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SPECTRAL ANALYSIS OF ALBENDAZOLE IN FORENSIC CHEMISTRY

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Abstract.Conditions for the analysis of albendazole by spectral methods: UV-spectrophotometry and thermal desorption surface ionization spectroscopy (TDSIS) were developed. Qualitative and quantitative analysis of albendazole isolated from biological fluids was performed under the developed analysis conditions. According to the results, 38.74% of albendazole was isolated from blood by UV spectrophotometry, and 43.00% from urine, while 47.46% from blood and 64.72% from urine by TDSIS.

Keywords: Albendazole, UV spectrophotometry, thermal desorption surface ionization spectroscopy (TDSIS), spectra, biological fluids, extraction.

There are more than a thousand types of worms in the world today. Some of them also have virus-carrying properties. It is estimated that about 90 percent of the world's population today has worm disease. Human helminthiasis such as enterobiosis, hymenolepidosis, ascaridosis, trichocephalus, teniarinhosis, echinococcosis are most common in the territory of the Republic of Uzbekistan [1,2].

Albendazole is a broad-spectrum anthelmintic. It affects the intestinal and tissue types of worms and has anti-worm effects on different types of parasites (eggs, larvae, adults) [3]. Albendazole is used to treat intestinal parasites, including nematodes (Ascaris lumbricoides, Trishiurus trishiura, Enterobius vermicularis, Ancylostoma duodenale, Necator americanus, Strongiloides stercoralis, Cutaneous Larva Migrans), cestodes (Hymenolepsis niver, Taenia solium, Taenia saginata). Albendazole is also effective against tissue parasites, including echinococci (Echinococcus granulosus and Echinococcus multilocularis) [4,5].

Such drugs, in addition to relieving vomiting, in some cases can lead to cases of severe poisoning as a result of overdose [6]. According to the literature, given the insufficient chemical and toxicological study of albendazole, the study of the composition of albendazole in biological objects and biological fluids in accordance with the requirements of chemical and toxicological studies is one of the most sensitive, rapid and high-precision spectral methods: UV spectrophotometry and thermal desorption surface ionization spectroscopy.

Research methods. 1. Determination of albendazole by UV spectrophotometric method. The experiments were performed on a spectrophotometer 8453E Spectroscopy System from Agilent Technologies. To do this, 0.01 g (p.h.) of albendazole is weighed, placed in a 100 ml volumetric flask, dissolved in 0.1 M hydrochloric acid, and the volume is brought to the mark of the volumetric flask with 0.1 M hydrochloric acid. To determine the optical value of albendazole, a layer thickness of 10 mm and a wavelength of 220 to 350 nm are carried out. 0.1 M hydrochloric acid was obtained as the reference solution [7].

Research results. Based on the experimental results, it is confirmed that a solution of albendazole in 0.1 M hydrochloric acid has a high light absorption index at a wavelength of 291 nm. The results of the analysis are shown in Figure 1.

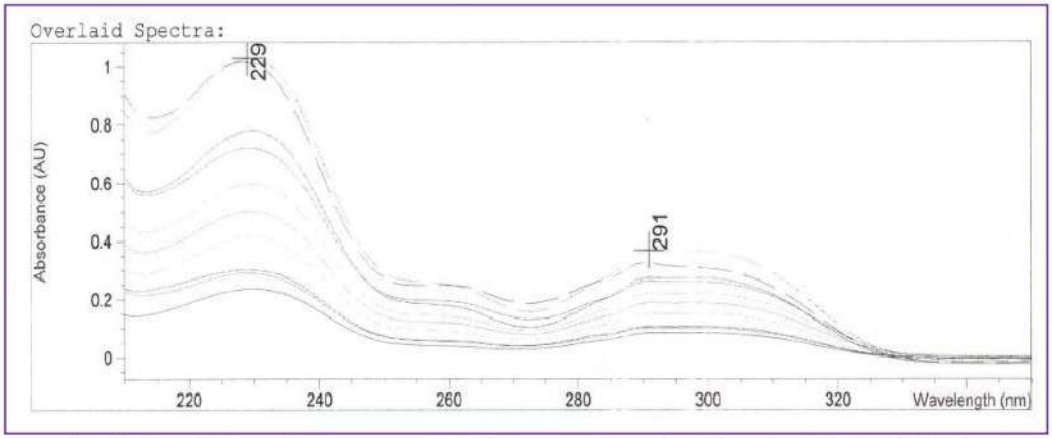

Figure 1. Light absorption spectrum of a solution of albendazole in 0.1 M hydrochloric acid

Quantitative analysis of albendazole by UV spectrophotometric method is calculated using a calibrated graph. To do this, 0.01 g (p.h.) of albendazole is weighed, placed in a 100 ml volumetric flask, dissolved in 0.1 M hydrochloric acid, the volume is brought to the mark of the measuring flask with 0.1 M hydrochloric acid, and from this solution the working standard of albendazole 1; 2; 3; 4; 5 μg / ml solutions are prepared, the optical densities of which are determined on a spectrophotometer 8453E Spectroscopy System Agilent Technologies at a wavelength of 291 nm. Take 0.1 M hydrochloric acid as the reference solution anduse them to draw a calibration diagram (Fig. 2).

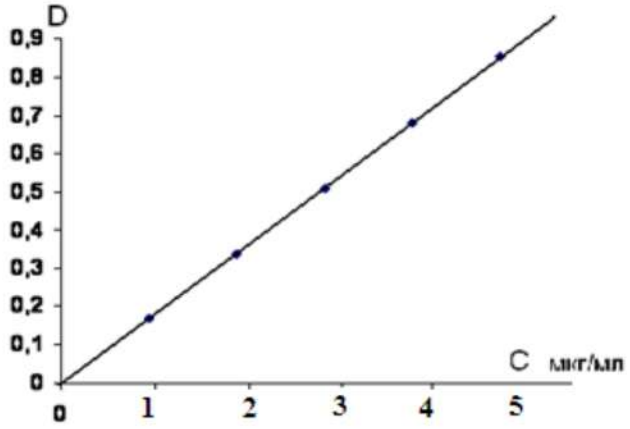

Figure 2. To the concentration of the optical density of albendazole connection diagram

Based on the experimental data, the specific and molar light absorption values of albendazole are calculated. The results of the analysis are presented in Table 1.

Table 1

Comparison of albendazole and molar light absorption
Results of determination of indicators (n = 5)

Amount of substance, μg / ml	Optical density (D)	Comparison light absorption index (E)	Molar absorption index (ε)
1	0,103	1030,0	27336,2
2	0,250	1250,0	33175,0
3	0,302	1006,6	26699,2
4	0,413	1032,0	27389,2
5	0,503	1006,0	26689,4
Average		1064,9	28263,2

Quantitative analysis of albendazole is performed in order to verify the accuracy of the analytical conditions developed during the experiment and to test the quantitative analysis.

For this, 5 samples were prepared from a 5 μg / ml solution of albendazole, and then the optical value of the solutions was determined spectrophotometrically at a wavelength of 291 nm. The amount of albendazole is determined based on a calibrated drawing. Metrological report of the obtained results is calculated on the basis of SPh.XI edition.

Table 2

The amount of albendazole by UV spectrophotometric method the results of the analysis obtained

Dosage of the drug, μg / ml	Found		Results of metrological analysis
	μg / ml	%	
5,00 5,00 5,00 5,00 5,00	5,01 5,00 4,89 4,95 5,11	100,2 100,0 97,8 99,0 102,2	$\overline{X}$ =99,84 S^2 =2,648 S=1,627 Sx=0,729 ΔX=3,824 $\Delta\overline{X}$ =1,710 ε=4,53% $\overline{\varepsilon}$ =2,02%

Spectrophotometric analysis of albendazole resulted in = 99.84%, mean relative error = 2.02%. The fact that the values of relative and absolute errors given in the table are at the level of the requirements for the analysis shows that the results are reliable and that the developed method can be used to determine the amount of albendazole.

2. Determination of albendazole by thermal desorption surface ionization spectroscopy.

The surface ionization indicator PII-N-S "Iskovich-1" recommended by the Institute of Electronics named after UA Arifov of the Academy of Sciences of the Republic of Uzbekistan is used in the spectroscopic analysis of thermal desorption surface ionization albendazole. The essence of the method is based on the programmed evaporation of the temperature of the molecules of the substance and their recording in the form of thermal desorption spectra in the surface ionization detector [8,9].

Thermal desorption surface ionization spectroscopic analysis of albendazole is carried out under the following conditions:

- emitter - oxidized molybdenum with iridium input,
- emitter voltage - 405 V,
- emitter temperature - 390 - 420^{0}C,
- evaporation temperature - 505^{0}C above room temperature,
- air flow - 50 l / h (compressor voltage 12 V)
- volume of the test sample taken for analysis - 1.0 μl;
- Analysis duration -3 minutes.
- Spectra recording is performed directly using a computer program.

The determination of the authenticity of the substances (standard method) is carried out on the effective desorption temperatures. Comparison of the separation of a biological sample with respect to any substance is carried out by comparing the obtained spectrum with the reference spectrum in a computer database.

Albendazole is weighed to 0.01 g (p.h.), dissolved in a 10 ml volumetric flask with 95% ethyl alcohol and made up to 95% ethyl alcohol to the volume mark. From this solution a working standard solution of albendazole 100 μg / ml is prepared, injected into a cylindrical

cavity in the evaporator tape of the apparatus PII-N-S "Iskovich-1" in the amount of 1 µl using a microspray and obtain thermal desorption surface ionization spectra of albendazole.

The obtained thermal desorption spectra are recorded in the computer's database as a reference spectrum.

Thermal desorption surface ionization spectroscopic studies of albendazole show that its solution in 95% ethyl alcohol forms a linear peak of ~ 382 ± 10 °C (Fig. 3).

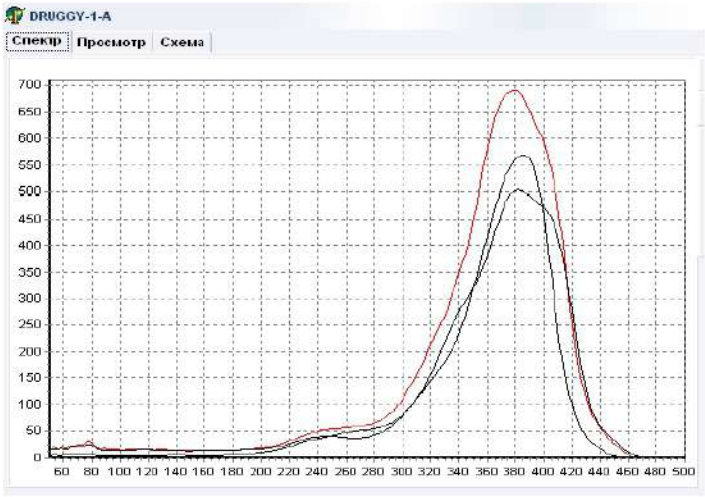

Figure 3. TDSI spectra of albendazole obtained at different concentrations

Emitter temperature (T) along the abscissa line, 0S; Current strength value along the ordinate line (I), A.

Quantitative analysis of albendazole by TDSI spectroscopy is carried out on the basis of a calibration diagram based on standard sample solutions of precise concentration.

For drawing up a calibration graph, standard samples containing 10; 20; 30; 40; 50 µg/ml of albendazole prepared in a 96% solution of ethyl alcohol was injected into a cylindrical well in the evaporator tape of the PII-N-S Iskovich-1 apparatus using a microsyringe, and the analysis was carried out under the indicated above conditions. Their mean values (taking into account the peak at albendazole ~ 382 ± 10 °C) are calculated and a calibration diagram is drawn. The results of the analysis are presented in Table 3 and Figure 4.

Table 3

Results of the study of the linearity of the developed TDSI spectroscopic analysis conditions (albendazole ~ 382 ± 10 °C, n = 5)

Solution concentration, µg / ml	Albendazole content,µg	TDSI spectrum height (current value)($Ix10^{-12A}$)
10	10	155
20	20	314
30	30	468
40	40	615
50	50	776

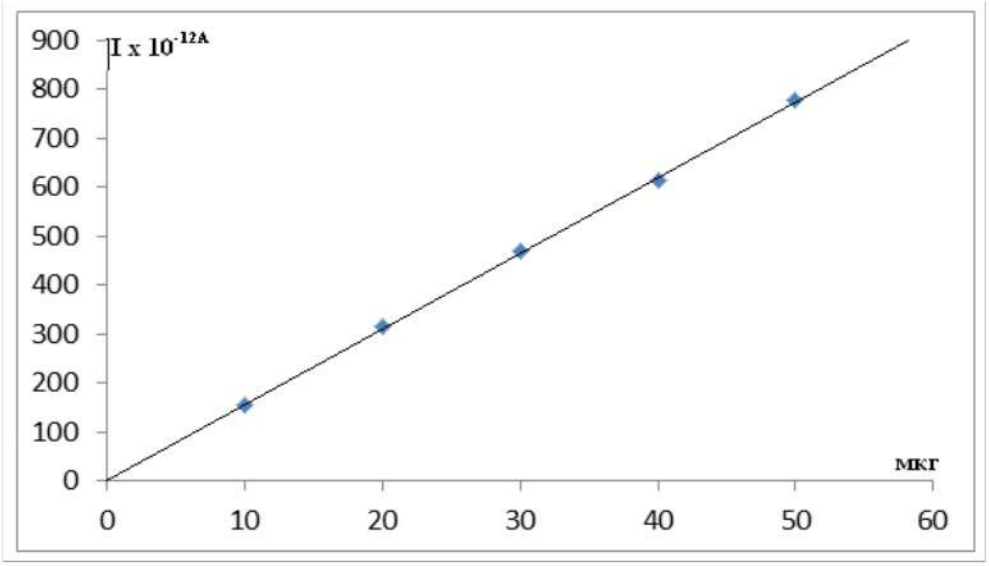

Figure 4. Diagram of the dependence of the peak height of albendazole on the solution concentration under the conditions of TDSI spectroscopic analysis

The linear range of detection for albendazole of the method is 10–50 μg and the sensitivity is 0.5 μg.

In order to study the accuracy and reversibility of the developed TDSI spectroscopy method, working standard solutions of albendazole in a certain concentration of 95% ethyl alcohol were prepared and analyzed under the recommended conditions. The amount of albendazole was determined using its calibration chart and then the metrological report was analyzed. The results obtained are presented in Table 4.

Table 4

TDSI spectroscopic analysis of albendazole the results of the study of accuracy and reversibility

Amount of substance, μg / ml	Determined amount		Results of metrological analysis
	μg	%	
20	20,1	100,5	$\overline{X}$ =99,62 % S^2=1,250
20	19,9	99,5	S=0,9203 S_x=0,411
20	21,2	100,6	ΔX=2,558 $\Delta\overline{X}$ =1,144
20	19,7	98,5	ε=2,568% $\overline{\varepsilon}$ =1,148 %
20	19,8	99,0	

The results in Table 4 show that the TDSI spectroscopic analysis of albendazole showed = 99.62%, = 1.148%.

Conditions for extraction of albendazole from biofluids: Take 25 ml of urine and 5 ml of blood (1 mg of albendazole) with 2 M hydrochloric acid to pH = 4.0-5.0 and add 10 ml of organic solvent hexane in a mechanical shaker for 10 minutes. shaken. The protein in the mixture is then centrifuged for 5 minutes (3000 rpm) to precipitate. The hexane layer is separated from the aqueous layer, the remaining aqueous layer is extracted with 5 ml of hexane and the hexane is poured. The hexane extracts were combined and 5 g. passed through filter paper containing anhydrous sodium sulfate salt. The filter is washed with 5 ml of hexane. The organic solvent from the filtrate is evaporated at room temperature, the residue is dissolved in 5 ml of ethyl alcohol and albendazole is purified from foreign substances by a thin layer chromatography, followed by analysis by UV spectrophotometry and thermal desorption surface ionization spectroscopy [10].

Purification from extractive substances by the method of thin layer chromatography.

To do this, chromatographic "Silufol UV 254" is dripped from the alcohol solution taken on the starting line of the plates in the form of a line, on one side as a confirmation of the working standard solution of albendazole and dried at room temperature. The mixture of organic solvents is placed in a chromatographic chamber filled with chloroform - ethyl alcohol - formic acid (8: 1: 1) and saturated with their vapor. In order to determine the place of accumulation of the substance on the chromatographic plate, it is determined using a lamp UV-254, or the side of the abrasive sorbent layer is closed and sprayed with Dragendorf reagent on the dripping part of albendazole. (Rf = 0.70-0.72) The part of albendazole formed by stains is determined, the sorbent layers are scraped off and analyzed by UV-spectrophotometry and thermal desorption surface ionization spectroscopy.

1. UV-spectrophotometric analysis of albendazole isolated from biological fluids. The resulting dry residue is dissolved in 3-4 ml of 0.1 M hydrochloric acid solution and transferred to a measuring vessel (using a syringe) and the volume of the solution is increased to 10 ml using 0.1 M hydrochloric acid solution. The optical density at a wavelength of 291 nm is then determined in a cuvette with a layer thickness of 10 mm. 0.1 M hydrochloric acid is obtained as the reference solution. The results of the analysis are presented in Table 5.

Table 5

Isolated from biological fluids

results of quantitative analysis of albendazole

Determined amount		Results of metrological analysis
μg	%	
blood		
0,384 0,375 0,390 0,401 0.387	38,40 37,50 39,00 40,10 38,70	$\overline{X}$ =38,74 S^2=0,893 S=0,940 S_x=0,421 ΔX=2,62 $\Delta\overline{X}$ =1,17 ε=6,78% $\overline{\varepsilon}$ =3,03%

urine		
0,426	42,60	$\overline{X}$ =43,00 S^2=1,195
0,432	43,20	S=1,093 S_x=0,48
0,440	44,00	ΔX=3,038 $\Delta\overline{X}$ =1,358
0,436	43,60	ε=6,71% $\overline{\varepsilon}$ =3,01%
0,428	42,80	

The table shows that under the recommended extraction conditions, albendazole was isolated from UB-spectrophotometry by 38.74% of blood and an average of 43.00% of urine.

2. TDSIS analysis of albendazole isolated from biological fluids. To do this, the dry residue removed from foreign substances is dissolved in 1-2 ml of ethyl alcohol, transferred to a measuring vessel (using a syringe) and the volume of the solution is brought to 10 ml using ethyl alcohol. This solution is poured into a cylindrical funnel in a 1 μl evaporator belt using a microship. Thermal desorption spectra of substances are obtained and their quantitative analysis is carried out by means of qualitative analysis and structured calibration drawing by comparison with the reference spectra in the computer database. The results of the analysis are presented in Figure 5 and Table 6.

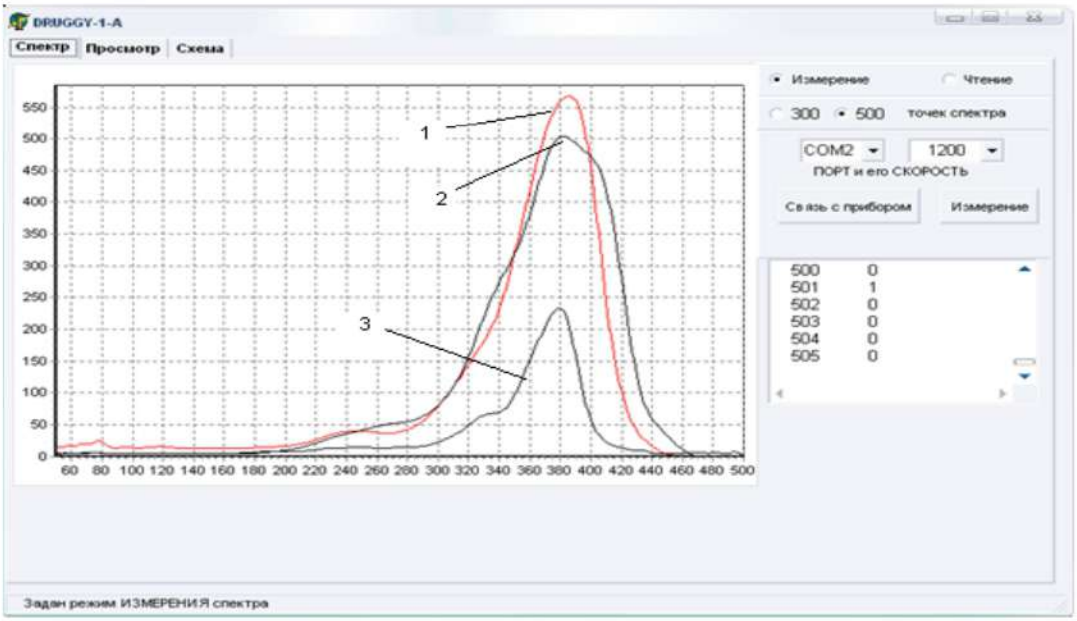

Figure 5. TDSI spectra of albendazole: 1 working standard sample of albendazole, 2 urine excreted albendazole, 3 blood excreted albendazole

Table 6

Isolated from biological fluids

results of quantitative analysis of albendazole

Determined amount		Results of metrological analysis
μg	%	
blood		
0,474 0,485 0,467 0,491 0,459	47,41 48,56 46,75 49,18 45,94	$\overline{X}$ =47,56 S^2=1,823 S=1,350 S_x=0,603 ΔX=3,753 $\Delta\overline{X}$ =1,678 ε=7,89% $\overline{\varepsilon}$ =3,53%
urine		
0,647 0,675 0,624 0,631 0,659	64,74 67,56 62,75 49,18 45,94	$\overline{X}$ =64,72 S^2=4,282 S=2,069 S_x=0,925 ΔX=5,752 $\Delta\overline{X}$ =2,572 ε=8,88% $\overline{\varepsilon}$ =3,975%

The table shows that under the recommended extraction conditions, 47.56% of albendazole was excreted from the blood and an average of 64.72% from the urine.

These results showed that the proposed method can be used to isolate it from biological fluids (blood, urine) in cases of acute poisoning with albendazole. Rapid methods of isolation and analysis of albendazole allow rapid detection of the substance and prompt medical care of poisoned patients.

Conclusions.

1. The conditions for the analysis of albendazole by UV spectrophotometry were studied. It was found that solutions of albendazole in 0.1 M hydrochloric acid had the highest absorption wavelength in the UV light field at 291 nm for derosal, with a high light absorption index, and its specific and molar absorption values were 1064.9 and 28263.2, respectively. values.

2. For the first time, the thermal desorption surface ionization spectroscopic analysis of albendazole was studied. In this case, albendazole was found to form linear peaks at ~ 382 ± 10°C. The method was found to have a linear range of determinations for albendazole of 10–50 μg and a sensitivity of 0.5 μg. 3. Separation of albendazole from biofluids (blood and urine) was carried out on the basis of the conditions developed. 4. Under the recommended extraction conditions of albendazole by UV-spectrophotometry, 38.74% of the sample was removed from the blood and 43.00% from the urine. 5. TDSIS method was used to separate 47.56% of albendazole from model blood and 64.72% from urine.

References.

[1] Arkhipov I.A. Anthelmintics: pharmacology and application. - M., 2009 .-- 405 p.
[2] Mashkovkiy M.D. Medicines Volume II. - M .; LLC New Wave. - 2002, - P.296
[3] Clarke's isolation and idevtification of drugs, London, 2000. P. -323.
[4] Mikhailitsyn FS, Lebedeva MN, Sadikov T., Aripov Kh.N. and others. Development and implementation of a new domestic anthelmintic albendazole. Medical parasitology and parasitic diseases, 2001.- №3.-P.49-51
[5] Pilyugin B.C. Synthesis and biological activity of 2-aminobenzimidazole derivatives. Obtaining new anthelmintic and fungicidal preparations // M .: Chemistry.
[6] 2003, - 297 p
[7] Dayan A.D. Albendazole, mebendazole and prazi quantel // Review of non clinical toxicity and pharmacokinetics // ActaTropica. 2003. - V.86. - P. 141-159.
[8] 7. Abdullaev Sh. The use of UV spectrophotometry for the analysis and standardization of drugs based on benzimidazole // Chemistry and pharmacy, 2002, No. 2, pp. 14-19.
[9] 8. Ibragimova M.M. Atlas of thermal desorption surface ionization spectra of toxicologically significant substances and some drugs. –Tashkent, 2015, -145p.
[10] 9. F.S.Jalilov. Spectroscopic analysis of carmazepine by thermal desorption surface ionization. Journal of Pharmacy. - Tashkent, 2012. - №1. - P.46-49
[11] 10.Usmanalieva Z.U., Tojiev M.A. Isolation and analysis of albendazole from biofluids. Journal of Pharmacy. - Tashkent, 2015. - №1. - P.77-80.