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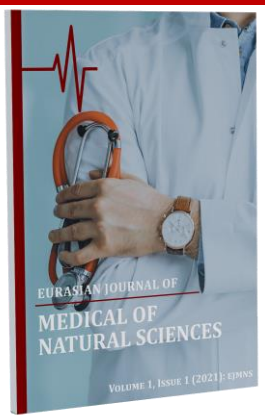


# QUANTITATIVE ANALYSIS OF THE COMBINED PREPARATION OBTAINED FROM PLANT RAW MATERIALS

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**ABSTRACT**

*Quantitative analysis of the active ingredients of the combined original preparation containing glycyrrhizic acid and dry oak bark extract was carried out. When analyzing the active ingredients of the combined dosage form, HPLC and titration were used. The metrological characteristics of the results of the quantitative determination of active substances that meet the regulatory requirements have been calculated.*

Combined drugs are more stable drug systems with effective technological, biological and consumer characteristics. This is achieved by selecting the optimal composition of excipients or dosage form. This fact determines their advantage over other dosage forms. For example, the components of combined preparations are used in moderate doses, which allows good tolerability, minimal side effects and increased effectiveness of treatment. In this regard, in order to obtain a more stable, effective, easy-to-use, cost-effective drug system, we obtained a combined preparation in the form of a capsule containing the monoammonium salt of glycyrrhizic acid and a dry extract obtained from oak bark, which has hepatoprotective, antioxidant and antiviral properties [ 1-3].

**Purpose of the study**. The aim of the study was to develop a quantitative determination of the active substances of the obtained capsules. The quantitative content of the monoammonium salt of glycyrrhizic acid in the resulting dosage form was determined by high performance liquid chromatography. The quantitative content of tannins in the composition of the combined capsule was carried out by titration.

**Experimental part. Determination of the quantitative content of the monoammonium salt of glycyrrhizic acid**. The analysis was carried out on an Agilent 1100 series liquid chromatograph with a UV detector. In the separation of the test substance by HPLC, reversed-phase columns and mobile phases with organic solvents were used. When selecting the optimal mobile phase for analysis, we used systems of organic solvents, which are shown in Table 1.

**Table 1**

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**Solvent systems used for the analysis of the monoammonium salt of glycyrrhizic acid in capsules**

| № | Mixtures of organic solvents | Solvent ratio |
|---|---|---|
| 1 | Purified water-acetonitrile-acetic acid | 307:190:3 |
| 2 | Purified water-acetonitrile | 50:50 |
| 3 | Purified water-acetonitrile-acetic acid | 309:190:1 |

Chromatography conditions were selected taking into account the obtained spectral data (Fig. 1) on a chromatograph with a UV detector and the optimal separation of the monoammonium salt of glycyrrhizic acid using a system consisting of a mixture of purified water-acetonitrile-acetic acid in the ratio (307:190:3). The most suitable chromatographic column turned out to be Zorbax Eclipse XDB-C18, USA, 200 mm x 4.6 mm, filled with silica gel with a particle size of 5.00 µm, the flow rate of the mobile phase was 1.5 ml/min. Detection was carried out at a wavelength of 254 nm.

The volume of the injected sample is 20 µl. The analysis time is about 10 min. The column thermostat temperature is room temperature.

To develop a method for the quantitative analysis of the monoammonium salt of glycyrrhizic acid in capsules by HPLC, we used a solution of the working standard sample (Working Standard Sample) of the monoammonium salt of glycyrrhizic acid and the test solution.

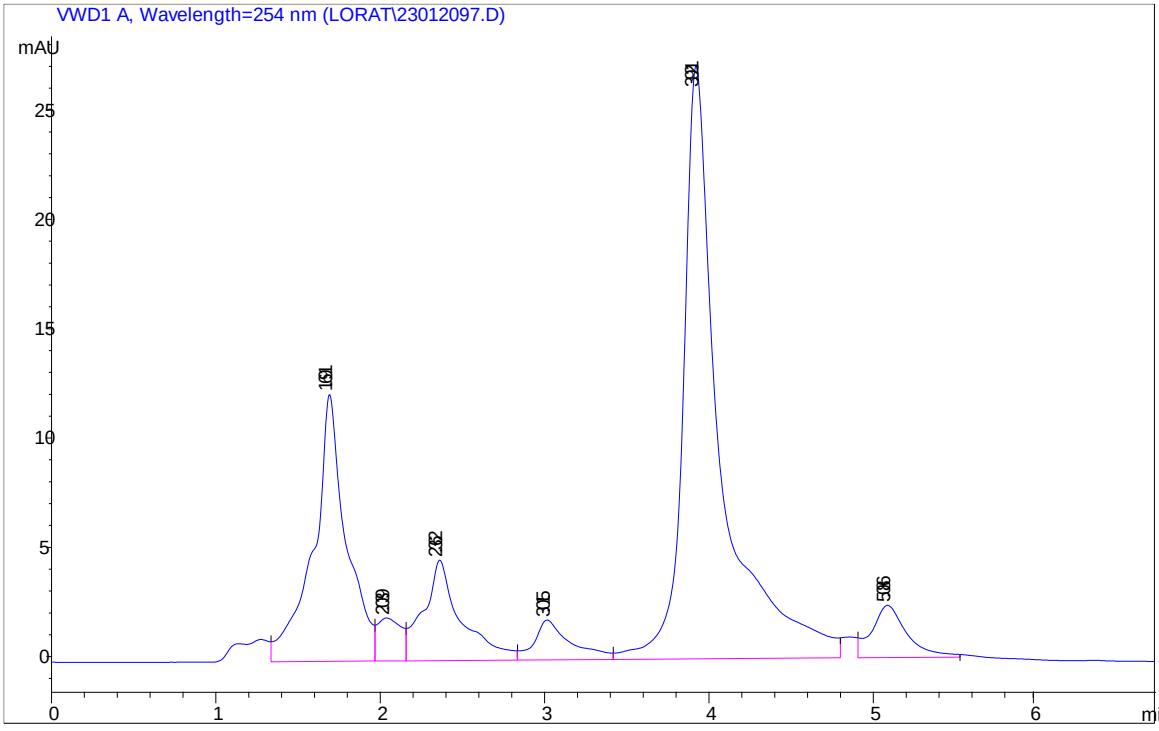


**Fig.1. Chromatogram of a standard sample of the monoammonium salt of glycyrrhizic acid**

**Preparation of the test solution of the monoammonium salt of glycyrrhizic acid.** To prepare the test solution, 50 mg (accurately weighed) of the drug is placed in a volumetric flask with a capacity of 50 ml, 20 ml of the mobile phase are added and sonicated for 15 minutes. Then the volume of the solution is adjusted to the mark with the mobile phase, mixed, filtered. The initial portion of the filtrate 5 ml is discarded, the resulting solution is

placed in a flask with a capacity of 25 ml and brought to the mark with the mobile phase. Mix, the resulting solution is filtered through a membrane filter "Millipore" with a pore size of 0.45 μm.

**Preparation of a working standard sample (WSS) of the monoammonium salt of glycyrrhizic acid**. When preparing the solution, the Working Standard Sample of the monoammonium salt of glycyrrhizic acid, a sample of about 20 mg (accurately weighed) is placed in a volumetric flask with a capacity of 50 ml, 20 ml of the mobile phase are added and sonicated for 15 minutes until complete dissolution. Then the volume of the solution is adjusted to the mark, the resulting solution is mixed and filtered through a Millipore membrane filter with a pore size of 0.45 μm. The initial portion of the filtrate in a volume of 5 ml is discarded. 1 ml of the resulting solution is placed in a 10 ml volumetric flask and the volume of the solution is adjusted to the mark with the mobile phase.

The results of the analysis showed that the method developed by us makes it possible to identify and quantify the monoammonium salt of glycyrrhizic acid in the developed casulae. Chromatograms of the solution of the Working Standard Sample of the monoammonium salt of glycyrrhizic acid and the test solution are shown in Figures 1, 2.

From the obtained chromatograms, it can be seen that the retention time of the solution of the Working Standard Sample of the monoammonium salt of glycyrrhizic acid and the test solution is 3, 3.921 and 3.920 minutes, respectively.

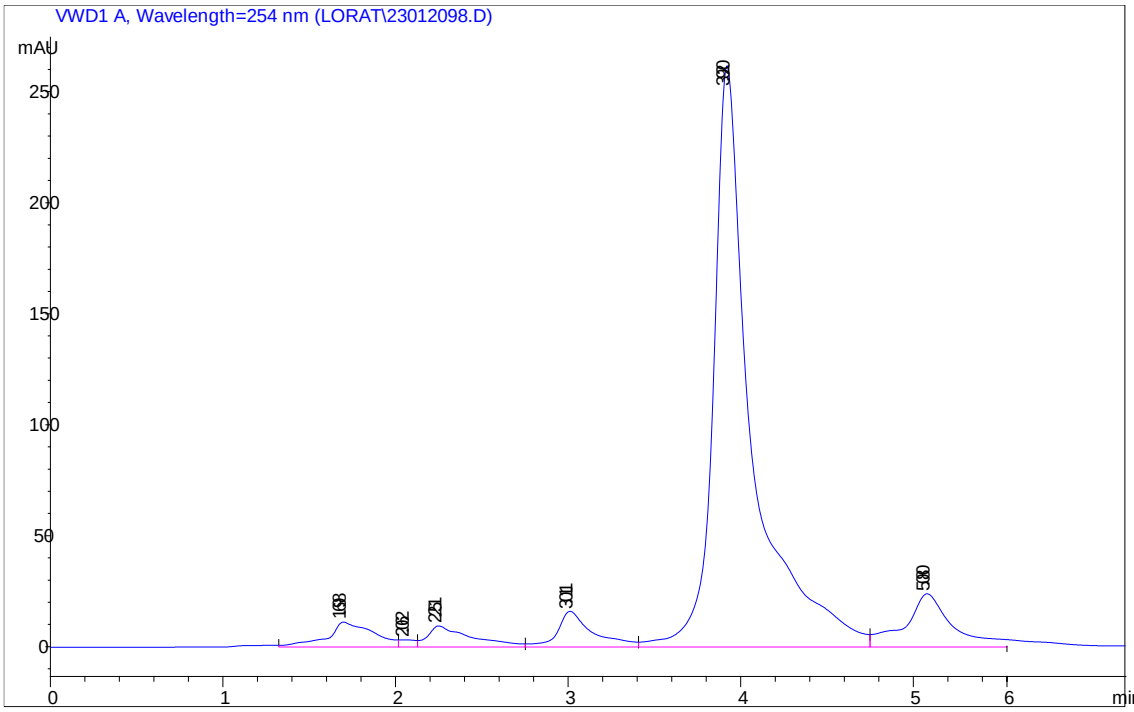


**Fig.2. Chromatogram of test solution (combination capsule)**

To check the suitability of the chromatographic system, the chromatography of the WSS solution of the monoammonium salt of glycyrrhizic acid was carried out at least 5 times. A chromatographic system is considered suitable if the following conditions are met:

- the efficiency of the chromatographic column, calculated on the chromatograms of the WSS according to the peak of the monoammonium salt of glycyrrhizic acid, is not less than 1500 theoretical plates;

- the degree of separation of the peaks, calculated for the monoammonium salt of glycyrrhizic acid on the chromatograms of the WSS, not less than 1.6;
- relative standard deviation calculated for the peak area of the monoammonium salt of glycyrrhizic acid on the chromatograms of the WSS solution, not more than 2%.

The content of the monoammonium salt of glycyrrhizic acid mg in combined capsules was calculated by the formula:

$$X = \frac{S_{исп} * m_{стд} * 50 * 1 * 25 * (100 - W) * P * 822{,}94 * b}{S_{стд} * m_{исп} * 50 * 5 * 10 * 100 * 100 * 839{,}97}$$

Where,

$S_{исп}$- the value of the peak area of the monoammonium salt of glycyrrhizic acid on the chromatogram of the test sample;

$S_{стд}$ - the value of the peak area of the monoammonium salt of glycyrrhizic acid on the chromatogram of the WSS;

$W$ – humidity of the standard substance;

$m_{исп}$ - sample weight, mg;

$m_{стд}$ is a sample of the WSS of the monoammonium salt of glycyrrhizic acid, mg;

$P$ - the content of WSS monoammonium salt of glycyrrhizic acid,%;

822,94 molar mass of glycyrrhizic acid;

839,97 molar mass of the monoammonium salt of glycyrrhizic acid.

Based on the data obtained, the norm of the content of the monoammonium salt of glycyrrhizic acid in capsules is set at least 45-55 mg. Metrological characteristics of the quantitative determination of the monoammonium salt of glycyrrhizic acid in combined capsules are shown in table 2.

**Table 2. The results of the quantitative determination of the monoammonium salt of glycyrrhizic acid by HPLC**

| The content of monoammonium salt of glycyrrhizic acid, mg | Metrological characteristics |
|---|---|
| 51,85<br>52,05<br>52,14<br>52,32<br>52,41 | R = 0,56<br>Q1= 0,3571<br>Qn = 0,16071<br>X cp.=52,154<br>S2=0,0491; S=0,2216; SX=0,0991<br>n = 5; f= 4<br>T(95%,4)=2,78<br>$\sum = 1{,}1815\%$<br>$\sum = 0{,}5283\%$ |

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**Quantitative analysis of tannins in the studied capsules.**

**The content of tannins**. The quantitative content of tannins in the composition of the combined capsule was carried out by titration with constant stirring with a 0.02M solution of potassium permanganate until yellow coloration.

To prepare the test solution, 2 g (accurately weighed) of the drug is placed in a volumetric flask with a capacity of 500 ml, adding 250 ml of hot purified water, boil with constant stirring for 30 minutes. The resulting solution is cooled to room temperature and the volume is adjusted to 250 ml. 25 ml of the resulting solution is placed in a 750 ml volumetric flask, 500 ml of purified water and 25 ml of indigo sulfonic acid solution are added. The resulting solution is titrated with constant stirring with a 0.02M solution of potassium permanganate until a yellow color.

In parallel, conduct a control experiment. 1 ml of 0.02 M solution of potassium permanganate corresponds to 0.004157 g of tannins in terms of tannin.

The content of tannins (X) in mg combined capsules is calculated by the formula:

$$X = \frac{(V_1 - V_0) * T * K * 250 * 100}{a * 25}$$

Where,

$V_1$ – volume of potassium permanganate solution used for titration, ml;

$V_0$ – the volume of potassium permanganate solution used for titration in the control experiment, ml;

$T$ – titer 0.004157;

$K$ – correction factor to 0.02 M potassium permanganate solution;

$a$ – sample of the drug, g.

Metrological characteristics of the quantitative determination of tannins (in relation to tannin) in combined capsules are shown in table 3. As can be seen from table 3, the content of tannins in the combined preparation ranges from 17.4-17.7 m3, respectively, the norm for the content of tannins was established not less than 17.5 mg.

**Table 3**

**The results of the quantitative determination of tannins (in relation to tannin) by the titration method**

| The content of tannins, mg | Metrological characteristics |
|---|---|
| 17,4<br>17,5<br>17,6<br>17,5<br>17,7 | R = 0,3<br>Q1= 0,333<br>Qn = 0,333<br>X cp.=17,54<br>S2=0,013; S=0,11401; SX=0,0509<br>n = 5; f= 4<br>T(95%,4)=2,78<br>$\sum = 1,8071\%$ $\sum = 0,8081\%$ |

**Conclusions**: Quantitative analyzes of the active ingredients of the combined capsules were carried out. The metrological characteristics of the results of the quantitative determination of active substances are calculated, which meet the regulatory requirements.

The quantitative analyzes of the active ingredients of the obtained combined capsules provide an opportunity for an objective assessment of the medicinal product and can be used in its standardization and execution of the corresponding draft Provisional Pharmacopoeia Article and other regulatory documentation.

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