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DEVELOPMENT AND ESTIMATION OF VALIDATION CHARACTERISTICS FOR THE QUANTITATIVE DETERMINATION OF GLYCOALKALOIDS IN *SALSOLA COLLINA L.* EXTRACTS

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ABSTRACT

In the course of the research the presence of glycoalkaloids in aqueous and aqueous-alcoholic extracts of *Salsola collina L.* has been determined. The method for extraction-photometric determination of glycoalkaloids in the aerial part of *Salsola collina L.* equivalent to salsoline has been developed. It is based on formation of ion associates of alkaloids with bromothymol blue and subsequent determination of their optical density at the wavelength of 422 nm. The quantitative content of alkaloids in the extracts of *Salsola collina L.* has been determined using the method developed. The validation characteristics of the method such as stability of solutions and linearity have been studied; which confirm the method's correctness and allow to recommend it for use when analysing alkaloids of tetrahydroisoquinoline series.

Key words: Quantitative determination, Glycoalkaloids, *Salsola collina L.*

INTRODUCTION

Natural alkaloids that are numerous and varied by their structure increasingly attract the attention of scientists. The interest to this group can be explained by a wide distribution in nature and a large range of the pharmacological action, such as antiviral, antibacterial, antifungal, antidiabetic, antiulcer, sedative, anticonvulsive, analgesic, neuroprotective, anticancer, antioxidative, hepatoprotective action, etc[1-5].

When studying the chemical composition of *Salsola collina L.* (hill-growing saltwort) it has been found that the plant contains steroids, flavonoids, sugars, glycoalkaloids, etc[1]. Salsoline and salsolidine are the main representatives of glycoalkaloids in the raw material under research.

The literature describes many methods for quantitative determination of alkaloids, in particular the method of high performance liquid chromatography[6,7], high-performance thin-layer chromatography[8], capillary isotachophoresis, which can separate and determine aglycones, but does not provide separation of individual glycoalkaloids differing by sugar part[9]. There is also the method of electrochemical determination of glycoalkaloids using carbon nanotubes of benzeneboronic acid[10].

The aim of our research is to develop the alternative method for quantitative determination of glycoalkaloids of *Salsola collina L.* by the extraction-photometric method based on formation of ion associates with a dye.

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EXPERIMENTAL

The object of research is the air dry powdered aerial part of *Salsola collina L.* of *Chenopodiaceae* family. The raw material was harvested in 2012 in Barnaul in the period of the utmost flowering of plants, and aqueous or aqueous-alcoholic (30%, 50% and 70%) extracts were prepared from the plant raw material in the ratio of 1:20.

Measuring glassware of class A and reagents meeting the requirements of the State Pharmacopoeia of Ukraine (SPhU), "AXIS" balances, Evolution 60S spectrophotometer, TLC plates with the layer of silica gel GF_{254} were used for the work.

The method for quantitative determination of glycoalkaloids in the raw material: Evaporate 5 mL of the extract on a water bath to a dry residue. After cooling transfer the residue quantitatively into a separating funnel, add 10 mL of a buffer solution with pH 7.5, 1.5 mL of bromothymol blue solution, 10 mL of chloroform and shake for 3 min. Filter the chloroform extraction through a filter wetted by chloroform into a 50 mL volumetric flask. Repeat the extraction twice with 10 mL of chloroform filtered through the same filter into the same flask. Into the flask add 10 mL of boric acid solution, dilute to the volume with 96% ethanol, mix and measure the optical density of the solution obtained on the spectrophotometer at the wavelength of 422 nm. The compensation solution was prepared similarly as the test solution, without adding bromothymol blue.

Preparation of the solution of the salsoline standard sample: Transfer quantitatively 0.117 g (an accurately weighed portion) of salsoline hydrochloride and 10 mL of water into a separating funnel, add 0.5 mL of the concentrated solution of ammonium hydroxide and extract consistently with 20, 15, 15 mL of chloroform while shaking for 3 min. Filter the chloroform extractions through a filter wetted by chloroform into a 100 mL volumetric flask and dilute the solution to the volume with chloroform.

Transfer 10 mL of the solution obtained into a 100 mL volumetric flask and dilute the solution to the volume with chloroform (solution A). 1.0 mL of the reference solution contains 0.0001 g of salsoline.

Transfer 3 mL of the solution A obtained into a separating funnel, add 7 mL of chloroform, 1.5 mL of bromthymol blue solution, 10 mL of phosphate buffer solution with pH 7.5 and shake for 3 min. Filter the chloroform extraction through a filter wetted by chloroform into a 50 mL volumetric flask. Repeat the extraction twice with 10 mL of chloroform filtered through the same filter into the same flask. Into the flask add 10 mL of boric acid solution, dilute to the volume with 96% alcohol and mix.

Preparation of boric acid solution: Dissolve 0.5 g of boric acid in the mixture of 25 mL of 96% ethanol and 20 mL of water when heating in a 250 mL volumetric flask, cool and dilute the solution to the volume with 96% ethanol.

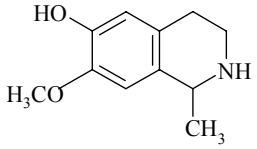
Test solution: Place 1 mL of the alcoholic extracts of *Salsola collina L.* into a 10 mL volumetric flask and dilute to the volume with 96% alcohol.

Reference solution: Solution A of salsoline prepared for quantitative determination.

Apply 20 mcl of the test solution and 20 mcl (10 mcg) of the reference solution as bands with the length of 2 cm on the starting line of the chromatographic plate with the size of 20 x 20. Dry the plate with the samples applied in the air for 15 min, place into the chamber with the mixture of such solvents as butanol *R* – glacial acetic acid *R* – water *R* (4:1:2) and chromatograph using ascending technique. Develop over a path of 15 cm from the starting line, get the plate out of the chamber, dry in the air for 30 min, treat with the solution of potassium tetraiodobismuthate, dry in the flow of warm air and examine.

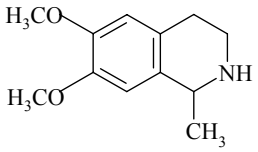
RESULTS AND DISCUSSION

Alkaloids essentially affect the pharmacological properties of plants and their drugs; besides they can increase toxicity, therefore, normalization of their content is required. It is known that hill-growing saltwort contains alkaloids, among which such glycoalkaloids as salsoline and salsolidine are in a large number[2-4]. They are found in the plant as a mixture of optically active and racemic forms. Moreover, the optical form of salsoline is dextrorotary, and that of salsolidine is laevorotatory.



I

Salsoline



II

Salsolidine

By their chemical properties the glycoalkaloids isolated are strong secondary bases, that is why they readily form salts with acids. The presence of a phenolic hydroxyl and a methoxy group in molecules is responsible for the ability to oxydate. The presence of alkaloids was determined in the extracts under study by the typical reactions of identification and by the method of thin-layer chromatography. Positive reactions with Mayer's reagent and Wagner's reagent indicate about the presence of alkaloids in the raw material[5].

Determination of alkaloids by the method of thin-layer chromatography was carried out in such systems of solvents as butanol-glacial acetic acid (100:5) or toluene-acetone-concentrated ammonia-ethanol (40:52:2:6) using the solution of potassium tetraiodobismuthate (Dragendorff's reagent) for development. Chromatograms were examined before and after development of the reagent. The sample of salsoline hydrochloride was used as a reference standard.

On the chromatogram of the test solution, there were the areas corresponding to the areas on the chromatogram of the reference solution by coloration. In normalization of the raw material identification the presence of other areas of different size and coloration is permitted.

Since the method of quantitative determination must be simple, sensitive and rather accurate, we tried to develop the method for quantitative determination of glycoalkaloids of *Salsola collina L.* by the extraction-photometric method based on formation of ion associates with dyes. As a reference standard salsoline isolated for the first time from *Salsola Richteri Kar*. was used.

To develop the method of quantitative determination at first the test for standard sample of salsoline hydrochloride was performed. Under the action of ammonia, salsoline hydrochloride was transferred into the base extracted with an organic solvent (chloroform), then reactions of the ion associates formation with such dyes as methyl orange and bromthymol blue were carried out. The absorption spectrum of the ion associate obtained with methyl orange at pH 5.5 was recorded in the range from 350 nm to 500 nm in the cuvette with the layer thickness of 1 cm.

As can be seen from Fig. 1, a rather wide flat maximum is observed on the absorption spectrum in the range of 423 nm; it can be used as an analytical band. It has been determined that when conducting the reaction in more alkaline medium (in the buffer solution with pH 6.0) the optical density in the maximum slightly increases, and it testifies the impact of pH on formation of an ion associate. With further research it has been found that the optical density of the solutions obtained sharply decreases already in 15 min, and it may indicate the instability of the ion associates obtained.

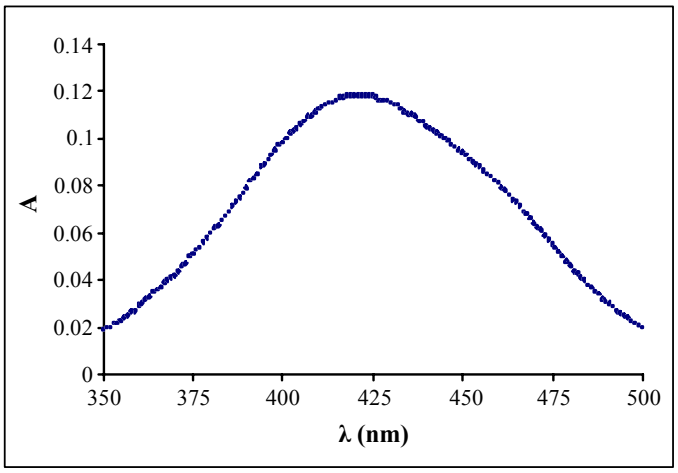


Fig. 1: The absorption spectrum of the chloroform solution of the ion associate of salsoline with methyl orange

In the absorption spectrum of the chloroform solution of the ion associate of salsoline with bromthymol blue two rather flat maxima were observed at the wavelengths of 333 nm and 422 nm. The maximum at the wavelength was more specific and it was selected as an analytical band (Fig. 2).

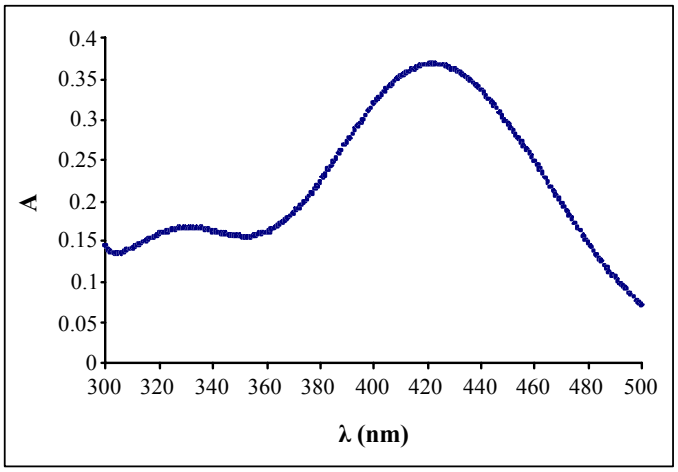


Fig. 2: The absorption spectrum of the chloroform solution of the ion associate of salsoline with bromothymol blue

Therefore, it was decided to use bromothymol blue as a dye for further research (Fig. 2).

To determine the quantitative content of alkaloids, at first the extracts were evaporated on a water bath, the reaction of the ion associates formation with bromothymol blue was carried out, extracted with chloroform, and the absorption spectrum of the solutions obtained was studied in the range from 350 nm to 500 nm in the cuvette with the layer thickness of 1 cm (Fig. 3).

The experimental data obtained (Fig. 3) testify that in all extracts studied (aqueous, alcoholic with the alcohol concentration of 30%, 50% and 70%) rather wide flat maxima are observed in the range from 418 nm to 424 nm, they are close to the wavelength corresponding to the absorption maximum of the ion associate of the reference solution-salsoline. Thus, the method can be used for analysis of extracts from *Salsola collina L*.

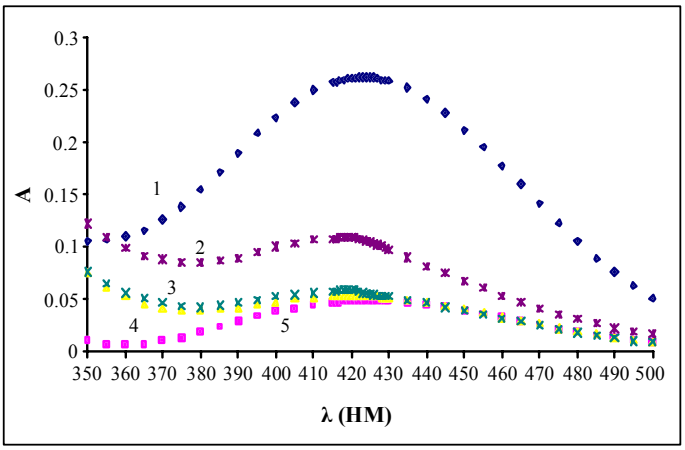


Fig. 3: The absorption spectrum of chloroform solutions of the ion associates with bromothymol blue and: 1 – salsoline; 2 – 70% alcoholic extract of hill-growing saltwort; 3 – 50% alcoholic extract of hill-growing saltwort; 4 – 30% alcoholic extract of hill-growing saltwort; 5 – aqueous extract of hill-growing saltwort

To check the effect of other BAS of the raw material the chloroform extraction from the extract of *Salsola collina L.* was prepared and its absorption spectrum was recorded in the range from 300 nm to 500 nm (Fig. 4).

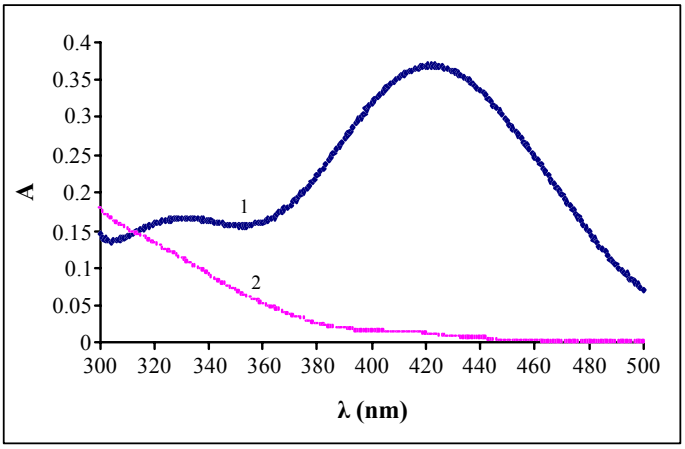


Fig. 4: The absorption spectrum of 1 – the chloroform solution of the ion associate of salsoline with bromthymol blue; 2 – the chloroform extract from hill-growing saltwort extract

The data in Fig. 4 indicate that other BAS of the raw material affect the absorption spectrum to a certain extent; therefore, we recommend to use the chloroform extract from the raw material without a dye as a compensation solution.

During the study, it has been found that the content of glycoalkaloids equivalent to salsoline is 0.0156% for the aqueous extract, for the extract prepared from 30% alcohol – 0.0176%, from 50% alcohol – 0.0192%, from 70% alcohol – 0.0384%. Thus, the largest number of alkaloids is extracted with 70% alcohol.

The number of validation characteristics of the method has been also studied. The stability test for chloroform solutions of ion associates was performed within 60 min for the test sample and the reference standard. The optical density was measured every 15 min three times with removing the cuvette. The mean values of the optical density are given in Table 1.

Table 1: The stability study of the analytical solution

No. of solution	Term of stability studies, t (min)					Mean
	0	15	30	45	60	
A	0.118	0.118	0.118	0.118	0.117	0.118
A_{st}	0.294	0.294	0.294	0.294	0.293	0.294

It has been found that the analytical solution is stable within an hour, and it is sufficient for determining the optical density.

Linearity of the method was studied using 70% extract of *Salsola collina L.* containing a larger number of alkaloids, for 9 separate weighed portions within the range of concentrations from 80% to 120%. For each weighed portion the reaction of the ion associate formation was carried out, extracted with chloroform, and the optical density of the chloroform solutions obtained was measured three times with removing the cuvette. The results of the linear dependence determination obtained for chloroform solutions of ion associates of salsoline with bromothymol blue are given in Table 2 and Fig. 5.

Table 2: The results of studying linearity of the quantitative determination method for alkaloids in the extract

No. of the test solution	Introduced in % to the concentration of the reference solution (Xi_{act} %)	Optical densities Ai (Ast = 0.142)	Found in % to the concentration of the reference solution (Yi%)	Found in % to the introduced Zi = 100 (Yi/Xi)
1	80	0.114	80.28	100.35
2	85	0.122	85.92	101.08
3	90	0.129	90.85	100.94
4	95	0.135	95.07	100.07
5	100	0.143	100.70	100.70
6	105	0.148	104.23	94.75
7	110	0.155	109.15	94.92
8	115	0.162	114.08	95.07
9	120	0.171	120.42	100.35
Mean, Z%				98.69
Relative standard deviation, Sz%				2.8528
Relative confidence interval Δ as % = t (95%.8)*Sz				5.3048
Critical value for convergence of results Δ as %				2.24%
Systematic error δ				-1.31
Criterion of the systematic error insignificance 1) δ ≤ Δ as /(g)^ 0.5 = 0.72/√9. 2) if it is not satisfied 1), then δ ≤ 0.72				0.24
The overall conclusion of the method				Correct

The results obtained testify the presence of the linear dependence within the range of concentrations of glycoalkaloids in the extract from 80% to 120%, the slope coefficient of the linear dependence (b) equals 0.9695, and the free member of the linear dependence (a) – 3.1299. The presence of the linear dependence is characterized by the value of the correlation coefficient (r), which is 0.9989. The value RSD_o characterizes the standard deviation of the straight line $Y_i = b \cdot X_i + a$ in relation to the nominal values of concentration and the value to be measured. The standard deviation RSD_{range} characterizes scatter of points X_i and Y_i from the mean values, respectively, and equals 13.6931.

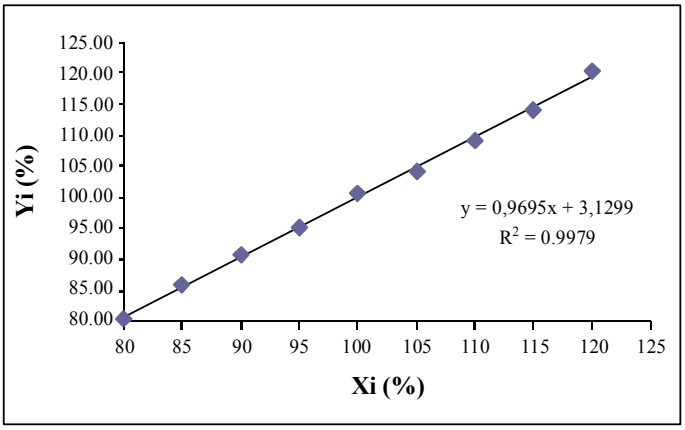


Fig. 5: The plot of the linear dependence of the quantitative determination method for alkaloids in the extract

The validation characteristics studied confirm correctness of the method chosen.

CONCLUSION

(i) The presence of glycoalkaloids in aqueous and aqueous-alcoholic extracts of *Salsola collina L.* has been determined by chemical reactions and TLC method.

(ii) The method for extraction-photometric determination of glycoalkaloids in the overground part of *Salsola collina L.* equivalent to salsoline has been developed. It is based on formation of ion associates of alkaloids with bromthymol blue and subsequent determination of their optical density at the wavelength of 422 nm.

(iii) The validation characteristics of the method such as stability of solutions and linearity have been studied; they allow to recommend this method for use when analysing alkaloids of tetrahydroisoquinoline series.

(iv) The quantitative content of alkaloids in the extracts of *Salsola collina L.* studied has been determined by the method developed.

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