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Development of a liquid chromatography method for the analysis of oil extract of artemisia cina

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Abstract

Introduction. The problem of development and implementation of own drugs in Kazakhstan today is acute and urgent. The solution to this problem is possible through the use of own resources – domestic medicinal plant raw materials. A promising medicinal raw material is Artemisia cina – an endemic medicinal plant of the of the Turkestan region of Kazakhstan.

Aim. Designing a liquid chromatography methodology to analyze the oil extract obtained from Artemisia cina.

Materials and methods. Standard sample (SS) of santonin, Artemisia cina's oil extract. The substance was identified and quantitatively determined using an Agilent Technologies 1200 liquid chromatograph (USA) equipped with ChemStation software.

Results and discussion. The main active substance of the oil extract was analyzed using high-performance liquid chromatography (HPLC) method. Choosing the separation conditions for the column involves determining the best composition of the mobile phase and the rate of elution. There were used a reversed-phase system with sorbent C18 and a mobile phase consisting of acetonitrile and a phosphate buffer with a pH of 6.8. Chromatographic studies of the substance were carried out in a gradient mode at an analytical wavelength of 237 nm. The retention time of the standard sample (SS) of santonin matched the retention time (t_R) of santonin isolated from the oil extract of Artemisia cina and amounted to 14.3 minutes.

Conclusion. A liquid chromatography method has been established for the identification and quantitative determination of the oil extract, focusing on the main active substance, santonin.

Keywords: oil extract of artemisia cina, santonin, identification, assay, high-performance liquid chromatography, validation

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Hosseini Shirazi Farshad developed the concept of the study and was responsible for planning the experiment, and participated in the data analysis. Gaukhar Bakhytkyzy and Aigul D. Serikbayeva performed the liquid chromatography experiments and participated in data processing and interpretation. Saule K. Ordabayeva made significant corrections to the text of the article, edited it, and prepared the final version for publication. All authors were fully involved in the development of the analytical method and statistical processing of the data.

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Разработка методики жидкостной хроматографии для анализа масляного экстракта полыни цитварной

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Резюме

Введение. Проблема разработки и внедрения отечественных препаратов в Казахстане на сегодняшний день является острой и актуальной. Решение данной проблемы возможно посредством использования собственных ресурсов – отечественного лекарственного растительного сырья. Перспективным лекарственным сырьем является полынь цитварная – эндемичное лекарственное растение Туркестанской области Казахстана.

Цель. Разработка методики жидкостной хроматографии для анализа масляного экстракта полыни цитварной.

Материалы и методы. Стандартный образец (СО) сантонина масляный экстракт полыни цитварной (МЭПЦ). Идентификацию и количественное определение вещества проводили на жидкостном хроматографе Agilent Technologies 1200 (США) с программным обеспечением ChemStation.

Результаты и обсуждение. Исследование хроматографического поведения основного действующего вещества масляного экстракта проводилось методом высокоэффективной жидкостной хроматографии (ВЭЖХ). Подбор условий разделения на колонке включает в себя выбор оптимального состава подвижной фазы и скорости элюирования. Использовалась обращенно-фазовая система с сорбентом С18 и подвижной фазой, состоящей из ацетонитрила и фосфатного буфера с pH 6,8. Хроматографические исследования субстанции проводили в градиентном режиме при аналитической длине волны 237 нм. Время удерживания стандартного образца (СО) сантонина совпало со временем удерживания (t_R) сантонина выделенного из масляного экстракта полыни цитварной и составило 14,3 мин.

Заключение. Разработана методика идентификации и количественного определения масляного экстракта по основному действующему веществу – сантонина методом жидкостной хроматографии.

Ключевые слова: масляный экстракт полыни цитварной, сантонин, идентификация, анализ, высокоэффективная жидкостная хроматография, валидация

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. Ш. Х. Фаршад разработал концепцию исследования и была ответственен за планирование эксперимента, а также принял участие в анализе данных. Г. Бахыткызы и А. Д. Серикбаева провели эксперименты по жидкостной хроматографии, участвовали в обработке и интерпретации данных. С. К. Ордабаева внесла значительные правки в текст статьи, занималась ее редактированием и подготовила окончательный вариант для публикации. Все авторы в полной мере занимались разработкой аналитического метода и статистической обработкой данных.

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INTRODUCTION

The problem of the evolution of diseases and increasing the resistance of pathological microorganisms, parasites to drugs is becoming more and more widespread due to the unfavorable global dynamics of the ecological situation. According to the WHO expert assessment, diseases caused by helminths rank third globally among the most significant infectious and parasitic diseases. Official statistics indicate that approximately 2 billion people worldwide are infected with helminths [1, 2].

The demand for anthelmintic agents in the Kazakhstani market is predominantly met by imported drugs [3-6]. The problem of development and implementation of own drugs in Kazakhstan today is acute and urgent. The solution to this problem is possible through the use of own resources – domestic medicinal plant raw materials. A promising medicinal raw material is Artemisia cina – an endemic medicinal plant of the of the Turkestan region of Kazakhstan. For many years, citvar seed and santonin have been an effective anthelmintic. Sesquiterpenoid of Artemisia cina – santonin has a wide spectrum of pharmacological action: anthelmintic, anti-inflammatory, antipyretic, hemolytic, cardiotonic, immunomodulatory, including its own antitumor activity [7–10].

The department of pharmaceutical and toxicological chemistry of the South Kazakhstan medical academy has developed a method for obtaining Artemisia ci-

na's oil extract. Preliminary studies indicate that santonin is the primary active ingredient of the oil extract. Therefore, the study of quality indicators, standardization of oil extract according to santonin and the development of a draft regulatory document for oil extract is relevant in order to further introduce it into medical practice [11–14].

The efficacy of pharmaceutical analysis is intricately linked to the application of physicochemical research methodologies. The development of techniques for evaluating medication quality constitutes a multifaceted endeavor that can be solved using the HPLC method, which allows combining the stages of separation, identification and quantitative determination. The determination of santonin in the oil extract was hampered by the presence of extraneous chromatographic peaks of related substances. Thus, we optimized the chromatography conditions and developed optimal sample preparation conditions for the maximum transfer of santonin from the oil extract to the test solution [15–17].

Aim

Standard sample of santonin (α-santonin, purity >99.9 %, Sigma-Aldrich, USA), Artemisia cina's oil extract (ACOE), acetonitrile for HPLC (purity >99.9 %, Honeywell, USA), deionized water (obtained at the Milli-Q D-8 installation Water purification system, USA), phosphate buffer (850 ml of orthophosphoric acid is dissolved in 250 ml of deionized water, and the pH is corrected with a solution of 0.1 M sodium hydroxide).

MATERIALS AND METHODS

Chromatography equipment and conditions

Identification and quantitative determination was carried out on an Agilent Technologies 1200 liquid chromatograph (USA) with ChemStation software equipped with a G1322A degasser and a variable wavelength spectrophotometric detector MWD G1314. Separation was carried out on a column with a reversed phase Agilent C18. 5μ, (4.6 × 150 mm) with a pre-column (Agilent C18. 5 μ). Also, a concentrator centrifuge (Concentrator plus eppendorf, USA) and an ultrasonic bath (Elmascript S., Germany) were used for sample preparation of oil extract of Artemisia cina.

Analytical determinations were carried out under the following conditions: the flow rate is 1.0 ml/min, the volume of the injected sample is 20 μl, the column temperature is 40 °C. Chromatography was passed under conditions of gradient elution with acetonitrile (A) and phosphate buffer with pH 6.8 (B): 0 min – 35:65 (A:B); 5 min – 20:80; 10 min – 20:80; 15 min – 20:80; 20 min – 35:65. Optimization of chromatographic separation conditions was carried out according to the system suitability parameters: resolution, number of theoretical plates, peak asymmetry factor. Different variants of the mobile phase, stationary phase, elution modes, and ionization modes were tested. Acetonitrile was used as the mobile phase, which is characterized by low viscosity, mixes with water in any ratio, does not create pressure at the inlet to the column and a phosphate buffer pH 6.8. Chromatographic studies of the substance were run in gradient mode at an analytical wavelength of 237 nm.

Identification of santonin peaks on the chromatogram was pursued by comparative analysis of chromatographic characteristics with a standard sample.

Preparation of standard sample solution of santonin

About 1.0 mg (exact weight) of a standard sample of santonin is placed in a vial, 1 ml of acetonitrile is added, shaken on a shaker and centrifuged at 2000 rpm for 15 minutes (solution A).

100, 250, 500, 750 of a standard sample of santonin solution are placed in 1000 μl volumetric flasks, diluted to the mark with acetonitrile and mixed (solutions B).

Preparation of samples

Approximately 5.0 grams (exact mass) of the oil extract from Artemisia cina are introduced into a 25 ml conical flask, followed by the addition of 10 ml of acetonitrile. To ensure complete transfer of santonin into the acetonitrile phase, the resulting mixture is subjected to ultrasonication for 15 minutes at 60 °C. Subsequently, centrifugation is performed at a rate of 14.000 rpm for 10 minutes. The acetonitrile layer is separated using a micropipette and transferred to a 50 ml chemical beaker and tightly closed. Extraction is carried out 2 more times. The resulting extracts are combined and centrifuged for 10 minutes. Then, they are placed in a vacuum concentrator until the extractant completely evaporates. The dry residue is dissolved in 10 ml of acetonitrile and placed in an ultrasonic bath for 15 minutes at a temperature of 60 °C (test solution).

About 1.0 ml (exact weight) of the test solution of Artemisia cina's oil extract is placed in a vial, shaken on a shaker for 5 minutes and chromatographed under the above conditions.

RESULTS AND DISCUSSION

Method validation

Validation of the developed methodology was conducted in accordance with the relevant provisions outlined in the General Pharmacopoeia Article of the State Pharmacopoeia of the Republic of Kazakhstan (SP RK), as well as modern recommendations from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) [18–20].

The method of quantitative determination is validated according to the main characteristics: specificity, linearity, precision and limit of quantitative determination (LOQ) [8, 19–54].

Specificity

The specificity of the assay was evaluated by analyzing five samples of a standard santonin solution within the concentration range of 100–1000 μg/ml. No extraneous peaks corresponding to the retention time of santonin isolated from the oil extract of Artemisia cina were observed on the chromatograms of the standard samples (figures 1–3).

Figures 1–3 show chromatograms of the tested santonin solution of Artemisia cina's oil extract and SS solution of santonin under the selected conditions of chromatographic analysis. The chromatogram shows the specificity and selectivity of the developed method for identifying the oil extract by santonin: the retention time of the obtained peak of the studied santonin solution isolated from the oil extract coincides with

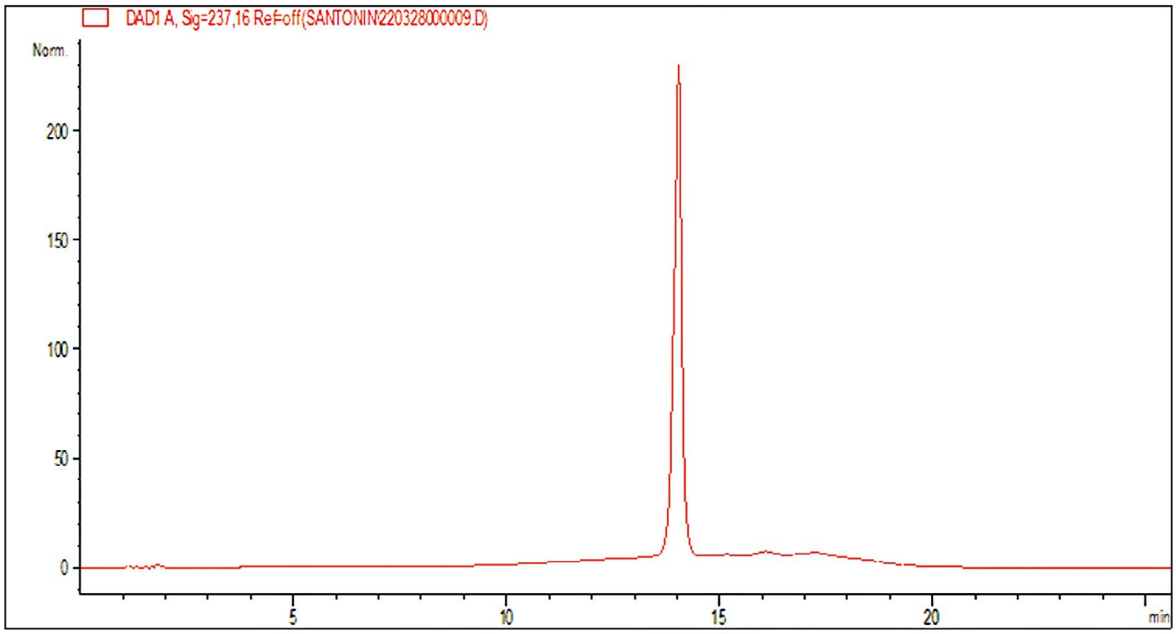

Figure 1. **Chromatogram of a standard sample of santonin**

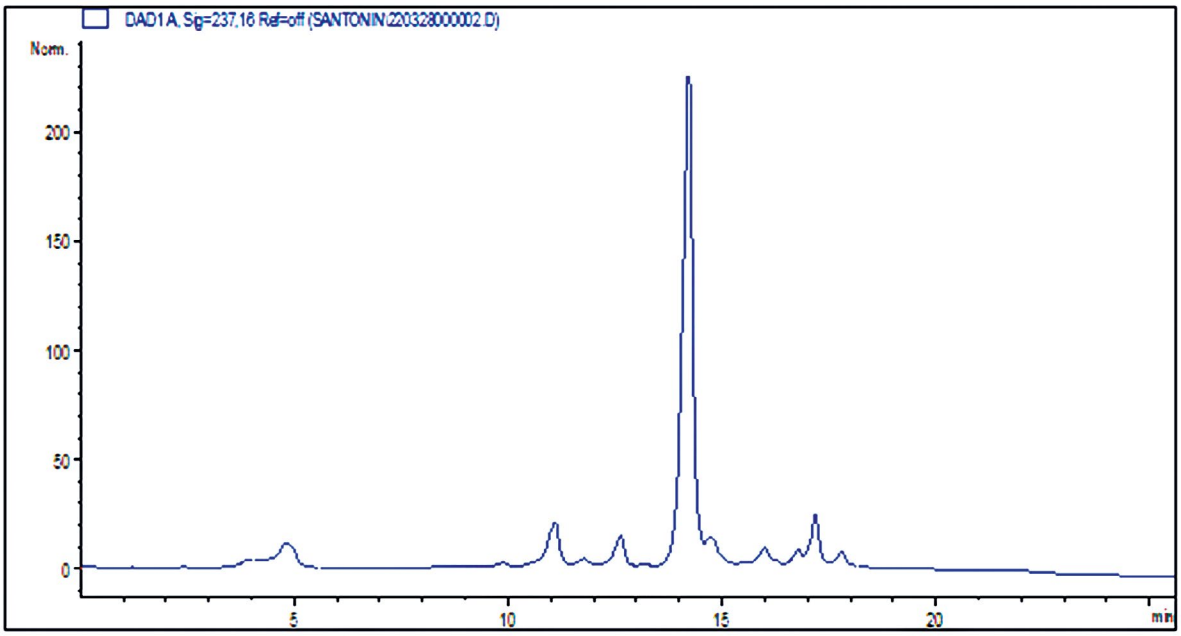

Figure 2. **Chromatogram of Artemisia cina's oil extract**

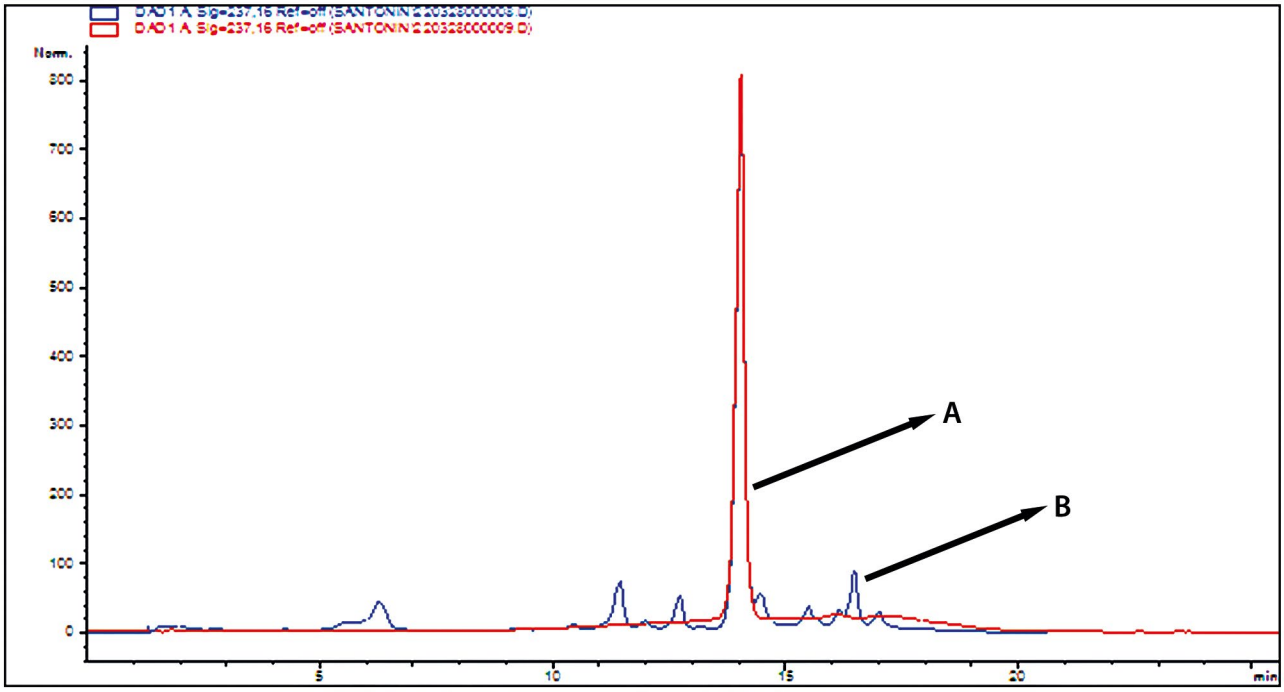

Figure 3. **Chromatogram:**
A – standard sample of santonin; B – oil extract of Artemisia cina

the retention time (t_R) of the peak of the SS santonin solution and is within 14.3 minutes with 99.9 % purity of the pharmaceutical substance. In order to show the separating ability of the system, we calculated the indicator R_s by the formula (1).

$$R_s = \frac{2 \cdot (t_{R1} - t_{R2})}{W_{s1} + W_{s2}} = 0.69. \quad (1)$$

Linearity

The linearity was evaluated according to the graph of the dependence of the chromatographic peak area on the concentrations of santonin in the sample: 100, 250, 500, 750, 1000 μg/ml. When statistically processing the linear dependence according to the equation $y = 30.41x + 555.4$, the correlation coefficient of the linear regression graph r was 0.99960. The results obtained support the assertion that the developed methodology exhibits linearity (figure 4, table 1).

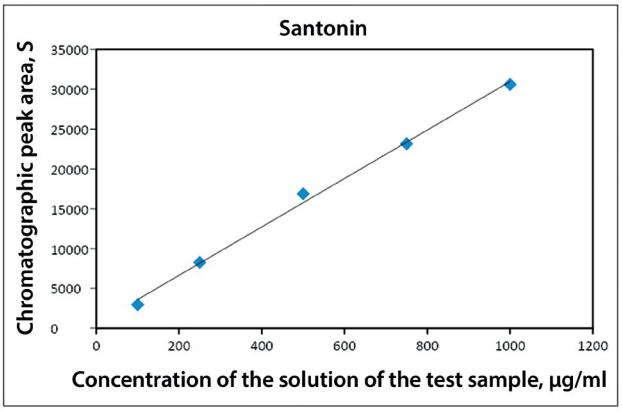

Figure 4. **Calibration graph of the linear relationship between the concentration of santonin and the area of chromatographic peaks**

Table 1. **Estimation of the linear relationship between the concentration of santonin and the area of chromatographic peaks**

№	Samples Concentration, μg/ml	Peak area, S	Metrological characteristics			
			SD	RSD	$X_{av.} \pm \Delta X_{av.}$	$\varepsilon_{av.}$, %
1	100.0	2944.6	246.3	1.891	1.93	0.9996
2	250.0	8265.2				
3	500.0	16878.7				
4	750.0	23161.0				
5	1000.0	30602.5				

Precision

Standard samples santonin solution with concentrations of 100, 500, 1000 μg/ml were analyzed. Each solution was chromatographed 6 times. The study was conducted during day 1 (intra-day) and 2 day (inter-day). Standard deviation (SD), relative standard deviation (RSD) and relative error of the method (ε, %) of the obtained concentration values.

The relative error of the method was observed in the range of 1.02–1.91 % for sequence No. 1 and in the range of 1.01–1.63 % for sequence No. 2 and does not exceed the permissible standard deviation, which confirms the precision and correctness of the developed method (tables 2 and 3).

95 % confidence intervals were calculated. The true values fall within the obtained intervals, which confirms the precision and correctness of the developed method.

Table 2. Precision (intra-day). Sequence № 1

Conc, μg/ml	Peak area *S*	Obtained		Metrological characteristics						
		μg/ml	%	$\bar{X}$	S^2	$S\bar{x}$	$\Delta\bar{X}$	SD	RSD	CI (95 %)
100	2944.6	99.11	99.11	98.97	0.450	0.274	0.704	0.671	0.006	98.97 ± 0.82
	2957.6	97.93	97.93							
	2978.3	99.61	99.61							
	2896.2	98.93	98.93							
	2963,1	98.27	98.27							
	2947.7	99.98	99.98							
500	16878.7	496.3	99.26	498.0	2.179	0.602	1.548	1.476	0.016	498.00 ± 1.23
	16882.8	498.6	99.72							
	16879.3	497.2	99.45							
	16879.5	498.3	99.66							
	16881.4	497.9	99.58							
	16896.5	499.7	99.95							
1000	30602.5	998.3	99.83	999.0	0.759	0.355	0.914	0.871	0.009	999.05 ± 0.80
	30611.2	999.3	99.93							
	30605.3	998.9	99.89							
	30601.2	998.1	99.81							
	30703.9	999.7	99.97							
	31062.8	1000.0	100.0							

Table 3. Precision (inter-day). Sequence № 2

Conc, μg/ml	Peak area, S	Obtained		Metrological characteristics						
		μg/ml	%	$\bar{X}$	S^2	$S\bar{x}$	$\Delta\bar{X}$	SD	RSD	CI (95 %)
100	2943.9	97.5	97.55	98.42	1.872	0.558	1.431	1.368	0.015	98.42 ± 1.16
	2977.6	99.8	99.81							
	2958.5	99.7	99.78							
	2946.2	97.6	97.69							
	2943.1	97.4	97.43							
	2947.7	98.5	98.56							
500	16888.7	498.9	99.78	498.2	1.265	0.272	1.18	1.125	0.012	498.20 ± 0.85
	16892.8	499.3	99.86							
	16799.3	497.1	99.42							
	16879.1	498.2	99.64							
	16861.2	497.6	99.52							
	16876.3	498.1	99.62							
1000	30592.5	998.2	99.82	999.28	0.745	0.352	0.907	0.863	0.009	999.28 ± 0.79
	31351.2	1000.0	100.0							
	30685.3	998.8	99.88							
	30601.4	998.9	99.89							
	31056.3	999.9	99.99							
	30962.6	999.9	99.99							

The stability

The stability of the standard solutions of santonin and tested solutions of Artemisia cina's oil extract according to santonin for chromatographic analysis was investigated. Short-term stability was studied within 24 hours after sample preparation and during the next day of analysis. The study of long-term stability showed that during chromatography of the studied solutions for 14 days when stored in a refrigerator from 2 to 8 °C, the standard solutions of santonin and tested solutions of the oil extract were stable. At the same time, the area of the chromatographic peak did not change more than 10 % during repeated analyses.

Reproducibility

There were prepared 9 test solutions to determine the reproducibility criterion and analyzed. The data obtained are shown in table 4. The method exhibits high reproducibility, as evidenced by the relative error of 1.67 %.

Limit of detection and limit of quantification

The analytical range of the technique of quantitative determination of santonin in the oil extract of Artemisia cina is determined, based on the accuracy, linearity and precision of the technique. The detection limit was 50 µl, the limit of quantitative determination was 100 µl, and the range of quantitative detection is in the range of 100–1000 µl.

We carried out a quantitative determination of laboratory samples (LS) of the oil extract of Artemisia cina for santonin, which was calculated using the formula (2):

$$X = \frac{S_1 \cdot 1000 \cdot 1000 \cdot a_{CT} \cdot 100 \cdot W\%}{S_2 \cdot a \cdot 100 \cdot 1000 \cdot 1000} = \frac{S_1 \cdot a_{CT} \cdot W\%}{S_2 \cdot a}. \quad (2)$$

The content of santonin in the oil extract is presented in Table 5.

Validation of the developed method of quantitative determination by the method of high-performance liquid chromatography showed that it is specific, characterized by correct accuracy and reproducibility in the analytical domain in relation to the established working concentration of santonin in solution, which allows it to be used for a reliable assessment of the quality of the oil extract by santonin.

CONCLUSION

A liquid chromatography technique has been developed for the identification and assay of the oil extract from Artemisia cina, focusing on its main active substance, santonin. validation of the developed technique revealed a linear relationship between the concentration of santonin and the area of chromatographic peaks, with a correlation coefficient (r) of 0.9996 Across a span of concentrations from 100–1000 µg/ml; the relative standard deviation of the technique is within 0.097–0.018 % (inter day) and within 0.096–0.272 % (int-

Table 4. Evaluation of the reproducibility of the method for the quantitative determination of santonin in the oil extract of Artemisia cina

№	Santonin content in model mixtures of oil extract, %	Metrological characteristics (SP RK v.1, p.100)						
		n	$x_{av.}$	*S*	$\Delta x_{av.}$	P_f	$x_{av.} \pm \Delta x_{av.}$	$\varepsilon_{av.}$, %
1	0.612	9	0.61	0.009	0.010	0.372	0.61 ± 0.01	1.67
2	0.597							
3	0.619							
4	0.614							
5	0.599							
6	0.619							
7	0.620							
8	0.621							
9	0.616							

Table 5. Quantitative content of santonin in oil extract

№	Santonin substance in oil extract	Metrological characteristics						
		n	$x_{av.}$	*S*	$\Delta x_{av.}$	*Sx*	$x_{av.} \pm \Delta x_{av.}$	$\varepsilon_{av.}$, %
1	0,620	6	0,61	$5{,}7 \cdot 10^{-3}$	$8{,}8 \cdot 10^{-3}$	$3{,}4 \cdot 10^{-3}$	0,61 ± 0,0088	1,45
2	0,615							
3	0,599							
4	0,612							
5	0,619							
6	0,593							

ra day) the relative error is within 1.01–1.91 % confirms the reliable reproducibility and accuracy of the developed methodology. Quantitative determination of laboratory samples of Artemisia cina's oil extract by santonin using the developed liquid chromatography technique was about 0.6 %.

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