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DETERMINATION OF THE DRY RESIDUE, HEAVY METALS AND GLYCYRRHIZIC ACID CONTENT IN A COMPLEX LIQUID EXTRACT BASED ON LICORICE ROOTS, ECHINACEA GRASS AND ROSEHIP FRUITS

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ABSTRACT

The article presents the results of research on standardization of a liquid extract with expectorant effect, containing licorice roots, echinacea herb and rosehip fruit. The studies were conducted in accordance with the requirements of the State Pharmacopoeia of the Republic of Uzbekistan. Studies were conducted to determine heavy metals, density, dry residue, and quantified the content of alcohol and glycyrrhizic acid (GA).

Introduction. In recent years, there has been a significant increase in interest in medicinal plants and herbal remedies, due to their complex biological effects, relative safety, and the possibility of prolonged use. Plant extracts are widely used in the composition of medicines, dietary supplements (DS), and functional food products. In this regard, quality control and safety assurance of products containing plant-derived components has acquired particular importance.

One important area of pharmaceutical analysis is the study of the physicochemical and biologically active components of plant extracts. Various analytical methods are used to confirm the quality and standardization of herbal preparations, enabling determination of active substance content as well as detection of possible impurities and contaminants.

Of particular importance in evaluating the quality of herbal preparations is the determination of dry residue, which characterizes the total content of dissolved substances in the extract and indirectly reflects the concentration of biologically active compounds. This parameter is one of the key standardization indicators for plant extracts and is widely used in monitoring their manufacturing processes.

An equally important safety indicator for herbal preparations is heavy metal content. Medicinal plants are capable of accumulating such toxic elements as lead, cadmium, mercury, and arsenic, which enter the plant from soil, water, or the surrounding environment. The presence of heavy metals in plant raw materials and preparations may pose a potential health risk; therefore, their content must be strictly controlled in accordance with established

pharmacopoeias and sanitary-hygienic standards.

In addition to general quality indicators, quantification of specific biologically active compounds characteristic of particular plant species plays an important role. One such compound is glycyrrhizin acid, the principal active component of licorice root. This compound exhibits pronounced anti-inflammatory, antioxidant, and immunomodulatory properties and is frequently used in pharmaceutical practice.

Consequently, quantitative determination of glycyrrhizic acid is an important step in the standardization of preparations and dietary supplements containing Glycyrrhiza glabra, allowing control of the biologically active substance content in the product under investigation.

The object of the present study is a liquid plant extract containing Glycyrrhiza glabra, Echnacae spp. and Rosa spp.. These plants are widely used in phototherapy owing to their immunomodulatory, anti-inflammatory, and antioxidant properties.

The aim of this study is to determine the dry residue, heavy metal content, and quantitative glycyrrhizic acid content in the liquid plant extract under investigation, in order to assess its quality and safety.

Experimental Section. Prior to conducting the analytical studies, the samples were subjected to preliminary preparation; the liquid extract was thoroughly mixed to obtain a homogeneous system. Mineralization of samples was carried out for heavy metal determination. Mineralization was performed using concentrated nitric acid with subsequent heating until complete destruction of the organic matrix. The resulting clear solution was cooled and brought to a defined volume with distilled water in a volumetric flask. The prepared solutions were used for further analysis by atomic emission spectrophotometry. This method is based on measuring the emission intensity of atoms of chemical elements upon excitation in a high-temperature plasma.

When the sample solution is introduced into the plasma, atomization of elements occurs and atoms transition to an excited state. Upon returning to the ground state, atoms emit electromagnetic radiation at a characteristic wavelength for each element. The intensity of this radiation is proportional to the concentration of the element in the sample under study.

Calibration solutions of reference standards were used for quantitative determination of elements. Heavy metal content was calculated based on the calibration relationship between spectral line intensity and element concentration.

The results obtained are presented in Table 1.

Table 1

Results of Heavy Metal Analysis by Atomic Emission Spectrophotometry

Element	Sample 1 (µg/L)	Sample 2 (µg/L)	Note
Arsenic (As)	<20	<19	below detection limit
Cadmium (Cd)	<1.1	<1.1	below detection limit

Element	Sample 1 (µg/L)	Sample 2 (µg/L)	Note
Lead (Pb)	<30	<29	below detection limit
Mercury (Hg)	<0.82	<0.80	below detection limit
Nickel (Ni)	<4.0	<3.9	below detection limit
Chromium (Cr)	<3.4	<3.3	below detection limit
Copper (Cu)	<13	<12	below detection limit
Zinc (Zn)	<1.0	<1.0	below detection limit

The determination of heavy metal content in the liquid plant extract was carried out by inductively coupled plasma atomic emission spectrometry (ICP-AES). Concentrations of toxicologically significant elements, including lead, cadmium, arsenic, and mercury, were determined in the course of the analysis.

The results obtained showed that the concentrations of these elements in the samples under investigation were below the method's detection limits, indicating the absence of significant heavy metal contamination. Furthermore, the content of other elements (Ni, Cr, Cu, Zn) was found to be at trace levels and did not exceed the permissible sanitary-hygienic standards.

Determination of Dry Residue

The dry residue content in the liquid extract was determined by the method of drying to constant mass. This parameter characterizes the total content of dissolved substances in the extract under investigation.

The test was performed according to the following procedure. A weighing dish (crucible) previously dried at 100–105 °C to constant mass was charged with 5.0 mL of the liquid extract, evaporated to dryness on a water bath, dried in a drying oven for 3 hours at 100–105 °C, cooled in a desiccator (over anhydrous silica gel, anhydrous calcium chloride, or another suitable desiccant) for 30 minutes, and weighed. The result is expressed in mass-to-volume percent. The dry residue content must meet the requirements specified in the pharmacopoeial monograph.

The dry residue content was calculated according to the formula:

$X = (m_2 - m_1) / m \times 100$

where:

X — dry residue content, %

m_1 — mass of the empty dish, g

m_2 — mass of the dish with dry residue, g

m — mass or volume of the sample under investigation, g (or mL)

Result:

X = (26.7363 – 26.5483) / 4.7795 × 100 = 3.9335%

Determination of Density (State Pharmacopoeia XI, Issue 1, p. 24)

A clean dry pycnometer is weighed to an accuracy of 0.0002 g, filled with distilled water slightly above the graduation mark using a small funnel, closed with a stopper, and kept in a thermostat maintaining a constant water temperature of 20 °C (accurate to 0.1 °C) for 20 minutes. At this temperature, the water level in the pycnometer is brought to the mark by rapidly removing the

excess water with a pipette, or with wax in such a quantity as to occupy 1/3–1/2 of the pycnometer volume. The pycnometer is placed for one hour without its stopper in hot water, then cooled to 20 °C and weighed; brought to the mark with distilled water at 20 °C, dried, and reweighed. In both phases and at their interface there must be no air bubbles.

The density is calculated by the following formula:

$$X = \frac{(m_2 - m_1)}{m} \times 100$$

where:

m — mass of the empty pycnometer, g

m_1 — mass of the pycnometer with purified water, g

m_2 — mass of the pycnometer with the test liquid, g

0.99703 — density of water at 20 °C (in g/cm^3, accounting for air density)

0.0012 — density of air at 20 °C and barometric pressure 1011 hPa (760 mmHg)

Result obtained:

ρ = (26.7333 – 17.0611)/(26.9703 – 17.0611) × 0.99703 + 0.0012 = 0.985 g/cm^3

Quantitative Determination of Glycyrrhizic Acid in the Liquid Extract by HPLC

High-performance liquid chromatography (HPLC) was used for the analysis. The monoammonium salt of glycyrrhizic acid (96%) was used as a secondary reference standard.

Test solution: 5.0 mL of the liquid extract is placed in a 50 mL volumetric flask, the volume is brought to the mark with mobile phase, and mixed. The resulting solution is filtered through a membrane filter with a pore size of 0.45 µm.

Standard solution: 5.0 g is placed in a 50 mL volumetric flask, the volume is brought to the mark with mobile phase, and mixed. The resulting solution is filtered through a membrane filter with a pore size of 0.45 µm.

20 µL aliquots of the test and working reference standard solutions are injected into the injector of a Shimadzu Agilent 1200 Series liquid chromatograph equipped with a UV detector and an isocratic pump; not fewer than 3 chromatograms are obtained for each solution under the following conditions:

– Liquid chromatograph equipped with an isocratic pump and a variable-wavelength spectrophotometric detector — Shimadzu RF-20AXS 1200 Series;

– Chromatographic column — Shim-pack Velox C18 (4.6x150 mm);

– Detector wavelength — 254 nm;

– Mobile phase — acetonitrile: water: acetic acid pH = 3.0 (±0.05) (190:307:3), previously degassed and filtered through a membrane filter with a pore size of 0.45 µm;

– Mobile phase flow rate — 0.5 mL/min;

– Injection volume — 10 µL;

– Analysis time — 15 minutes.

The glycyrrhizic acid content (X) in the liquid extract, expressed as a percentage, is calculated by the formula:

$$X = \frac{S_{исп} \times a_{стд} \times 50 \times P \times 100 \times 82294}{S_{стд} \times a_{исп} \times 25 \times 100 \times 839,97} = \frac{S_{исп} \times a_{стд} \times 2 \times P \times 82294}{S_{стд} \times a_{исп} \times 839,97}$$

where:

S_{test} — peak area of glycyrrhizic acid in the chromatogram of the test solution;

S_{std} — peak area of glycyrrhizic acid in the chromatogram of the working reference standard solution;

a_{std} — weighed sample of the reference standard (WRS) of monoammonium glycyrrhizinate, g;

a_{test} — volume of the test sample aliquot, 5 mL;

822.94 — molecular weight of glycyrrhizic acid;

839.97 — molecular weight of the monoammonium salt of glycyrrhizic acid;

P — content of monoammonium glycyrrhizinate in the WRS sample, %.

HPLC Results

Equal volumes of the secondary reference standard solution of monoammonium glycyrrhizinate and the test liquid extract (10 µL each) were separately injected into the chromatograph. Chromatograms were recorded and the areas of the main peaks were measured. The retention time of the secondary reference standard of monoammonium glycyrrhizinate was 6.8 min, which corresponds to the peak obtained in the chromatogram of the liquid extract at 6.8 min. The quantitative content (%) of glycyrrhizic acid in the liquid extract was calculated. The glycyrrhizic acid content in the liquid extract was found to be 0.89502 mg/mL.

The chromatograms obtained are presented in Figures 1 and 2.

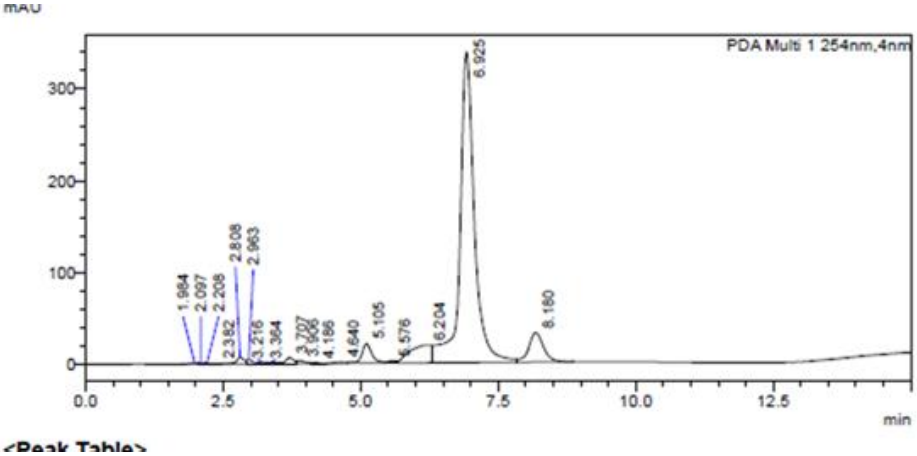

Fig. 1. Chromatogram of the working reference standard of monoammonium glycyrrhizinate

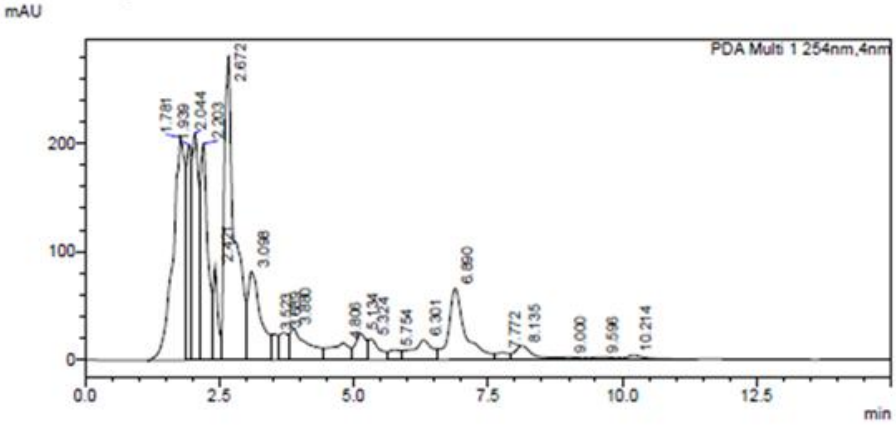

Fig. 2. Chromatogram of the test liquid extract sample

Determination of Alcohol Content by Gas Chromatography (GC)

Using the gas chromatography (GC) method, the alcohol concentration was determined. The tests were carried out under the following conditions: Shimadzu gas chromatograph (HS10); capillary column, 150 × 0.4 cm, packed with Porapak Q polymer sorbent, particle size 100–120 mesh; column temperature — 150 °C; injector temperature — 170 °C; detector temperature — 170 °C; carrier gas (nitrogen or helium) flow rate — 30 mL/min; flame ionization detector.

Test solution. An accurately measured amount of the test preparation sufficient to obtain a solution containing 4–6% ethanol by volume is placed in a 100 mL volumetric flask, 5.0 mL of propanol (internal standard) is added, mixed, the volume of solution is brought to the mark with water, and mixed. 10.0 mL of the resulting solution is placed in a 100 mL volumetric flask, the volume is brought to the mark with water, and mixed.

Reference standard solution. 5.0 mL of ethyl alcohol of not less than 95% (reference standard) and 5.0 mL of propanol (internal standard) are placed in a 100 mL volumetric flask, the volume is brought to the mark with water, and mixed. 10.0 mL of the resulting solution is placed in a 100 mL volumetric flask, the volume is brought to the mark with water, and mixed.

After the gas chromatograph was brought to operating conditions, 1–2 µL aliquots of the test solution and reference standard solution were successively injected into the injector, and not fewer than 3 chromatograms were recorded for each solution.

The reliability of the analytical results was verified by assessing the suitability of the chromatographic system with regard to:

– resolution (R) of the ethyl alcohol peaks (not less than 2.0);

– asymmetry factor (T) of the ethyl alcohol peak (must not exceed 2.0);

– relative standard deviation (RSD) (must not exceed 2.0).

Identification of ethyl alcohol in chromatograms of the test samples was performed by comparing retention times with those of the reference standard. In the chromatogram, the peak with a retention time of 5.95 min corresponds to the ethyl alcohol WRS. The results of the study are presented in Figures 3 and 4.

The ethyl alcohol content in the liquid extract was calculated by the formula:

$$X = \frac{S \cdot S'_0 \cdot 5{,}0 \cdot P}{S_0 \cdot S' \cdot V_{пр}},$$

where:

S and S' — peak areas of ethyl alcohol in the chromatograms of the test solution and reference standard solution, respectively;

S_0 and S'_0 — peak areas of propanol in the chromatograms of the test solution and reference standard solution, respectively;

Vprep — volume of the preparation taken for analysis, mL;

P — ethyl alcohol content in the reference standard, %.

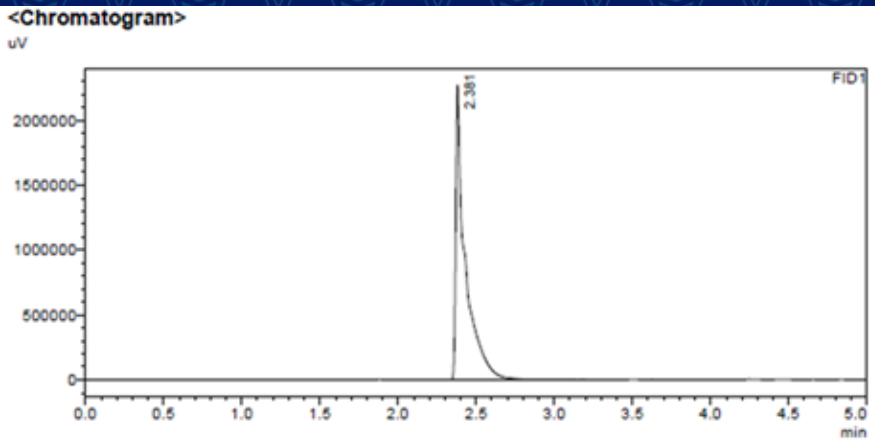

Fig. 3. Chromatogram of the ethyl alcohol reference standard

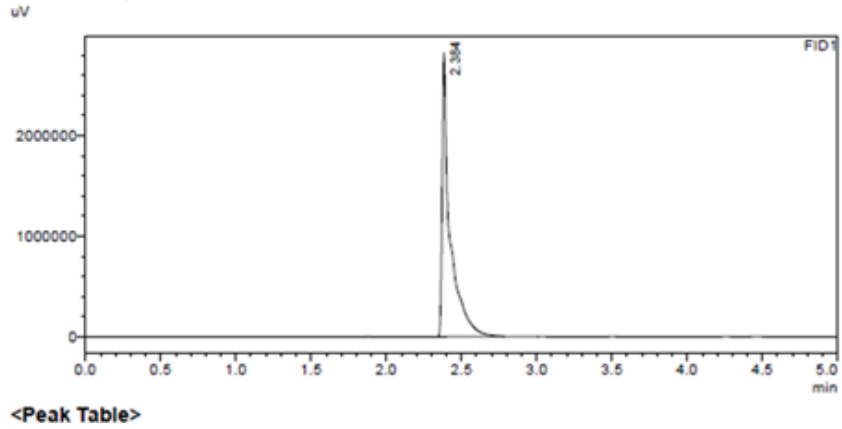

Fig. 4. Chromatogram of the test sample

Upon completion of the test, the alcohol concentration was calculated and found to be 22–23%.

Conclusions

In the course of the study, the principal quality indicators of the liquid plant extract containing echinacea herb, licorice root, and rosehip fruit were examined. Determination of dry residue demonstrated a sufficient content of dissolved substances, confirming that the extract meets the requirements for herbal preparations and indicating the preservation of biologically active components.

The results of the heavy metal analysis performed by inductively coupled plasma atomic emission spectrometry (ICP-AES) showed that the concentrations of toxicologically significant elements (Pb, Cd, Hg, As) are below the detection limits of the method and do not exceed the permissible sanitary-hygienic standards. Quantitative determination of glycyrrhizic acid was carried out by HPLC, and the alcohol content was determined by GC.

The results obtained indicate that the liquid extract under investigation meets the established quality and safety requirements and may be considered a promising candidate for further pharmacological and phytochemical studies.

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