2. Using the HPLC method, an identification was made and the content of GA in the medicine was determined. The content of GA was 13.03mg per gram of the nasal gel.
3. For the methodic of determining the GA, as one of the validation characteristics, specificity is determined.
4. The methodic of identification and quantitative determination of essential oils of pine and eucalyptus in the nasal gel by the gas chromatography method are proposed. Their contents ranged from 8.5mg to 11.5mg and 17.0mg to 23.0mg respectively.

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