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Quantitative Analysis of Cyclosieversioside F from Astragalus Pterocephalus

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ABSTRACT

Astragalus pterocephalus Bunge growing in Uzbekistan are a source of triterpene glycosides. The main triterpene glycoside, is a cycloartane type - cyclosieversioside F (astragaloside IV). To obtain a biologically active compound cyclosieversioside F with 95% purity, a proposed method involves extraction with ethanol. The optimal conditions for the isolation and separation of the amount of extract have been tried to obtain cyclosieversioside F. Quantitative analysis of the glycoside was carried out by high-pressure liquid chromatography, HPLC-ELSD.

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Introduction

The flora of Uzbekistan includes 275 plant species belonging to the genus Astragalus from the family Leguminosae (Fabaceae). The number of species belonging to the genus is 650 in all Central Asian Countries [1]. Plants of the genus Astragalus have been used in folk medicine since ancient times. In the literature, traditional use of this genus and biologically active compounds isolated from Astragalus is not enough.

Cardiovascular system diseases rank first in the world in terms of both prevalence and threat to life. Most of these diseases (coronary heart disease, myocardial infarction) are accompanied by metabolic disorders in the myocardium and blood vessels. For the treatment of patients with cardiac pathologies, cardioprotector drugs are used, these drugs contribute to the normalization of impaired metabolic processes in the body.

It is an urgent task for Uzbekistan to research the lice of the flora and the ingredients of these plants in terms of folk remedies used in traditional treatment. Cycloartane glycosides have cardiotonic activity, hypotensive, diuretic, sedative, analgesic and hypolipidemic effects, are non-toxic, and have a wide range of pharmacological effects.

The process of manufacturing drugs from natural plant materials is complex and multi-stage. Since as a result of plant extraction, not only the active substance, but also related substances (pigment dyes, chlorophylls, and others) pass into the solvent, the qualitative and quantitative yield of the substance will depend on the correctly chosen method for extraction and separation of plant materials.

Obtaining individual natural compounds with high yield and purity percentages is a requirement of the medical and pharmaceutical industries. Individual compounds are obtained by various chromatographic methods for the purification of biologically active compounds. Cyclosieversioside F (astragaloside IV) is a triterpene glycoside of the cycloartane series known in the literature (Fig. 1).

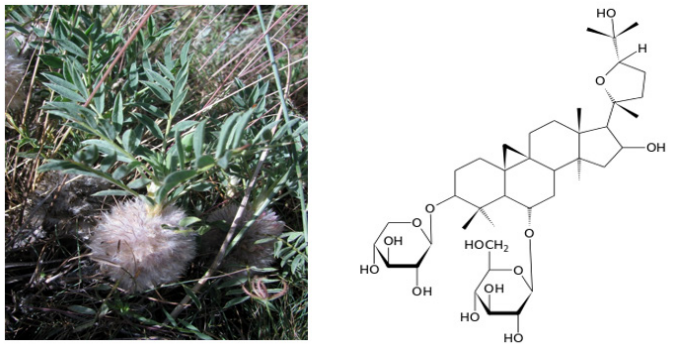

Figure 1: Chemical Structure of Cyclosieversioside F (Astragaloside IV) from Astragalus Pterocephalus Bunge

The glycoside was isolated from Astragalus pterocephalus of the Leguminosae family with a yield of more than 1% by weight of the air-dry raw material Cyclosieversioside F showed several

physiological activities: antiinflammotory it has pronounced antihypoxic properties when administered intravenously to patients with congestive heart failure, improves the contractile function of the left ventricle and slows down the heart rate and in vitro on a culture of human umbilical vein endothelial cells, stimulates proliferation and migration [2-6]. In studies conducted, cyclosieversioside F improves the condition of patients with acute myocardial infarction normalizes lipid metabolism disorders in atherosclerosis, and restores biochemical processes in myocardial cells [7, 8].

Cyclosieversioside F had an activating effect on the antioxidant system of the heart, contributed to a decrease in lipid peroxidation processes (the MDA level in the myocardium decreased by 40.7% under the action of the drug), as well as the optimization of nitric oxide metabolism in the heart muscle (the NO level increased 37.1%). It has been shown that cyclosieversioside F has a high hypocholesterolemic and hypolipidemic activity, contributes to a pronounced correction of the disturbed lipid metabolism in organs and tissues [9, 10].

Currently, research is being carried out to develop a method for obtaining the substance of cyclosieversioside F with 90% purity under technological conditions [11] . The study aimed to develop a quantitative method for determining the substance of cyclosieversioside F using Liquid chromatography HPLC-ELSD analysis (Fig.2)

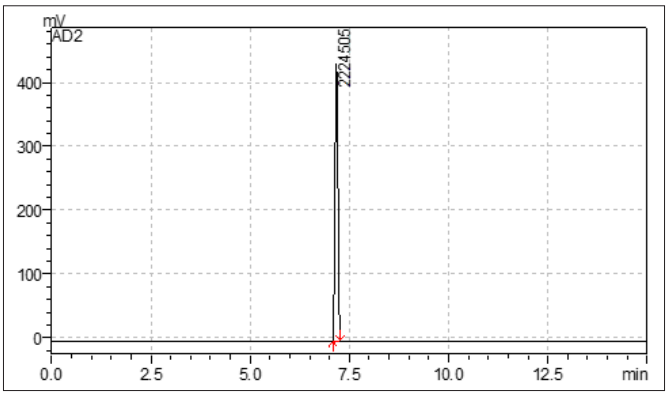

Figure 2: Cchromatography HPLC-ELSD Analysis of Cyclosieversioside F.

Materials and Methods

Chemicals

The chemicals Acetonitrile, methanol, chloroform, 2-Propanol, n-butanol, ethyl acetate, and ethanol were used of analytical grade.

Plant Material

Plant material A. pterocephalus was harvested during the flowering period in the Surkhandarya region of Uzbekistan. Crushed air-dry raw materials (4 kg) were extracted with 70% ethanol five times. Air-dry raw material A. pterocephalus was extracted at room temperature with a 70% alcohol solution. The solvent was distilled and concentrated to 1 liter, than diluted with an equal volume of water, and filtered to remove lipophilic substances. Next, the aqueous extract was treated with nonpolar solvents: chloroform, ethyl acetate, and n-butanol, successively extracting the number of extracts by liquid-liquid extraction.

In the laboratory of the chemistry of glycosides of the Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan, the foundations for the study of biologically active saponins have been laid [2]. Phytochemical studies of the species A. pterocephalus Bunge led to the isolation of the triterpenoid of the cycloartane series cyclosieversioside F which showed cardioprotective activity in animal experiments

The establishment of the structure of cyclosieversioside F was carried out by chemical and physicochemical methods: determination of the elemental composition, melting point Separation of an individual glycoside was carried out by chromatographic methods of analysis. By IR, 1H and 13C NMR, DEPT, COZY, HSQC, HMBC, and ROESY data were obtained, which made it possible to identify the chemical structure [7,9,10].

Liquid Chromatography HPLC-ELSD

Test solution. 2.5 mg of the dried extract dissolved in 1 mL of methanol. Reference solution (stock). 1.5 mg of standard cyclosieversioside F dissolved in 0.5 mL methanol and dilute with the same solvent to get 0.3, 0.45, and 0.6 mg/mL dilutions. Shimadzu HPLC System coupled with ELSD detector was used for the analysis of cyclosieversioside F. Column: size: l = 0.25 m, Ø = 4.6 mm, stationary phase: Octadecylsilyl silica gel (5 µm), temperature: 40 °C. – mobile phase A: water; mobile phase B: acetonitrile; (50:50), flow rate: 0.5 mL/min. Detection: Evaporative Light-Scattering detector; the following settings have been used for analysis: Carrier gas: nitrogen, pressure: 350 KPa, gain: 10, Evaporator temperature: 50 °. Injection: 2 µL of the test solutions and reference solutions Calculateing the percentage content of cyclosieversioside F using the equation from calibration curve obtained for the reference compound (Figure 2). In ELSD detectors, the concentration and peak area values are not always linear. Therefore, standard concentration values with a linear curve were used in the calculation of the calibration equation.

Results and Discussion

Qualitative analysis of cyclosieversioside F. When performing experiments on glycoside isolation, the authenticity of cyclosieversioside F is determined by thin layer chromatography (TLC) as follows: a sample of cyclosieversioside F (accurately weighed 0.05) is dissolved in 0.5 ml of chloroform-methanol (1:1) solvent system: From the prepared solution, a capillary is applied to a plate coated with silica gel with a 13% gypsum content. The driving phase of the chromatogram is the solvent system: chloroform-methanol-water (70:23:4). The substance on the chromatogram is shown by spraying with a 20% alcohol solution of phosphotungstic acid and followed by heating at 110 ºC in a thermostat for 5-7 minutes. Cyclosieversioside F appears as a brick red spot, which becomes brown after a while. R_f is 0.3

Table 1: Solvent System for Recrystallization of Cyclosieversioside F

№	Solvent system	The dry weight of extracts %	The content of cyclosieversioside F in the extracts, %
1.	Methanol-chloroform (1:9)	1,54	93,2
2.	Methanol-chloroform (1:9)	1,45	98,6
3.	Ethanol-chloroform (1:9)	1,33	92,4
4.	Ethanol - ethyl acetate (1:9)	1,20	96,5
5.	2-Propanol-ethyl acetate (1:9)	1,39	90,5

As can be seen from Table 1, data with a high percentage of the yield of an individual glycoside, cyclosieversioside F in the substance, were obtained in experiments № 2 and № 4. And the yield of the substance in experiment № 2 is greater than № 4. Therefore, for the recrystallization of the technical product - cyclosieversioside F - the solvent system methanol - ethyl acetate in the ratio (1:9) was chosen. Quantitative analysis of the determination of cyclosieversioside F. HPLC with evaporative light scattering detector ELSD. Samples

of the number of extracts obtained by isolating the glycoside in an ethanol extract, as well as obtained by elution with butanol and ethyl acetate from an aqueous extract of plant materials The percentage of cyclosieversioside F was calculated using the equation from the calibration curve obtained for the reference compound (Fig. 3).

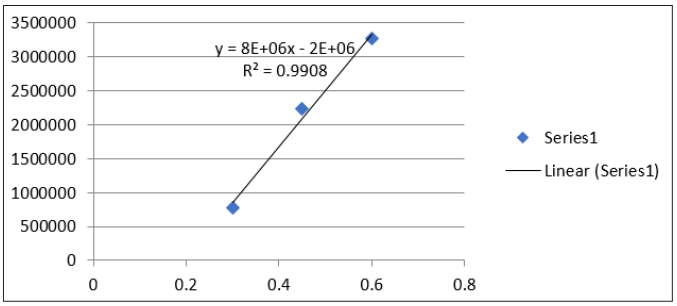

Figure 3: Calibration Curve of Standart Cyclosieversioside F

The elution was carried out in isocratic mode (acetonitrile: water, 50:50). On the HPLC-ELSD chromatogram, the peak corresponds to the retention time for the standard glycoside cyclosieversioside F. Seven tests of experiments were carried out to assess the quantitative composition of extracts by the method RP-HPLC with ELSD detector, which is shown in the Table 2.

Table 2: Liquid-Chromatographic Analysis of Cyclosieversioside F in Extracts/Fractions

Sample	cyclosieversioside F content, mg/100 mg_{extr}
Sample № 2	16,17
Sample № 3	6,89
Sample № 4	15,27
Sample № 5	11,09
Sample № 6	ND
Sample № 7	6,30
Sample № 8	6,27
ND: not detected	

Sample 2 - Butanol extract obtained by extraction in the system: aqueous extract – butanol/ Sample 3 - aqueous extract after fractionation with chloroform. Sample 4 - extract obtained by extracting the plant with 70% ethanol five times. Sample 5 - aqueous extract after fractionated with ethyl acetate. Samples 6 and 7 are extracts obtained by extraction with 70% ethanol.

Conclusion

To obtain cyclosieversioside F with a 95% yield and above, recrystallization from a solvent system: methanol-ethyl acetate (1:9) is necessary. A method was developed for the quantitative determination of cyclosieversioside F by HPLC with an evaporative light scattering detector ELSD in samples of the sum of extractive substances from the plant A. pterocephalus.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Supplements

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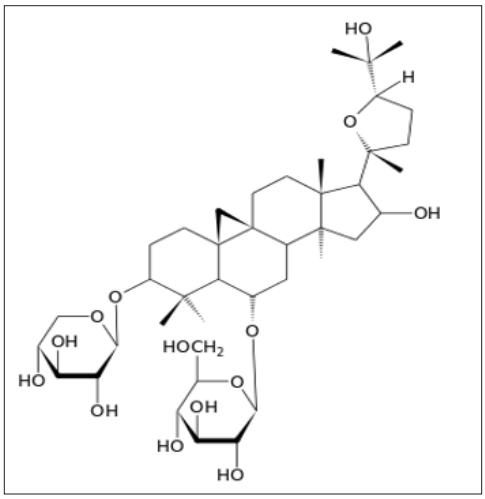


Figure 1: Chemical structure of cyclosieversioside F (Astragaloside IV)

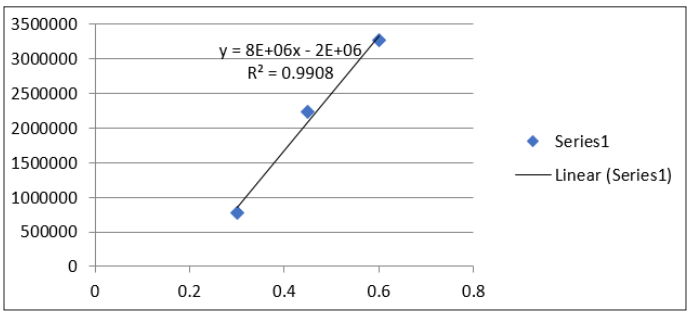
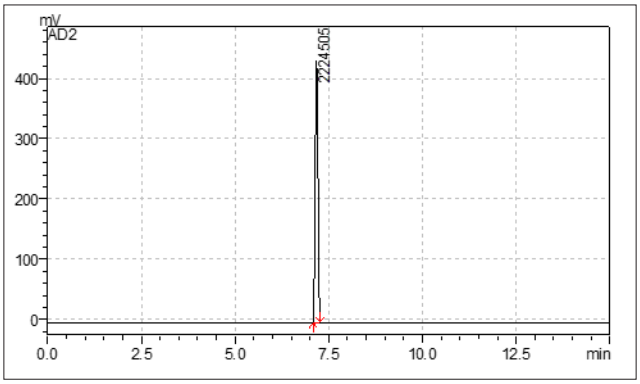
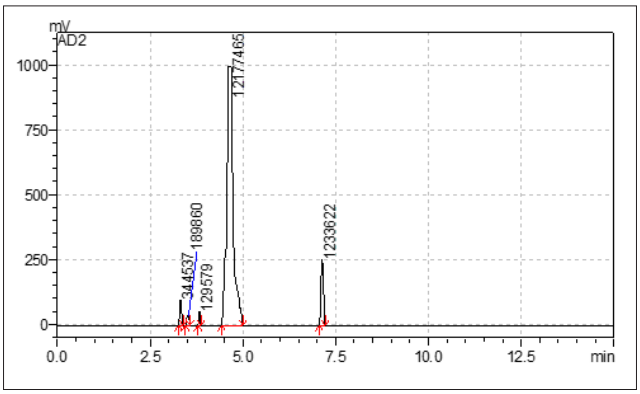


Figure 2: Calibration Curve of standart cyclosieversioside F



Cyclosieversioside F (Astragaloside IV)



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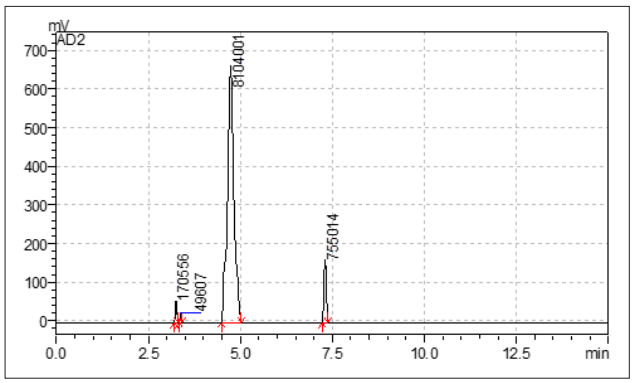

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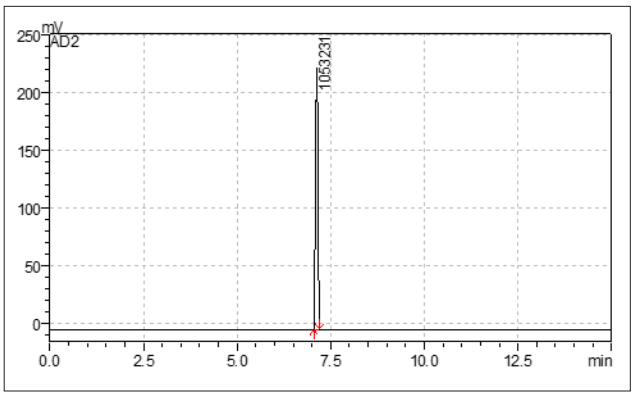

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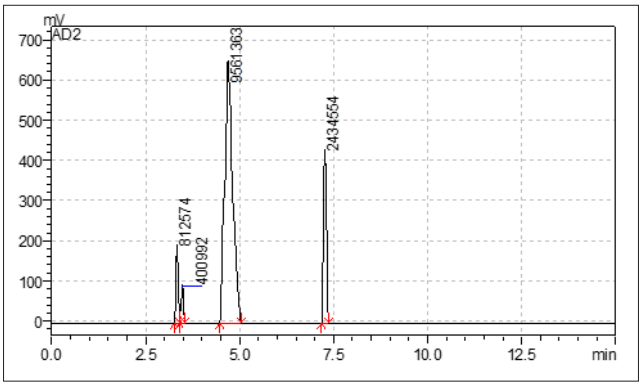

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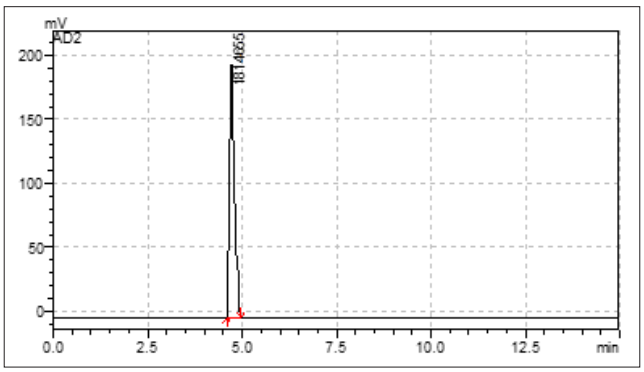

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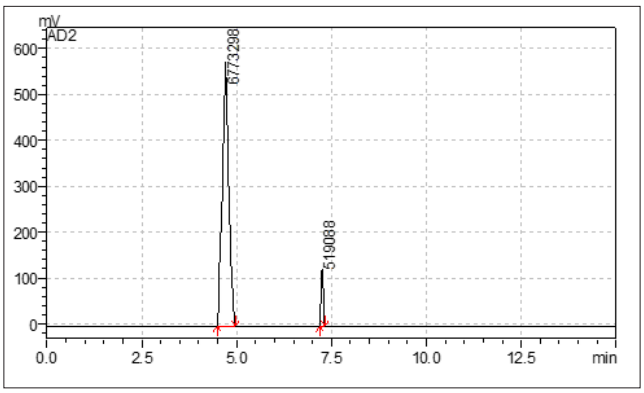

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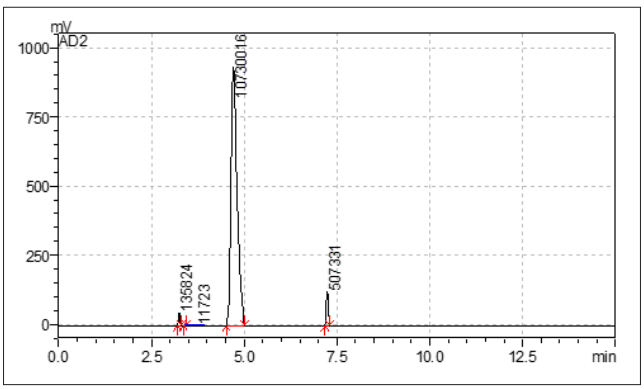

Sample No: 8

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