

DEVELOPMENT OF CONDITIONS FOR TRAMADOL ANALYSIS BY THE METHOD OF THERMODESORPTION SURFACE-IONIZING SPECTROSCOPY

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✓ **Resume**

The procedures for determination and identification of tramadol by the method of thermodesorption surface-ionizing spectroscopy have been developed. It was established that alcoholic solutions of tramadol had maximum absorption at $\sim 88 \pm 15^\circ\text{C}$ and $\sim 205 \pm 15^\circ\text{C}$. The linear-dynamic range is in the interval of 5-50 $\mu\text{g/mL}$ of substance concentration in the sample. Sensitivity of the method is 0,5 $\mu\text{g/mL}$. The developed method is recommended for analysis of tramadol in biological liquids.

Key words: thermodesorption surface-ionization spectroscopy, tramadol, biological fluids.

РАЗРАБОТКА АНАЛИЗА ТРАМАДОЛА МЕТОДОМ ТЕРМОДЕСОРБЦИОННОЙ ПОВЕРХНОСТНОЙ ИОНИЗИРУЮЩЕЙ СПЕКТРОСКОПИИ

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✓ **Резюме**

Разработаны методики определения и идентификации трамадола методом термодесорбционной поверхностно-ионизирующей спектроскопии. Установлено, что спиртовые растворы трамадола имеют максимальную ионизацию при $\sim 88 \pm 15^\circ\text{C}$ и $\sim 205 \pm 15^\circ\text{C}$. Линейно-динамический диапазон находится в интервале 5-50 мкг/мл концентрации вещества в пробе. Чувствительность метода 0,5 мкг/мл . Разработанный метод рекомендован для анализа трамадола в биологических жидкостях.

Ключевые слова: термодесорбционная поверхностно-ионизационная спектроскопия, трамадол, биологические жидкости.

TRAMADOLNI TERMODESORBSION SIRT IONLASHUV SPEKTROSKOPIYA TAHLIL USULINI ISHLAB CHIQLISH

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Toshkent farmatsevtika instituti
Respublika sud tibbiy ekspertiza ilmiy amaliy markazi Buxoro filiali

✓ **Rezyume**

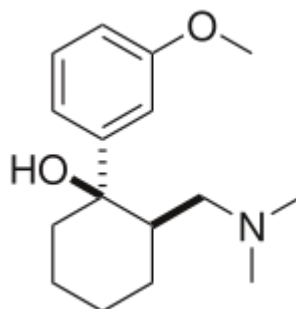
Tramadolni termodesorbsion sirt ionlashuv spektroskopiya tahlil usuli ishlab chiqildi. Tramadolning spirtli eritmaları $\sim 88 \pm 15^\circ\text{C}$ va $\sim 205 \pm 15^\circ\text{C}$ darajasida maksimal ionlanish darajasiga ega ekanligi aniqlandi. Namunada usulning chiziqliligi 5-50 mkg / ml tashkil qildi. Usulning sezgirligi 0,5 mkg / ml ni tashkil qildi. Ishlab chiqilgan usul tramadolni biologik suyuqliklardan aniqlashga tavsiya etildi.

Kalit so'zlar: termodesorbsion sirt ionlashuv spektroskopiya, tramadol, biologik suyuqliklar.

Relevance

Trade names: Plazadol, Protradon, Sintradon, Tramal, Tramolin, Tramundin retard. Chemical

formula: $\text{C}_{16}\text{H}_{25}\text{NO}_2$. Chemical name: 2 - [[Dimethylamino] methyl] -1- [3-methoxyphenyl] cyclohexanol.



Tramadol is a bluish-yellow crystalline powder substance. Insoluble in water, soluble in alcohol. Begins to liquefy at 183 ° C. Molecular mass: 326.8 [1,9].

Tramadol, sold under the brand name Ultram among others, is an opioid pain medication used to treat moderate to moderately severe pain. When taken by mouth in an immediate-release formulation, the onset of pain relief usually begins within an hour. It is also available by injection. It may be sold in combination with paracetamol (acetaminophen) or as longer-acting formulations [1,2,3].

As is typical of opioids, common side effects include constipation, itchiness, and nausea. Serious side effects may include seizures, increased risk of serotonin syndrome, decreased alertness, and drug addiction. A change in dosage may be recommended in those with kidney or liver problems. It is not recommended in those who are at risk of suicide or in those who are pregnant [2,3]. While not recommended in women who are breastfeeding, those who take a single dose should not generally stop breastfeeding [4]. Tramadol is converted in the liver to O-desmethyltramadol (desmetramadol), an opioid with stronger binding to the μ -opioid receptor [2,5]. Tramadol is also a serotonin–norepinephrine reuptake inhibitor (SNRI). [2,6]

Tramadol was patented in 1963 and launched under the name "Tramal" in 1977 by the West German pharmaceutical company Grünenthal GmbH. In the mid-1990s, it was approved in the United Kingdom and the United States. It is available as a generic medication and marketed under many brand names worldwide [6,7]. In 2018, it was the 25th most commonly prescribed medication in the United States, with more than 24 million prescriptions [8].

Tramadol belongs to the group of benzodiazepines and is widely used in medical practice in schizophrenia, tramadol-induced psychoses, anxiety, sleep disorders. Long-term and excessive use of the drug leads to fatigue, drowsiness, memory impairment, headache, dizziness, dry mouth, tachycardia, thirst, vomiting, fever [9]. Tramadol is associated with the increased suicide risk in different age groups: in

youth, adolescents and adults. In connection with this the study of tramadol in the biopharmaceutical and chemical and toxicological regard is the crucial task. The purpose of this study is to develop the analytical procedure for tramadol by thermodesorption surface-ionizing spectroscopy (TDSIS) method and the application in the study of biological liquids.

Experimental part. For the study the method of thermodesorption surface-ionization spectroscopy was used. The essence of the method lies in the temperature-programmed regime of evaporation of molecules of required substances in the extracts from biological samples with their subsequent entry the surface ionization detector, the signals of which are recorded in the form of thermodesorption spectra. These thermodesorption spectra are quite specific for certain test substances. The basis for registration is the principle of operation of surface-ionization detector. In the diode detector as the anode there is thermo emitter, and as the cathode – there is the collector of positive ions. While passing through the diode of the analyzed mixture, the molecules entering the surface of the emitter can be desorbed in the form of ions that are delivered to the collector by the electric field for registration [10,11].

In the detector, due to its high selectivity for the ionization potential the molecules of organic solvents (alcohols, ketones, aldehydes, esters, hydrocarbons, etc.) and simple gases are practically not ionized by surface ionization. The detector allows to register only the molecules of nitrogen bases, the derivatives of which are many narcotics, alkaloids and other synthetic nitrogen compounds [11,12].

The authenticity of the substances is established according to the effective temperature of desorption using standard samples of the studied drugs.

For the detection of tramadol by the method thermodesorption surface-ionization spectroscopy (TDSIS) the analysis was performed under the following conditions: the emitter – oxidized molybdenum, having iridium in its composition; emitter voltage – 405 V; emitter temperature – 390-420 °C; temperature of evaporation – 20-505

°C; air flow – 50 l/hour, (the voltage of the compressor is 12 V); volume of the test sample taken for analysis - 1.0 µl; analysis time – 3 minutes; recording of the spectra is performed directly by using computer program.

To conduct the study the standard solutions of tramadol were prepared. Accurately weighed quantity of 0,05 tramadol is dissolved in 96% ethyl alcohol in 10 ml flask. Solution is diluted with 96% ethyl alcohol to the mark. From this

solution working standard solution (50 µg/ml) was prepared and from which 1 µl of solution was taken by micro syringe and injected into cylindrical cavity of evaporative tape of PII-N-S “Iskovich-1” apparatus, and thermodesorption surface-ionization spectra were received. At the temperature of $\sim 88 \pm 15^\circ\text{C}$ and $\sim 205 \pm 15^\circ\text{C}$ the appearance of peaks was observed typical for tramadol (Figure 1.).

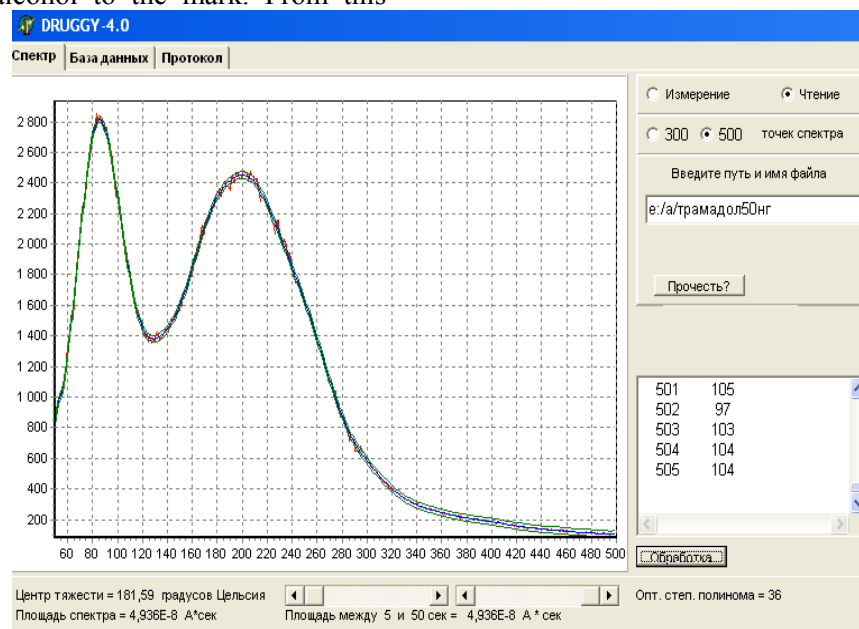


Fig 1. TDSI spectra of tramadol

The obtained thermodesorption spectra were recorded as the references to the database of the computer. The sensitivity of the method is within 0,5 µg/ml range.

Further, we examined the specificity of the conditions of tramadol analysis by TDSIS method.

The temperature of the surface-ionization spectrum of tramadol was differ from the above temperatures of the studied antidepressants. The results are shown in table 1.

Table 1.

The results of study of specificity of the conditions of tramadol analysis by TDSIS method.

Test substance	Temperature of maximum ionization, °C
Tramadol	$\sim 88 \pm 15^\circ\text{C}$ and $\sim 205 \pm 15^\circ\text{C}$
Heroin	$\sim 150 \pm 10^\circ\text{C}$
Morphine	$\sim 192 \pm 15^\circ\text{C}$
Codeine	$\sim 152 \pm 15^\circ\text{C}$
Haloperidol	$\sim 184 \pm 15^\circ\text{C}$

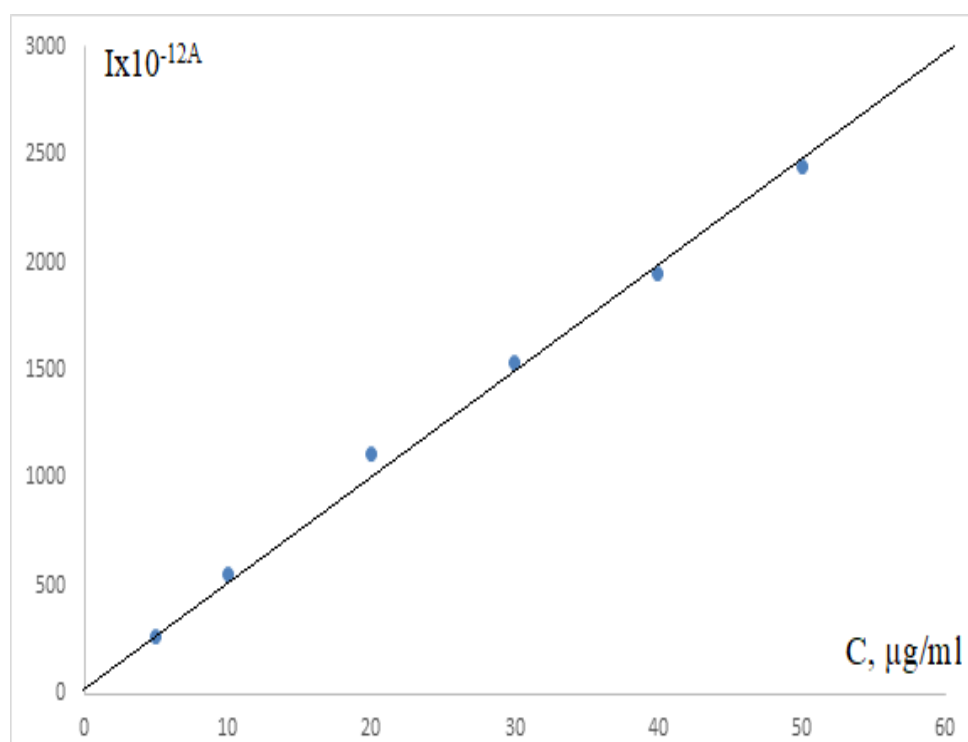
Quantitative determination was carried out according to calibration graph drawn up according to the exact concentration of the standard nominal solution. To construct the calibration curve 1 ml of standard alcohol solution in the concentrations of 5, 10, 20, 30, 40, 50 µg/ml, respectively by using

micro syringe is injected into the cylindrical cavity of the evaporative tape of PII-N-S “Iskovich-1” apparatus and their average values are obtained (based on the peak of tramadol at $\sim 205 \pm 15^\circ\text{C}$). The results of the analysis are given below in table 2 and on Fig.2.

Table 2.

The results of study of the linear dependence of tramadol analysis conditions by TDSIS analysis
(tramadol $\sim 205 \pm 15^\circ\text{C}$) $n=5$)

Solution concentration, $\mu\text{g/ml}$ C,	Amount of tramadol , ng	The height of TDSI spectra (at current intensity ($I \times 10^{-12}\text{A}$)).
5	5	261
10	10	551
20	20	1108
30	30	1536
40	40	1953
50	50	2441



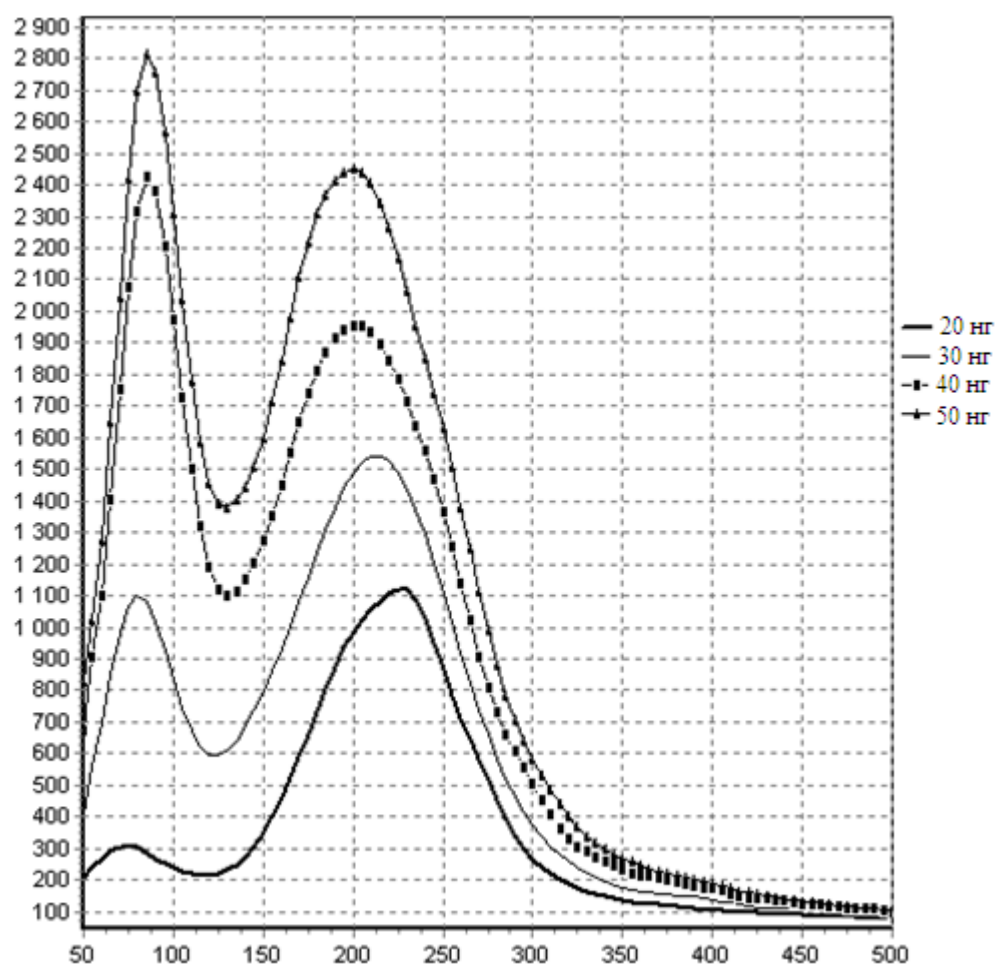


Fig. 2. Graph of TDSI tramadol spectra height dependence on the solution concentration.

After quantitative determination of tramadol by TDSIS method metrological calculation was conducted. The results are shown in table 3.

Table 3.

The results of study of accuracy and reproducibility of tramadol analysis conditions by TDSIS analysis

Preparation amount, $\mu\text{g/ml}$	Obtained amount		Temperature of maximum ionization, $^{\circ}\text{C}$	Results of metrological calculation
	ng	%		
30	30,12	100,40	205	$f = 5$ $T_{(95\%,5)} = 2,57$ $X_{\text{mean}} = 101,18$ $S^2 = 3,3630$ $S = 1,8338$ $S_x = 0,7487$
30	30,91	103,03	206	
30	31,07	103,56	206	
30	29,74	99,13	204	

30	30, 43	101,43	205	$\Delta X_{\text{mean}} = 1,9241$ $\varepsilon = 1,9017$
30	29, 85	99,50	204	

Thus, in the result of the analysis of tramadol TDSIS we have $X_{\text{mean}} = 101,18\%$, the average relative error is $\varepsilon = 1,9017 \%$.

In the next stage of study the verification of the developed procedure was conducted in the analysis of tramadol extracted from biological fluids (blood and urine).

Conclusions

The procedure of tramadol detection has been developed by the method of thermodesorption surface-ionization spectroscopy. Here it was established that alcoholic solutions of tramadol at $\sim 88 \pm 15^\circ\text{C}$ and $\sim 205 \pm 15^\circ\text{C}$ have maximum absorption.

2. The indicators such as specificity, accuracy, reproducibility, linear dynamic range and mean result accuracy have been studied. Linear dynamic range is in the interval of concentration of 5-50 $\mu\text{g/ml}$ in the sample. The sensitivity of the method is 0,5 $\mu\text{g/ml}$.

3. With the help of calibration curve the quantitative content of tramadol was calculated. It was established that quantitative determination of tramadol by TDSIS method makes up in average $X_{\text{mean}} = 101,18\%$ and $\varepsilon = 1,9017 \%$.

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