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EMPHASIZING AND PURIFICATION2B, 3B, 14A, 20R, 22R, 25 HEXAHYDROXY-5B (N) CHOLEST-7-EH-6-OH SERRATULA CARDUNCULUS (PALL.) SCHISCHK

With elaborate technology selected and investigated the optimal conditions for the isolation and purification of the respective pharmacological active natural polioksisteroida2β, 3β, 14α, 20r, 22r, 25 hexahydroxy -5β (n) cholest-7-EH-6-OHfrom a plant Serratula cardunculus (pall.) Schischk growing on the territory of the Republic of Kazakhstan.
Keywords: *serratula cardunculus, extraction, column chromatography, qualitivereactions.*

Introduction: In spite of the many tools that have a particular activity, there are certain classes of compounds, whose action on the body is determined, manifested most clearly and does not require proof. Alkaloids, glycosides, phenothiazines, benzodiazepines, etc.., In spite of the differences in the structure and nature of the substituents, is always to some extent will cause pharmacological effects. Of course, the list of such compounds is growing thanks to advances in pharmaceutical science and the latest developments in this field, but research and modernization of existing funds have not lost their relevance. Moreover, in-depth study of a particular substance has a physiological activity is always less time consuming and cheaper (and the results more predictable) than the continuous screening of unknown compounds. Some of such compounds are ecdysteroids - class of compounds having high pharmacological activity. To date,more than 150installedstructureof ecdysteroids. Ecdysteroidsare quite commonsteroid compounds. Plant sourcesof ecdysteroidsboth in compositionand quantityare far superioranimal organisms[7]. The exceptionisnot, and Serratula cardunculus (Pall.) Schischk.
The aim of thisstudy was to: Examine thedynamics of the70% ethanolextractingofecdysteroidscampiondepending on theof multiplicityandduration ofextractingandto develop a methodofpurificationusing a columnwith aluminum oxide.
Methods: As anofecdysteroids sourceusedcrusheda erial part Serratula Cardunculus (Pall.) Schischk.,collectedat the end ofof the growing season2015 (second half of August). Raw materialbeen subjected toa preliminary purification from the mineralim purities and was powdered passin gasieve having meshes of 1 mm.
The extraction of the leaves of ecdysteroids Serratula cardunculus conducted by the following method.
1. 10 g of raw material was extracted with 70% ethanol. Extraction was carried out 3 times, then.Ethanol was removed using a rotary evaporator. The residual extract is transferred to a separatory funnel and treated five times with 30 ml of chloroform. The chloroform layer was allowed to determine the amount of ballast substances. The purified extract was treated with chloroform-isopropyl alcohol (1: 1) five times. The organic portion was removed and pooled eluted at 40 0C to yield a light yellow amorphous mass.
2. The dry residue was dissolved in chloroform-ethanol system (1: 1) and chloroform-ethanol-acetone (6: 3: 1) in a volume of 3 ml and chromatographed on a column of 1 cm diameter and 20 cm long alumina (II at Brockmann).
3. From each eluate fraction, whose volume was 1 ml, 0.1 ml were collected, evaporated to dryness and reacted with acetyl chloride in glacial acetic acid to confirm the presence of ecdysone (reddish-brown staining).
4. The residue of the eluate was evaporated to dryness, dissolved in 95% ethanol and absorbance was measured at λ = 242 nm, the absorption spectrum in the shooting range of 220-360 nm. Total 100 samples were taken from each column.
The total content of ecdysteroids by .beta.-ecdysone in the feed was adjusted after exhausting 10-fold extraction of raw materials 70% -ethanol [6]. Depending on the total content of ecdysteroids revealed the dynamics of the output of the aerial part of ecdysteroids Serratula Cardunculus (Pall.) Schischk using the above-mentioned extractant. Output dynamics is shown in Fig. 3.
The absorption spectrum of the standard sample of β-ecdysone removed onan SF-46, using a solution thereo fin ethanol. According to these datato construct a calibration graph.To study the dynamics of the output of ecdysteroid sused spectro photometric method for measuring the concentration of ecdysteroids[1,5,3] n musing the following methods[1,2,3]:
1. Five grams of the crushed material was placed in a flask with ground joint capacity of 100 ml, 30 ml of 70% ethanol and stirred on a mechanical shaker at room temperature. The extract was filtered through cotton in a flask of 100 ml capacity so that the raw material particles do not fall to the filter. Wool was placed in the extraction flask and 30 ml of 70% ethanol. The extraction was repeated 5 times in the above-described conditions, extracting by filtering the same flask. The extracts were then combined and the solvent was distilled off on a rotary evaporator at a temperature of 40 0 C to a volume of 30 ml.
2. The resulting extract was placed in a separatory funnel with 100 ml 20 ml of chloroform was added and stirred for 10 minutes. The chloroform extracts were discarded. The operation was repeated five times under the conditions described above.
3. Purified lipophilic ballast material from the aqueous extract was added 20 ml of a mixture of chloroform and isopropyl alcohol (1: 1) and stirred for 5 minutes. The organic phase was transferred to a 100 ml flask. The extraction was repeated five times, filtering the extract in the same flask. stirred and then extracting the solvent was distilled off on a rotary evaporator to a volume of 10 ml.
4. 10 mkl of the solution were applied to the starting line "Sorbfil" plates in the form of a spot diameter of 5 mm, air dried and chromatographed in a solvent system of chloroform-acetone-ethanol (6: 3: 1) [1,3,4,5]. saturation time camera - 30 minutes. Then, the plate was removed, dried in air and removed in the zone of sorbent R f = 0,67 (band width "2 cm). Sorbent quantitatively transferred to a flask with 25 ml of 95% ethanol and stirred on a shaker for 5 hours. Then remove the optical density of the solution at λ = 242 nm. The concentration of ecdysteroids was calculated from the calibration curve.
Output from dynamics ecdysteroidal umina column determined depending on the solvent system chloroform-ethanol (1:1) and chloroform-acetone-ethanol(6:3:1) [1]. The content of β-ecdysone on dry feed stock as a percentage (X) was calculated using the formula:

$$X = \frac{A}{A^{1\%}_{1cm}} \cdot E \quad \text{, где}$$

Absorption coefficient of the pure20-hydroxyecdysone; A -the absorbance of the test solution; K-dilution factor.

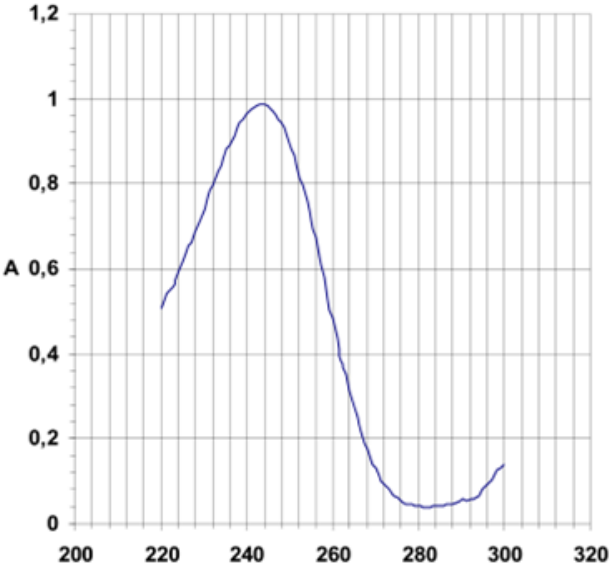


Figure 1 - The absorption spectrum β-ecdysone

Results and discussion: The absorption spectrum of β-ecdysone is a curve with a pronounced peak absorption at a wavelength of 242 nm. The calibration curve β-ecdysone is shown in Figure 2.

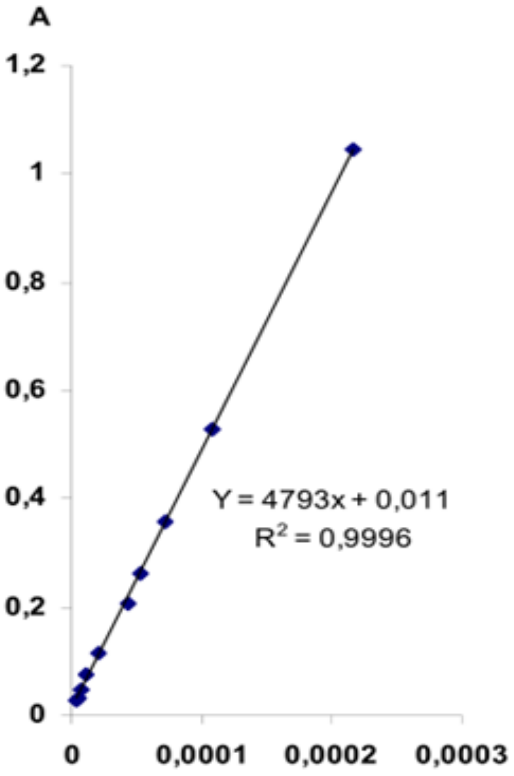
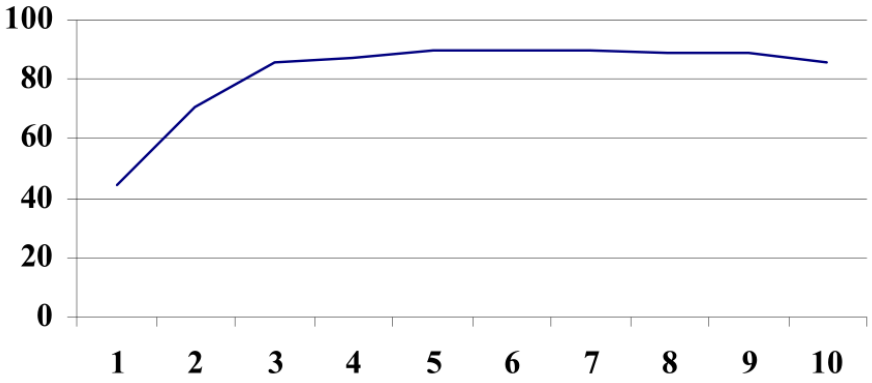


Figure 2 -The calibration curveβ-ecdysone



The multiplicity ofextraction

Figure 3 - Dynamics ofextractionof ecdysteroids, depending on the extractionratio

As can be seen from Fig. 3, a fairly complete output ecdysteroids observed after 3-fold extraction. Continued extraction gives significant yield of ecdysteroids, so in the future for the quantitative determination of ecdysteroids in the plant material using a 3-fold extraction.

Using a 3-fold extraction yield was studied ecdysone dynamics, depending on its duration. Dynamics ecdysteroid output depending on the duration of extraction was as follows: the first extraction - 300 minutes, the second extraction - 200 minutes, the third - 120 minutes. Increasing the duration of these phases does not lead to an increase in output of ecdysteroids (Figure 4,5 and 6).

Firstcontact

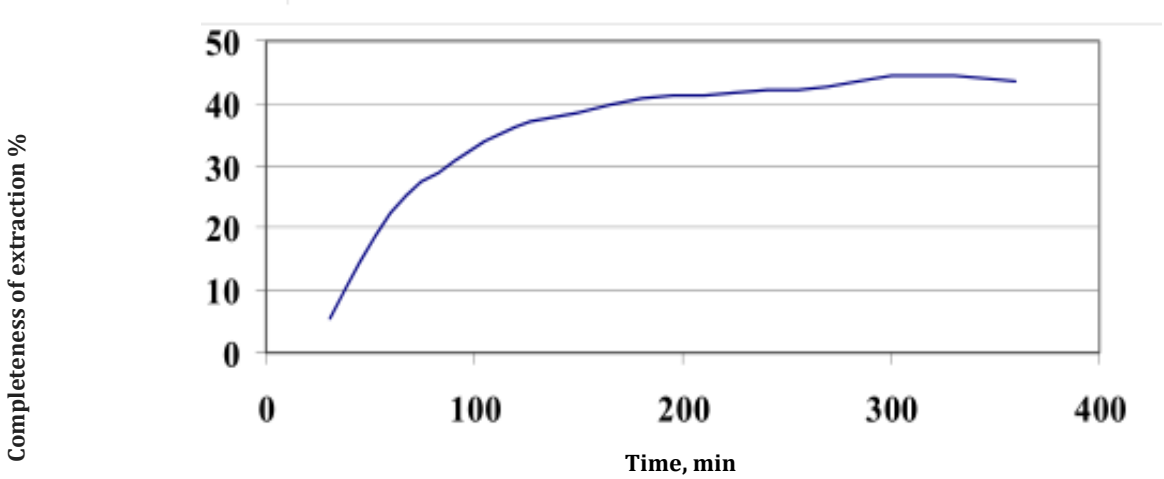


Figure 4 - The completeness ofthe extractionat the first contactwith the extractant

Thesecondcontact

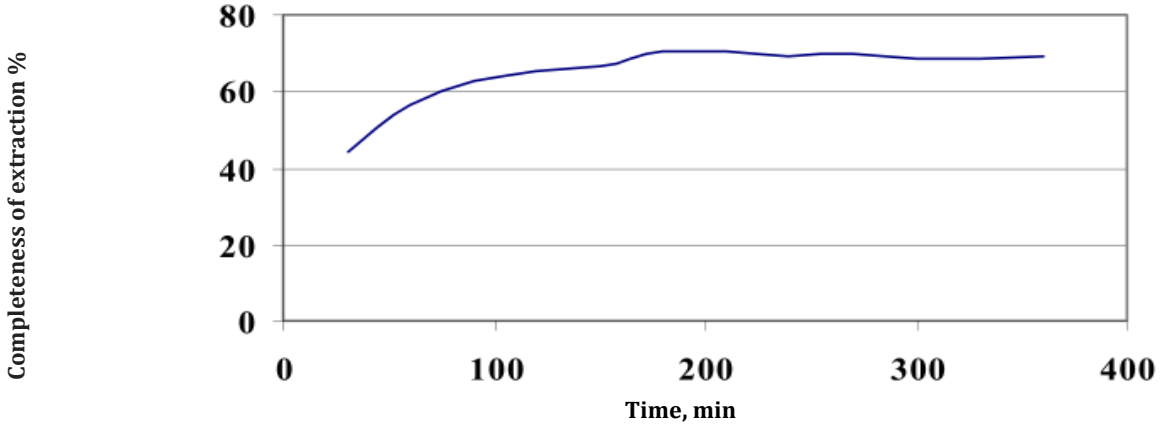


Figure 5 - The completeness ofthe extraction ofthe secondcontact with theextractant

Thethirdcontact

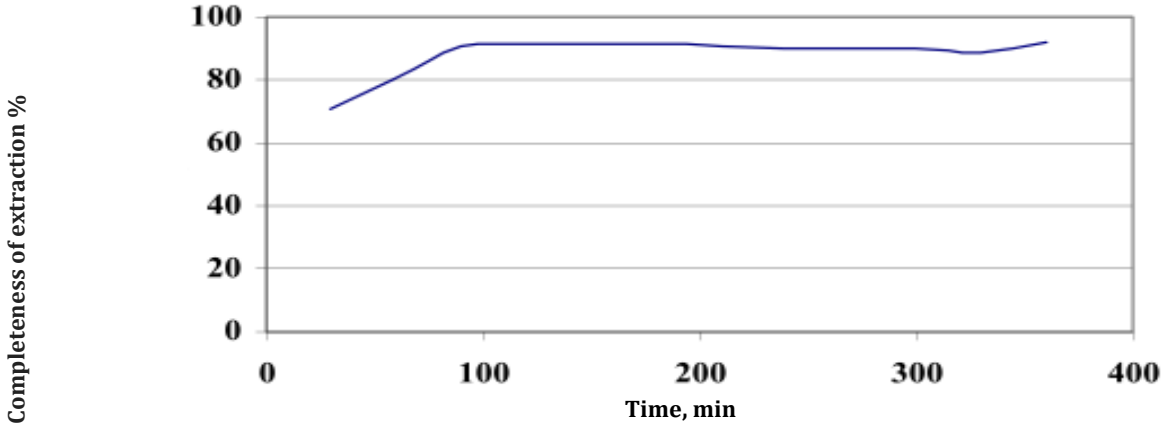


Figure 6 - The completeness ofextractionat the thirdcontact with theextractant

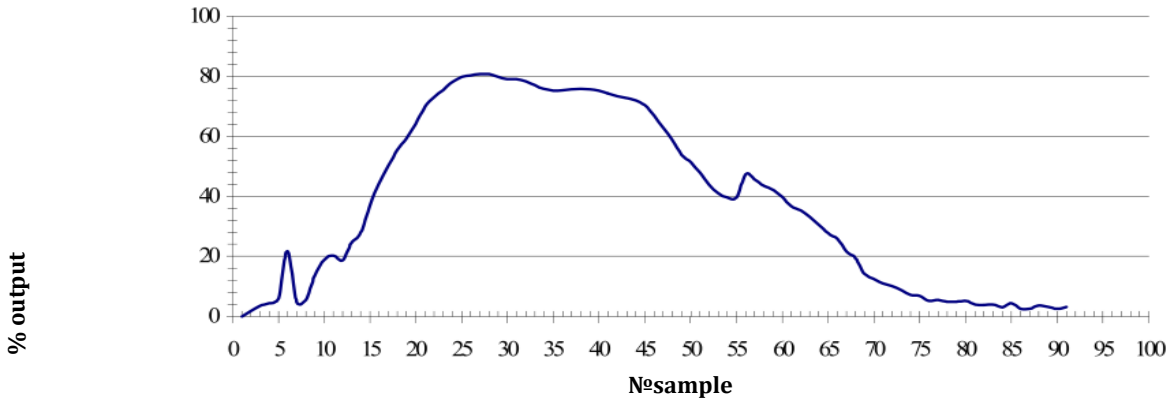


Figure 7 - Dynamicsecdysteroidoutputfromthe chromatography columnusingthe eluentchloroform-acetone-ethanol (6:3:1).

Isolated on total ecdysteroid compared with amended column degree calculated yield β-ecdysone percentage. It has been found that by using as the eluant chloroformetanol-acetone (6: 3: 1) to yield completeness was 80.7%, when using an eluent of chloroform-ethanol (4: 1) - 81.1%. However, when using the last extract and enough purified ecdysteroid along with fraction eluted colorants

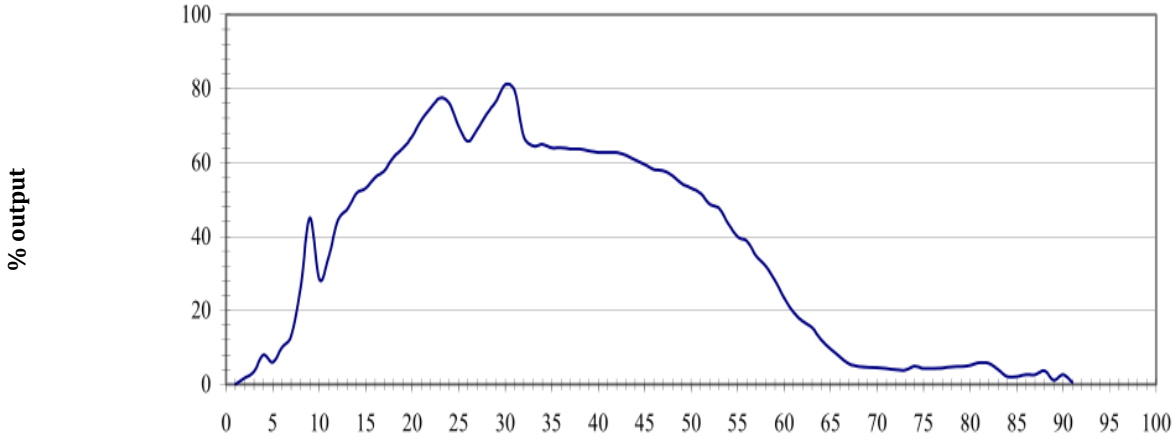


Figure 8 - ExitDynamics ofecdysteroidsfrom the chromatographiccolumn usingan eluentof chloroform-ethanol (4:1)

Conclusions:

1. A comprehensive exit ecdysteroids the extraction with 70% ethanol is observed after 3 times of extraction. Continued extraction leads only to flow the extractant;
2. Dynamics of release ecdysteroids depending on the duration of the extraction is as follows: first extraction - 300 minutes, the second extraction - 200 minutes, the third - 120 minutes. Increasing the duration of these phases does not lead to a significant increase in ecdysteroid output;

3. When using as eluent a mixture of chloroform-acetone-ethanol (6: 3: 1) yield was 80.7% completeness, using an eluent of chloroform-ethanol (4: 1) - 81.1%.
4. Dyeing and related substances eluted in the first 10-12 ml (chloroform-ethanol-acetone (6: 3: 1)), and the first 15 ml (chloroform-ethanol (4: 1));
5. Well eluent for column chromatography is a mixture of chloroform-acetone-ethanol (6: 3: 1), the most complete cleansing of the extract fiber.

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SERRATULA CARDUNCULUS (PALL.)SCHISCHK.2B, 3B, 14A, 20R, 22R, 25-ГЕКСАГИДРОКСИ-5B (H)-ХОЛЕСТ-7-ЕН-6-ОНҚОСЫЛЫСЫН БӨЛІП АЛУ ЖӘНЕ ТАЗАРТУ

Түйін: Технологиялық параметрлері іріктеле арнайы жасалған технологияны қолдана отырып Қазақстан аумағында өсетін Serratula cardunculus (pall.) Schischk өсімдігінен фармакологиялық күшті белсенділікке ие 2β, 3β, 14α, 20r, 22r, 25-гексагидрокси-5β (н)-холест-7-ен-6-он табиғи полиоксистероидың бөліп алудың оңтайлы әдістері мен жағдайлары таңдалып зерттелген.

Түйінді сөздер: Serratula cardunculus, экстракция, колонкалы хроматография, сапалы реакция

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ВЫДЕЛЕНИЕ И ОЧИСТКА 2B, 3B, 14A, 20R, 22R, 25-ГЕКСАГИДРОКСИ-5B (H)-ХОЛЕСТ-7-ЕН-6-ОНА ИЗ SERRATULA CARDUNCULUS (PALL.)SCHISCHK

Резюме: С помощью разработаной технологии подобраны и исследованы оптимальные условия по выделению и соответствующей очистке фармакологически активного природного полиоксистероида 2β, 3β, 14α, 20r, 22r, 25-гексагидрокси-5β (н)-холест-7-ен-6-она из растения Serratula cardunculus (pall.) Schischk,. произрастающего на территории Республики Казахстан.

Ключевые слова: Serratula cardunculus, экстракция, колоночная хроматография, качественные реакции