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O.S. Panteleieva[a], Ch.P. Akritidou[b], V.V. Boynik[b], V.I. Tkach[a]

AMPEROMETRIC METHOD OF DETERMINING THE SUM OF ALKALOIDS IN DRY EXTRACTS OF MULTIFOLIATE LUPIN (LUPINUS POLYPHYLLUS) SEEDS AND ROOTS USING 12-MOLYBDOPHOSPHATIC HETEROPOLY ACID AS AN ANALYTICAL REAGENT

[a] Ukrainian State University of Chemical Technology, Dnipropetrovsk
[b] National University of Pharmacy, Kharkov

The article describes the results of the research on the quantitative determination of the sum of alkaloids in dry extracts of lupin seeds and roots by means of amperometric titration using heteropolyanions (HPA) of Keggin structure as an analytical reagent. As Keggin heteropolyanions have particular properties (i.e. constant composition, ion-exchangeable capability and redox power), they can form stable ion-associates with organic nitrogen containing cations which are readily soluble in organic solvents and characterized by a very low solubility in water. The interaction between organic cations of alkaloid sum (ΣAlc^{1+}) and heteropolyanion $PMo_{12}O_{40}^{3-}$ of 12-molybdophosphatic heteropoly acid ($3\Sigma Alc^{1+}+PMo_{12}O_{40}^{3-}-(\Sigma Alc)_3PMo_{12}O_{40}\downarrow$) is investigated. To determine the composition of ion-associates formed, the ratio of reactants is specified using amperometric titration with endpoint indication according to the strength of the diffusion current of Keggin $PMo_{12}O_{40}^{3-}$ electroreduction. The results testify that the organic cations of alkaloid content in extracts of lupin seeds and roots interact with the heteropolyanion of 12-molybdophosphatic heteropoly acid in the molecular ratio $\Sigma Alk^{1+}:PMo_{12}O_{40}^{3-}=3:1$ and the reaction under study ($\Sigma Alk^{1+}+PMo_{12}O_{40}^{3-}\rightarrow(\Sigma Alk)_3PMo_{12}O_{40}\downarrow$) proceeds stoichiometrically in aqueous solution in the range of pH 4.4 to 5.7 for the extracts of lupin seeds and of pH 5.2 for the extracts of lupine roots, correspondingly, with the formation of stable and marginally soluble ion-associates. The developed methods of the quantitative determination of alkaloids sum in dry extracts of lupine seeds and roots by means of amperometric titration using 12-molybdophosphatic heteropoly acid as an analytical reagent are notable for their simplicity, speed and sufficient sensitivity.

Keywords: amperometric titration, quinolizidine alkaloids, sum of alkaloids, dry extracts of lupin seeds and roots.

Introduction

Raw material of lupin is a valuable source of phospholipids and digestible protein. The unique character of lupin is in the multiple functionalities of its application. It is a feeding, green manure and food crop as well as a decorative culture. Nowadays, lupin is considered to be prospective raw material for nutritional care and pharmaceutical production [7].

Alkaloids are found in all parts of lupin raw material with the largest amount found in seeds, flowers and leaves. The content of alkaloids in the plant organs vary over the vegetative period. By the period of ripening in seeds there are 5 to 10 times as many alkaloids as in herbage [1–2,6–7]. The content of alkaloids changes according to type of lupin; the main alkaloids are the following: lupanine ($C_{15}H_{24}ON_2$), lupinine ($C_{10}H_{19}ON$), sparteine and pachycarpine ($C_{15}H_{24}N_2$), they also contain anagirine ($C_{15}H_{20}ON_2$), isolupanine ($C_{15}H_{26}N_2$), isosparteine ($C_{15}H_{24}ON_2$), hydroxylupanine ($C_{15}H_{20}O_2N_2$) and others.To calculate the molar-mass average of alkaloids in the extracts of lupine seeds and roots, the four main alkaloids were chosen: lupanine – $C_{15}H_{24}ON_2$, lupinine – $C_{10}H_{19}ON$, pachycarpine – $C_{15}H_{24}N_2$, hydroxylupanine – $C_{15}H_{20}O_2$ N_2. Their total molar mass is 909 g/mol. To calculate the sum of alkaloids in lupine extracts, after the experimental data the molar-mass average of alkaloids was calculated which 909÷4=227.25 g/mol (M_{Salk}).

Currently, a great number of methods of quantitative determination of the sum of alkaloid in lupine are available, including gravimetry, colorimetry, high-performance liquid chroniatography (HPLC), high pressure gas chroniatography and others [7].

However, in most cases these methods are too much expensive, labour-intensive and inaccessible. We suggest a rapid (10–12 minutes), sensitive and simple method of quantitative determination of the sum of alkaloid in lupine through amperometric titration using heteropolyanions (HPA) of Keggin structure as an analytical reagent – standard solution of 12-molybdophosphatic heteropoly acid [3–4].

Experimental

The following chemical agents were used in the work:

1. MPA (12-molybdophosphatic heteropoly acid) $H_3PMo_{12}O_{40}{\cdot}26H_20$, reagent grade;

To prepare 100.00 ml of MPA solution with concentration 10^{-3} mol/l MPA sample weighing 0.2294 g was dissolved in distilled water in a 100.00 ml retort. The solution was heated in bain marie to achieve complete dissolution of the sample.

2. Dry extracts of lupine seeds and roots (the sum of the four main constituents of lupine alkaloids: lupanine – $C_{15}H_{24}ON_2$, lupinine – $C_{10}H_{19}ON$, pachycarpine – $C_{15}H_{24}N_2$, hydroxylupanine – $C_{15}H_{20}O_2N_2$).

To prepare 25.00 ml of dry extract of lupine seeds, a sample of the extract weighing 0.2502 g was dissolved in distilled water in a 25.00 ml retort.

To prepare 10.00 ml of dry extract of lupine roots, a sample of the extract weighing 1.007g was dissolved in distilled water in a 10.00 ml retort.

Results and discussion

The research provides quantitative determination of the sum of alkaloids in dry extracts of lupine seeds and roots through amperometric titration using heteropolyanions (HPA) of Keggin structure as an analytical reagent [3,4] which possess particular properties (constant composition, ion-exchangeable capability and redox power), are able to form firm ion-associates with organic nitrogen containing cations which are readily soluble in organic solvents and are characterized by very low solubility in water.

The interaction between the organic cations of the alkaloid sum (©Alc^{1+}) and heteropolyanion $PMo_{12}O_{40}^{3-}$ of 12-molybdophosphatic heteropoly acid was investigated

$$3\Sigma Alc^{1+}+PMo_{12}O_{40}^{3-}-(\Sigma SAlc)_3PMo_{12}O_{40}\downarrow$$

To define the composition of ion-associates formed, the ratio of reactants is studied using amperometric titration with endpoint indication according to the diffusion current strength of Keggin $PMo_{12}O_{40}^{3-}$ electroreduction [3,4].

The voltamperometric studies of electrochemical behaviour of the organic cations of the alkaloid sum ($SAlc^{1+}$) testified that they do not constitute an electroactive chemical compound during the cathodic polarization within an interval from +0.5 V to –0.5 V whereas the analytical reagent, heteropolyanion $PMo_{12}O_{40}^{3-}$, gives clear rise to a wave of electrochemical reduction of two molybdenum atom [4]:

$$PMo^{VI}{}_{12}O_{40}^{3-}+2\bar{e}=PMo^{V}{}_2Mo^{VI}{}_{10}O_{40}^{5-}$$

Assuming that a reaction forming slightly soluble compound occurs between the substance defined and titrant with the titrant being electroactive, it is possible to titrate amperometrically the organic cations of the alkaloid sum ($SAlc^{1+}$) with MPA aqueous solution with endpoint indication according to the strength of diffusion current of electroreduction of Keggin $PMo_{12}O_{40}^{3-}$.

The direct amperometric titration of the organic cations of the alkaloid sum ($SAlc^{1+}$) is conducted using the electrochemical cell which consists of the solution studied and electrode system: an indicator electrode, i.e. a frontal graphite rotating electrode, and a reference electrode, i.e. a saturated calomel half-cell. The aliquot of the prepared solution of the lupine dry extracts which contain the sum of the organic cations of the alkaloid sum ($\Sigma Alc1+$) (2.00 ml), is put into the electrochemical cell; with diluted acid solution (H_2SO_4) and alkaline solution (NaOH) the pH of the solution is adjusted to 4.00–5.50; superimposed voltage +0.05 V is applied to the electrodes and the value of zero current is fixed within 2–3 minutes. The titration is conducted with the preliminarily prepared 10^{-3} mol/l MPA aqueous solution in 0.2 ml portions. The value of the strength of diffusion current is fixed within 30–35 seconds after adding the titrant. The amperometric titration is completed after determining the constant value of the diffusion current and the volume of the titrant used for the titration is defined graphically on the titration curve (Figure1).

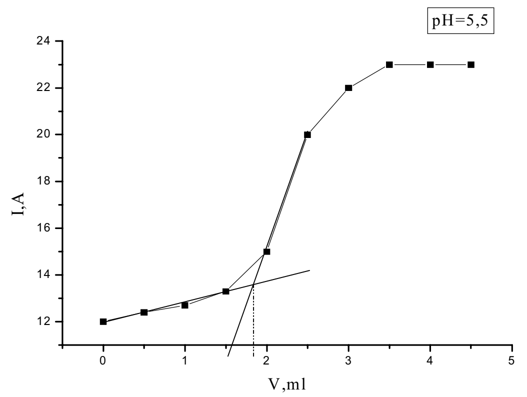

Figure 1. The curve of amperometric titration of the alkaloid sum in the lupine seed extract with MPA solution: $V_{\Sigma alk.}$=2.00 ml, $C_{MPA}=10^{-3}$ mol/l, solution pH 5.5

The calculation of the content of the alkaloid sum in the lupine seed extract was done after the following formula:

$$m=\frac{C_{MPA}\cdot V_{MPA}}{1000}\cdot 3\cdot M_{\Sigma alk.}$$

where C_{MPA} is the molar concentration of 12-molybdophosphatic heteropoly, mol/l; V_{MPA} is the volume of the titrant used for the titration, ml; $M_{\Sigma alk.}$ is the molar mass of the alkaloid sum which equals 227.25 g/mol; 3 is the stoichiometric coefficient of the reaction of interaction between HPA $PMo_{12}O_{40}^{3-}$ and the alkaloid sum

$$m=\frac{1{,}7\cdot 10^{-3}}{1000}\cdot 3\cdot 227.25=0.001160\text{g}.$$

m is the mass of the alkaloid sum in 2 ml.

$$x_1=\frac{25\cdot 0.0060}{2}=0.145\text{g}.$$

x_1 is the mass of alkaloids in 25 ml.

$$x_2=\frac{0.145\cdot 100}{0.2502}=5.795\%$$

x_2 is the percentage of the alkaloid sum in the lupine seed extract.

The results of the amperometric titration suggest that the organic cations of the alkaloid sum in the lupine seed extract interact with heteropolyanion of 12-molybdophosphatic heteropoly acid in molecular ratio $\Sigma Alk.^{1+}:PMo_{12}O_{40}^{3-}=3:1$ and the reaction studied occurs stoichiometrically in aqueous solution within the limits pH 4.4–5.7

$$\Sigma Alk.^{1+}+PMo_{12}O_{40}^{3-}\rightarrow(SAlk.)_3PMo_{12}O_{40}\downarrow$$

forming stable marginally soluble ion-associates.

The results of the quantitative determination of sum of alkaloids in dry extracts of lupine seeds through amperometric titration with pH=5.5 are given in Table 1.

Table 1

The metrological characteristics of the quantitative determination of the alkaloid sum in dry extracts of lupine seeds through amperometric titration with pH 5.5 (n=7, P=0.95)

Input, mg (theor.)	Found, mg	Found, %	Metrological characteristics
1.160	1.165	100.43	$\overline{X}$=99.83 S^2=0.61 S_x=0.23 S_r=0.09 x±Δ=99.83±0.57
	1.165	100.43	
	1.172	101.03	
	1.152	99.31	
	1.150	99.14	
	1.150	99.14	
	1.152	99.31	

The data obtained testify the accuracy of the results of the quantitative determination of sum of alkaloids in dry extracts of lupine seeds through amperometric titration and absence of constant bias.

There are few or hardly any alkaloids in the lupine roots. The same methods were applied to determine the alkaloid sum in the dry extract of the lupine roots as for the lupine seeds.

The direct amperometric titration of the alkaloid sum in the dry extract of the lupine roots is conducted using the electrochemical cell which consists of the solution studied and electrode system: an indicator electrode, a frontal graphite rotating electrode, and a reference electrode, a saturated calomel half-cell. The aliquot of the prepared solution of the lupine root (2.00 ml) is put into the electrochemical cell;

with the solution pH adjusted to 5.2 and superimposed voltage +0.05 V applied to the electrodes; the value of zero current is fixed within 2–3 minutes. The titration is conducted with the preliminarily prepared $5\cdot10^{-3}$ mol/l. The value of the strength of diffusion current is fixed within 30–35 seconds after adding the titrant. The amperometric titration is completed after determining the constant value of the diffusion current and the volume of the titrant used for the titration is defined graphically on the titration curve (Figure 2).

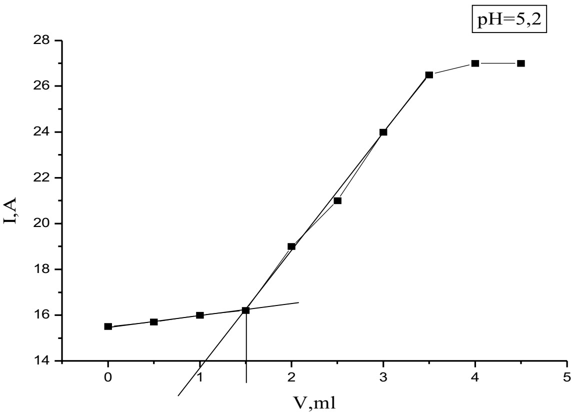

Figure 2. The curve of amperometric titration of the alkaloid sum in the lupine root extract with MPA solution: $V_{\Sigma alk.}$=2.00 ml, C_{MPA}=$5\cdot10^{-3}$ mol/l, solution pH 5.2

The calculation of the content of the alkaloid sum in the dry extract of the lupine roots was done after the following formula:

$$m=\frac{C_{MPA}\cdot V_{MPA}}{1000}\cdot 3\cdot M_{\Sigma alk.}$$

where C_{MPA} is the molar concentration of 12-molybdophosphatic heteropoly, mol/l ; V_{MPA} is the volume of the titrant which was used for the titration, ml; $M_{\Sigma alk.}$ is the molar mass of the alkaloid sum which equals 227.25 g/mol; 3 is the stoichiometric coefficient of the reaction of interaction between HPA $PMo_{12}O_{40}^{3-}$ and the alkaloid sum

$$m=\frac{1.5\cdot 5\cdot 10^{-3}}{1000}\cdot 3\cdot 227.25=0.005110g.$$

m is the mass of the alkaloids in 2 ml.

$$x_1=\frac{10\cdot 0.00511}{2}=0.025g.$$

x_1 is the mass of alkaloids in 10 ml.

$$x_2=\frac{0.025\cdot 100}{1.007}=2.482\%$$

x_2 is the percentage of the alkaloid sum in the dry extract of the lupine roots.

The results of the quantitative determination of sum of alkaloids in dry extracts of lupine roots through amperometric titration with pH 5.2 are given in Table 2.

Table 2

The metrological characteristics of the quantitative determination of the alkaloid sum in dry extracts of lupine roots through amperometric titration with pH 5.2 (n=7, P=0.95)

Input, mg (theor.)	Found, mg	Found, %	Metrological characteristics
5.11	5.061	99.06	$\overline{X}$=100.14 S^2=0.82 S_x=0.31 S_r=0.12 x±Δ=100.14±0.76
	5.140	100.58	
	5.181	101.38	
	5.079	99.39	
	5.079	99.39	
	5.140	100.58	
	5.140	100.58	

The data obtained testify the accuracy of the results of the quantitative determination of sum of alkaloids in dry extracts of lupine roots through amperometric titration and absence of constant bias.

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O.S. Panteleieva [a], ***Ch.P. Akritidou*** [b], ***V.V. Boynik*** [b], ***V.I. Tkach*** [a]

[a] **Ukrainian State University of Chemical Technology, Dnepropetrovsk, Ukraine**

[b] **National University of Pharmacy, Kharkov, Ukraine**

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Keywords: amperometric titration; quinolizidine alkaloids; sum of alkaloids; dry extracts of lupine seeds and roots.

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