Express Analysis in Acute Poisoning with Some Antihelminth Drugs

Usmanalieva Zumrad Uktamovna[1], Zulfikariyeva Dilnoza Alisherovna[2]

[1]Head of the Department of Toxicological Chemistry, Doctor of Pharmacy, Associate Professor. E-mail: usmanalieva1970@mail.ru

[2]Associate Professor of the Department of Toxicological Chemistry, Doctor of Pharmacy, Pharmaceutical Chemistry and Pharmacognosy Tashkent Pharmaceutical Institute, Tashkent City, Uzbekistan. E-mail: zulfidil@mail.ru

Abstract

In the practice of chemical-toxicological analysis, cases of poisoning of people with certain antihelmintic drugs have become more frequent. Some antihelmintics are on the WHO Model List of Essential Medicines, the World Health Organization's list of essential medicines, which lists the most effective, safest and most affordable medicines. Despite worldwide recognition, effectiveness, availability and low cost, they have an important drawback - serious side effects. In cases of poisoning, it is important to conduct an accurate analysis of blood and urine for emergency medical care. Chemical-toxicological analysis of biological fluids requires accurate and accessible methods. At the same time, the correct and clean extraction of a poisonous substance from biological fluids makes it possible to accurately and quickly determine it. Methods for isolating antihelmintic preparations from biological fluids in acute poisoning have been developed. The extracts were purified from ballast substances by elution by TLC. A chromatographic analysis of antihelmintic preparations isolated from biological fluids was carried out.

Keywords: Antihelmintic Drugs, Albendazole, Mebendazole, Levamisole, Biological Fluids, Isolation, Thin Layer Chromatography, High Performance Liquid Chromatography.

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INTRODUCTION

To date, about 250 species of such pests have been identified, of which 9 species have been found in children. Intestinal parasitosis is especially widespread. Many disorders of the gastrointestinal tract observed in clinical practice may be disguised forms of parasitic diseases. For the most part, this pathology is manifested by erased forms of ongoing parasitosis. Clinical manifestations of intestinal helminthiases are as follows: abdominal pain, loss of appetite, nausea, constipation, diarrhea, fatigue, anal itching, urticaria, helmintophobia. Helminthiases are dangerous with complications: abscess of the liver and pancreas, intestinal obstruction, intestinal perforation, blockage of the bile ducts and pancreatic ducts, iron deficiency anemia, myocarditis, CNS lesions. Over the past decade, significant progress has been made in the specific therapy of helminthiases. New effective and convenient drugs and methods of treatment have appeared. However, a complete cure is achieved only as a result of several courses of therapy with these drugs. There have been many publications about the presence of resistance in helminths to the action of certain drugs. Currently, various synthetic antihelmintic drugs are widely used to get rid of these parasites. Among these drugs, albendazole, mebendazole, and levamisole are often used in the treatment of diseases such as hookworm, ascariasis, trichuriasis, enterobiasis, necatoriasis, and others [1,2]. In the literature data, the authors show that the mechanism of action of these drugs is associated with inhibition of the fumarate reductase enzyme in helminth mitochondria, as a result of which glucose uptake is impaired. This process is 30 times more intense in parasite cells than in human cells. The drugs also inhibit the synthesis of the protein tubulin, which disrupts the structure of the microtubular apparatus of the parasite cells and this leads to its death. There are also many methods for analyzing the pharmacokinetics of antihelmintic drugs in the literature [11]. The authors of the article give the conditions for the analysis of imidazole derivatives by high performance chromatography. The method has been developed for the Food and Drug Administration (FDA) for the determination of drugs or in their dosage forms with high precision recovery, as well as clinically in human plasma, especially in relation to pharmacokinetic and bioequivalence studies [12].

In the practice of chemical-toxicological analysis, cases of poisoning of people with certain antihelmintic drugs have become more frequent. According to the literature, given the lack of chemical and toxicological studies of antihelmintic drugs, it is important to improve methods for their isolation and detection from biological fluids, as well as to develop optimal conditions, taking into account the factors that influence this.

OBJECTIVE

The aim of our research is to develop a method for isolating antihelmintic drugs from biological fluids (blood, urine) and their analysis by chromatographic methods.

MATERIALS AND METHODS

Albendazole extraction technique from bioliquids: 25 ml of urine sample or 5 ml of blood is taken, adjusted to pH 4.0-5.0 with a solution of 2 mol/l hydrochloric acid, 10 ml of hexane are added and shaken in a mechanical shaker for 10 minutes. The protein in the mixture is then centrifuged for 5 minutes (3000 rpm) to precipitate the proteins. 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 are combined and passed through filter paper containing 5 g of anhydrous sodium sulfate. 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 ethanol, albendazole is purified from impurities using thin layer chromatography, and then analyzed by HPLC methods [3,4,6,8].

Mebendazole Extraction Method from Biofluids: A solution of 25 ml of urine sample and 5 ml of blood, adjusted to pH 6.0-7.0 with ammonia solution, 10 ml of chloroform are added and shaken in a mechanical shaker for 10 minutes. Then, to precipitate proteins, the mixture is centrifuged for 5 minutes (3000 rpm). The chloroform layer is separated from the aqueous layer, the remaining aqueous layer is extracted with 5 ml of chloroform, and the chloroform is poured. The chloroform extracts are combined and passed through filter paper containing 5 g of anhydrous sodium sulfate. The filter is washed with 5 ml of chloroform. The organic solvent from the filtrate is evaporated at room temperature, the residue is dissolved in 5 ml of ethanol, mebendazole is purified from impurities using thin layer chromatography and analyzed by HPLC methods [5,7,10].

Levamisole Extraction Method from Biofluids: 25 ml of urine sample and 5 ml of blood are taken, adjusted to pH 3.5-4.0 with a solution of 0.1 mol / l sulfuric acid, 10 ml of chloroform are added and shaken for 10 minutes in a mechanical shaker. The mixture is then centrifuged for 5 minutes (3000 rpm) to precipitate the proteins. The chloroform layer is separated from the aqueous layer, the remaining aqueous layer is extracted with 5 ml of chloroform, and the chloroform is poured. The chloroform extracts are combined and passed through filter paper containing 5 g of anhydrous sodium sulfate. The filter is washed with 5 ml of chloroform. The organic solvent is evaporated from the filtrate at room temperature, the residue is dissolved in 5 ml of 95% ethanol, levamisole is purified from impurities using thin layer chromatography, and then analyzed by HPLC methods [9].

Chromatographic Purification from Ballast Substances and Detection of Antihelmintic Drugs in Extracts

TLC analysis is performed to confirm the detection of substances, their separation and removal from extractive substances.

To do this, on the starting line in three Silufol UV 254 chromatographic plates, the resulting alcoholic solutions of each test drug are applied separately in the form of a line, next, as a comparison, the standard working solution of albendazole, mebendazole, levamisole is applied in drops, respectively, and dried at room temperature. A mixture of organic solvents: chloroform - ethyl alcohol - formic acid (8:1:1) is used as the mobile phase for albendazole and mebendazole. For levamisole, a mixture of solvents chloroform - ethyl alcohol - formic acid is used in a ratio of 4: 2: 1. To identify the zones of localization of substances on chromatographic plates, a UV beam at a wavelength of 254 nm is used and the location of the spots formed is determined. with a glass plate and the part of the plate where the standard solutions are applied is sprayed with Dragendorf's reagent. The zone of formed spots is determined and the same zone in the closed part of the plate is scraped off and eluted with the appropriate eluant [14]. The eluate is then analyzed. The conditions for the analysis of antihelmintic drugs by TLC are presented in table 1.

Table 1: Recommended conditions for TLC analysis for investigational antihelmintic drugs

Substances	Solvent system	Developers	Rf meaning	Eluant
Albendazole	chloroform - ethanol - formic acid (8:1:1)	UV beam; Munje's modified Dragendorf reagent	0,66	0.1mol/l hydrochloric acid
Mebendazole			0,60	
Levamisole	chloroform - ethanol - formic acid (4:2:1)	Munje's modified Dragendorf reagent	0,50	0.1 mol/l sulfuric acid

Determination of antihelmintic drugs by high performance liquid chromatography.

Determination of Albendazole by High Performance Liquid Chromatography.

The study is carried out under the following conditions:

- Chromatographic column: 3x100 mm, sorbent - Eclipse XDV, particle size - 3.5 microns.
- Detection is carried out at a wavelength of 294 nm.
- Mobile phase: ammonium dihydrogen phosphate-methanol (300: 700),

- eluent flow rate - 1.0 ml / min.
- Column temperature - equal to room temperature.
- Analysis duration 15 minutes

Albendazole was weighed to 0.02 g (t. b.) and dissolved in a 50 ml volumetric flask in the mobile phase, and made up to the mark. Albendazole working standard solutions are prepared and analyzed from this solution. Under the HPLC conditions presented, the retention time of albendazole is 8.2 minutes (Fig. 1).

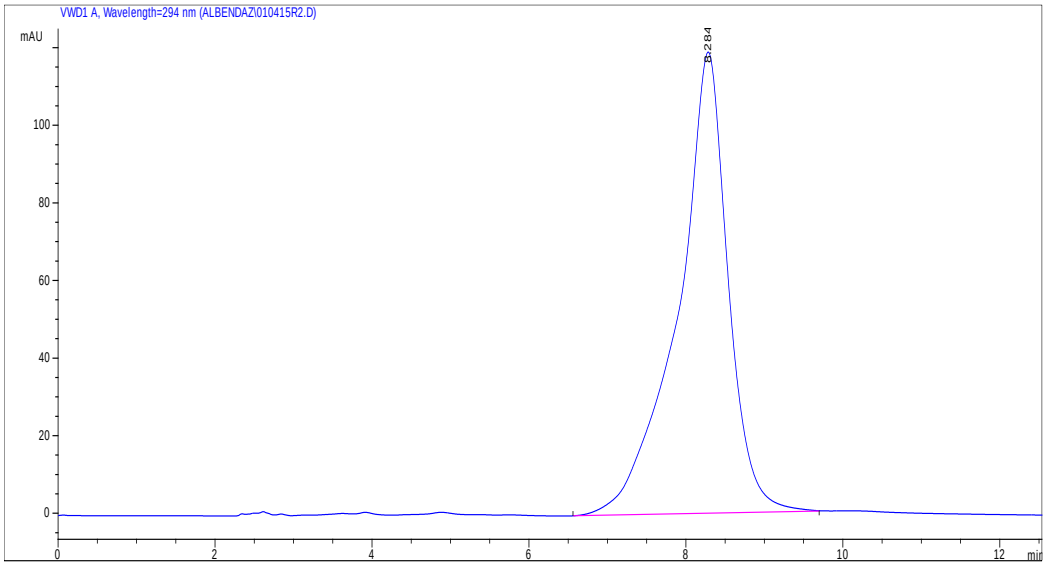

Figure 1: Chromatogram of albendazole standard sample solution

For quantitative determination, standard samples of solutions are prepared containing the standard substance albendazole in an amount of 1, 10, 20, 30, 40 µg/ml. Under the above HPLC conditions, the analysis is carried out and the resulting chromatographic peaks are counted. The results for albendazole are presented in Table 2.

Table 2: Albendazole Curve Results by HPLC

Solution concentration, µg/µl	Surface area of the chromatographic peak (S)
1.00	133.7
10.00	1386.8
20.00	2593.6
30.00	3710.4
40.00	5087.2

Under these conditions, the linear range of determination of albendazole by HPLC is 1–40 µg, and the sensitivity is 0.3 µg.

Based on the results, a calibration curve was drawn up for the quantitative determination of albendazole (Fig. 2).

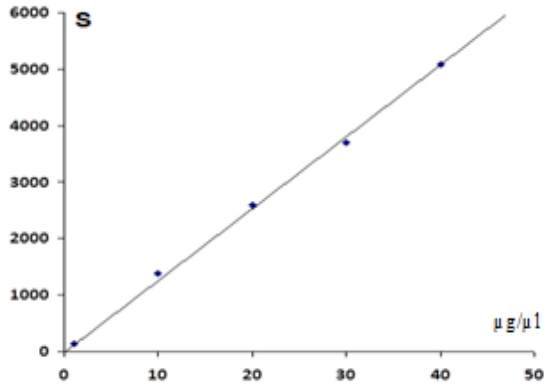

Figure 2. Calibration plot of albendazole under recommended HPLC analysis conditions

Based on the developed conditions for chromatographic analysis, it is possible to identify and determine the amount of albendazole isolated from biological fluids.

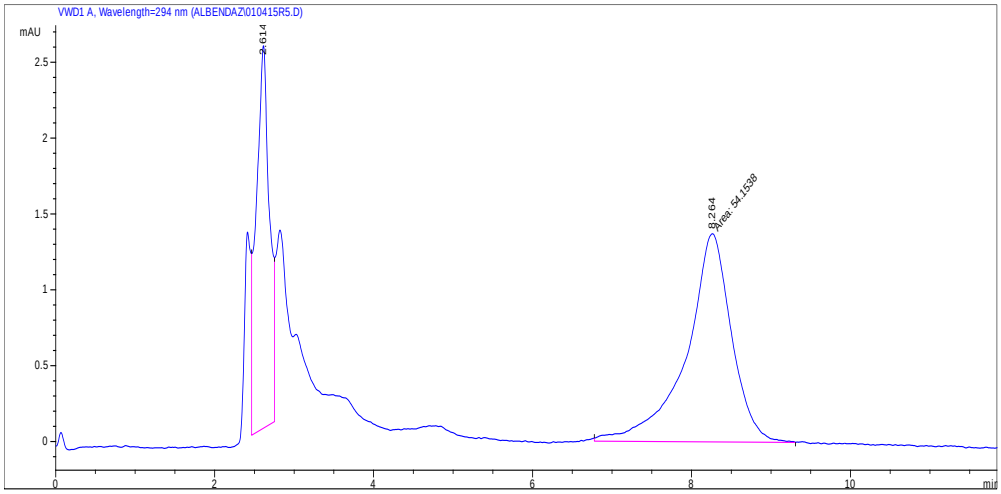

Figure 3: Chromatogram of albendazole isolated from blood

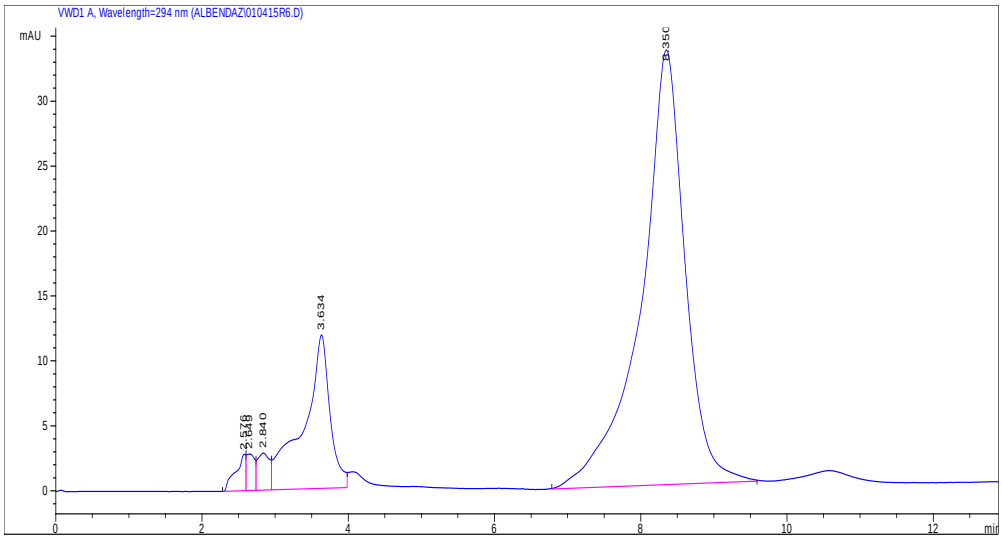

Figure 4: Chromatogram of albendazole isolated from urine.

As can be seen from the chromatograms, albendazole isolated from biological fluids according to the proposed method gives a chromatographic peak identical to the standard sample.

Determination of mebendazole by high performance liquid chromatography.

The study is carried out under the following conditions:

- Chromatographic column: 4.6 x 150 mm, sorbent - Eclipse ACE 5 C18 S / N-A82851, particle size - 5 microns.
- Detection is carried out at a wavelength of 210 nm.
- Mobile phase: ammonium dihydrogen phosphate-methanol (20:80),
- eluent flow rate - 1.0 ml/min.
- Column temperature - equal to room temperature.
- Analysis duration 10 minutes

Mebendazole is weighed 100 mg (t. N.), dissolved in 5 ml of 1% sulfuric acid solution in a 100 ml volumetric flask and brought to the mark with methanol. Take 1 ml of this solution into a 100 ml volumetric flask, dilute to the mark with methanol and analyze. Under these conditions, the retention time of mebendazole is 3.3 minutes (Fig. 5).

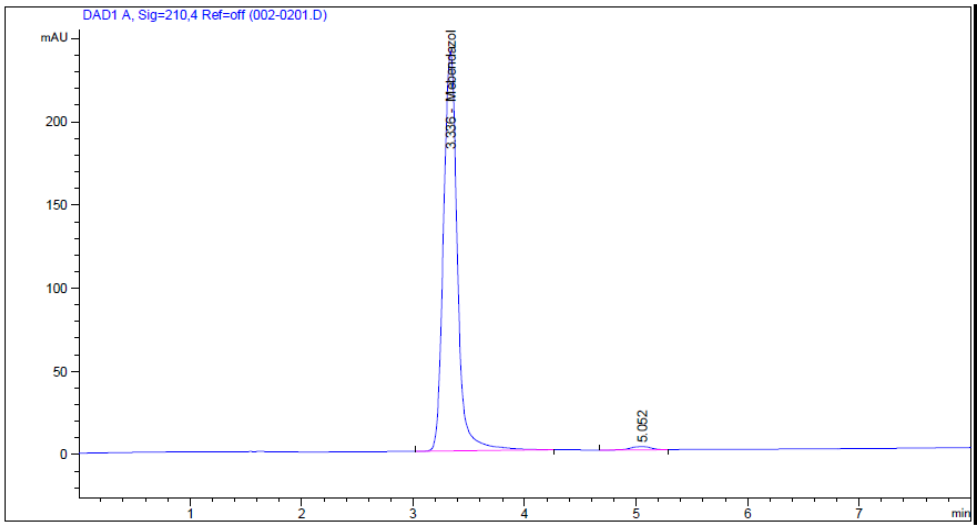

Figure 5: Chromatogram of mebendazole standard sample solution

For the quantitative determination of mebendazole, standard samples of solutions containing the standard substance of mebendazole in an amount of 10-50 µg/ml are prepared and analyzed by HPLC under the above conditions. The results are presented in table 3.

As a result of experiments with this method, the linear range of determination of mebendazole is 10-50 µg and the sensitivity is 0.3 µg.

Table 3: The results of the study of the linearity of detection of mebendazole by HPLC

Solution concentration, µg/µl	Surface area of the chromatographic peak (S)
10	2082,08
20	3903,2
30	5642,6
40	7417,2
50	9184,8

For the quantitative determination of mebendazole in different objects, a calibration curve was compiled (Fig. 6).

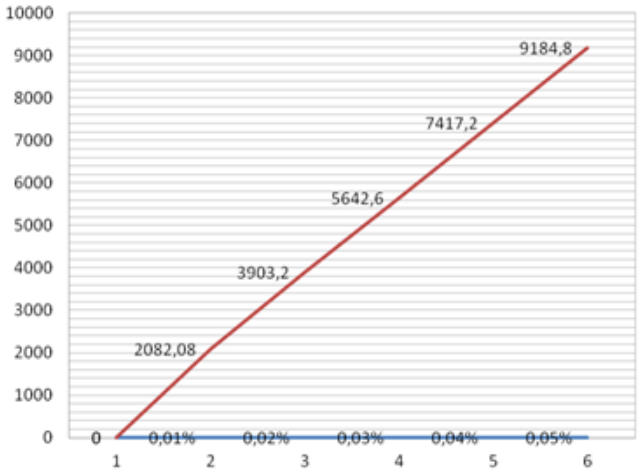

Figure 6: Mebendazole assay calibration curve under recommended HPLC assay conditions

Based on the developed conditions for chromatographic analysis, an analysis was carried out to identify and determine the amount of mebendazole isolated from biological fluids. The analysis was carried out in model samples of blood and urine.

As a result of the analysis of mebendazole extracted from blood and urine, chromatograms were obtained with a retention time of 3.3 minutes, which corresponds to the retention time of a standard sample of mebendazole.

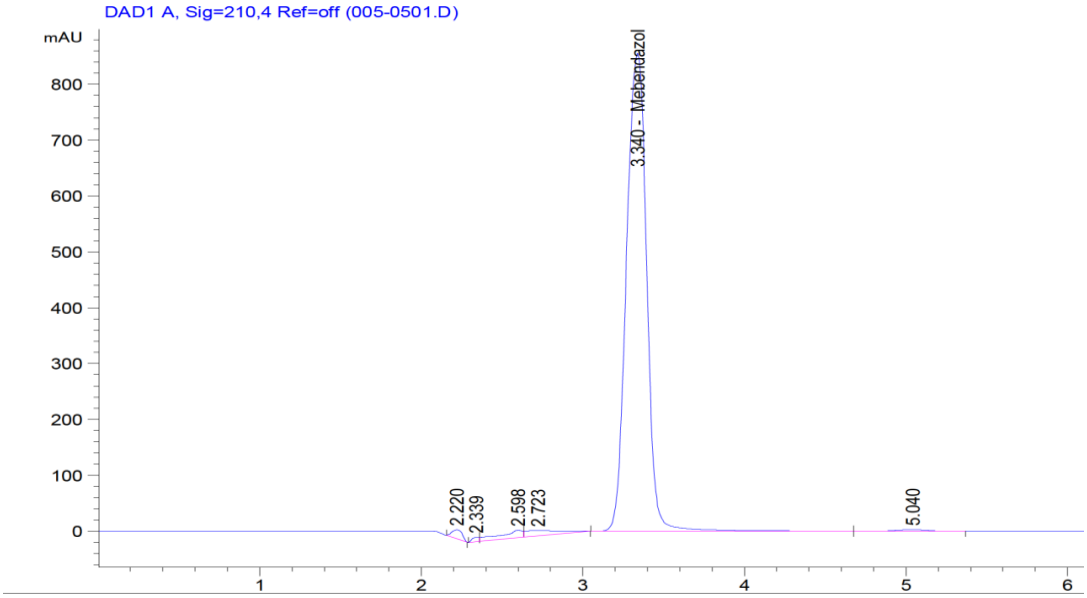

Figure 7. Chromatogram of mebendazole isolated from blood

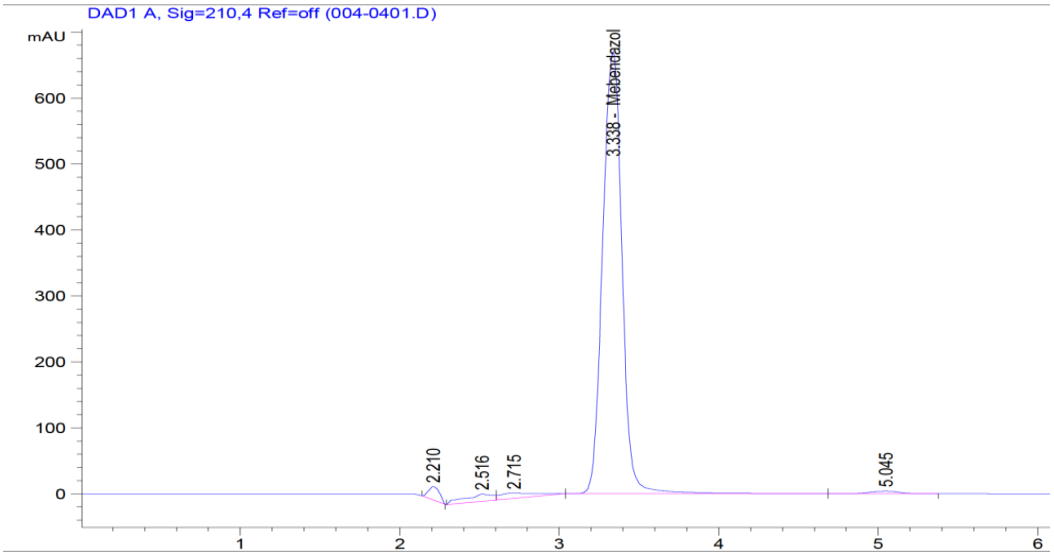

Figure 8. Chromatogram of mebendazole isolated from urine

Determination of levamisole by high performance liquid chromatography.

The study is carried out under the following conditions:

- Chromatographic column: 4.6 x 150 mm, sorbent - Eclipse XDB S-18, particle size - 3 microns.
- Detection is carried out at a wavelength of 215 nm.
- Mobile phase: methanol-distilled water (80:20),
- eluent flow rate - 2.0 ml / min.
- Column temperature - equal to room temperature.
- Analysis duration 10 minutes

Levamisole is weighed 25 mg (a.t.), dissolved in 5-10 ml of distilled water in a 25 ml volumetric flask and brought to the mark with methanol. Take 1 ml of this solution into a 100 ml volumetric flask, make up to the mark with methanol and analyze. Under these conditions, the retention time of levamisole is 3.9 minutes (Fig. 9).

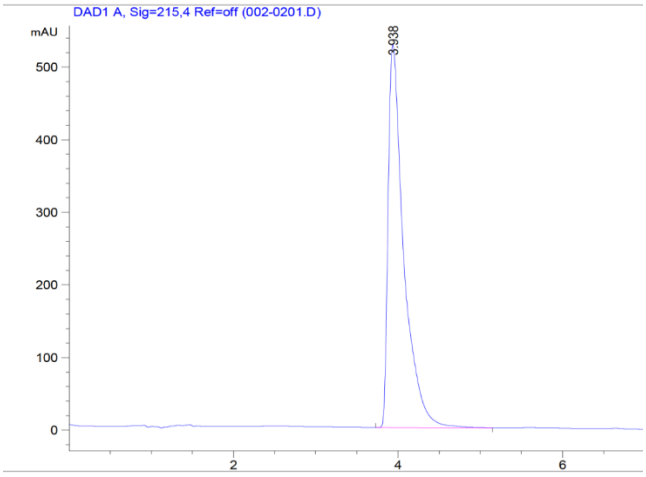

Figure 9: Chromatogram of levamisole standard solution

For the quantitative determination of levamisole, standard samples of solutions containing the standard substance levamisole in an amount of 0.015-0.075 µg/ml are prepared and analyzed by HPLC under the above conditions [9,13]. The results are presented in table 4.

Table 4: The results of the study of the linearity of detection of levamisole by HPLC

Solution concentration, µg/µl	Surface area of the chromatographic peak (S)
0,015	1657,5
0,030	3314,3
0,045	5125,7
0,060	6916,4
0,075	8727,1

As a result of the experiments, the linear range for determining levamisole by HPLC is 0.015–0.075 µg, and the sensitivity is 0.01 µg. For the quantitative determination of levamisole in different objects, a calibration graph was compiled (Fig. 10).

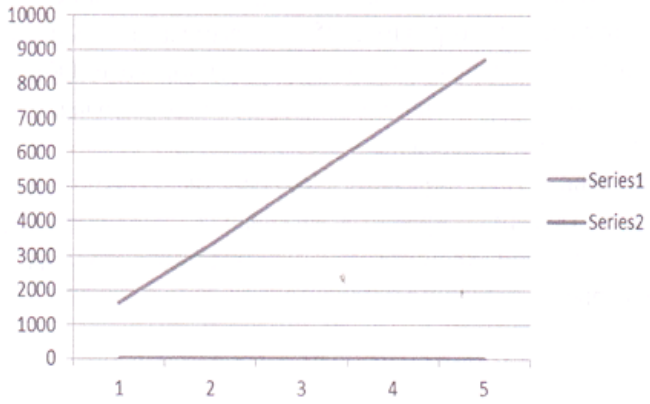

Figure 10: Levamisole quantitation calibration curve under recommended HPLC assay conditions

Based on the developed conditions for chromatographic analysis, an analysis of levamisole isolated from biological fluids was carried out. The results obtained showed the suitability of the developed methods for the detection and quantification of levamisole from blood and urine (Fig. 11-12).

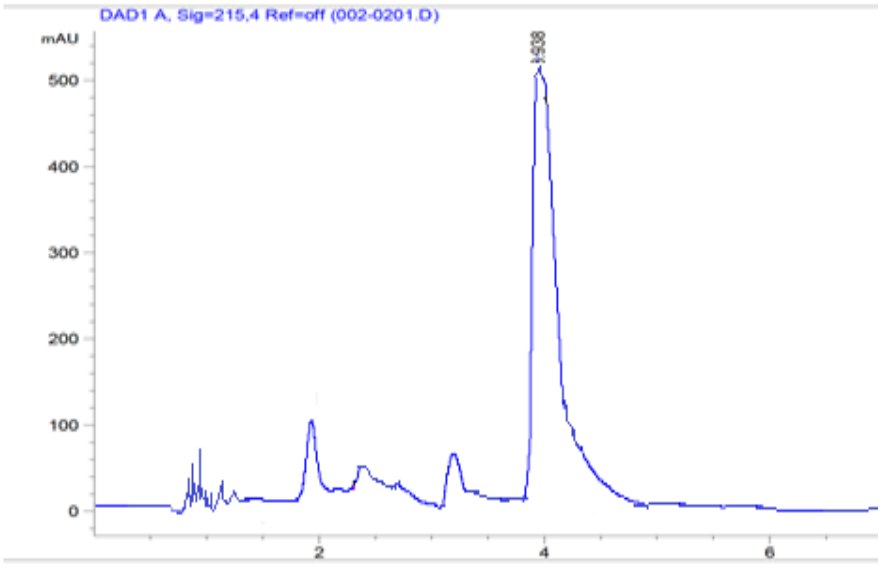

Figure 11: Chromatogram of levamisole isolated from blood

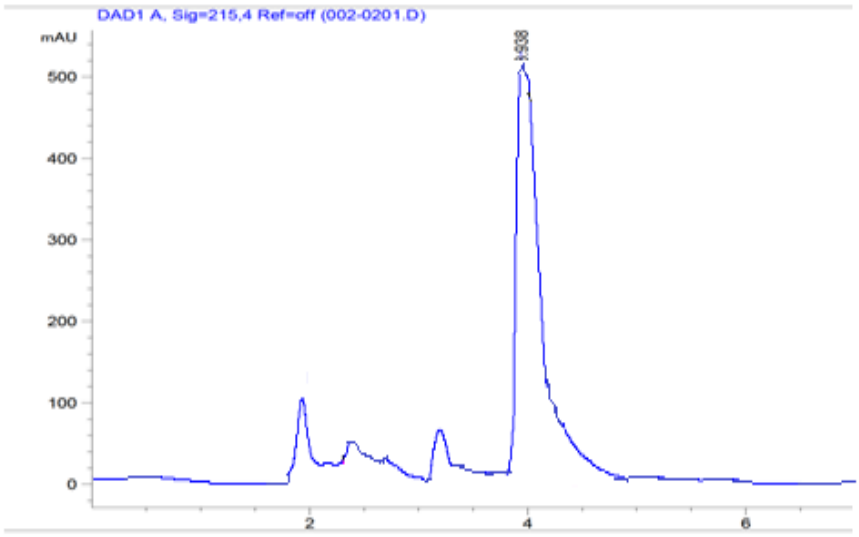

Figure 12: Chromatogram of levamisole isolated from urine

The number of antihelmintic drugs isolated from biological objects according to the HPLC method is calculated using the following formula:

$$X = \frac{Ssp \cdot Ast \cdot Vsp \cdot 100}{Sst \cdot Atest \cdot Vst}$$

where, Ssp. – peak area of the test sample;

Sst is the peak area of the standard sample;

Ast is the mass of the standard sample, µg;

Atest - mass of the test sample, mg;

Vst is the dilution of a weighed portion of the standard *sample, ml;*

Vsp. - dilutions of a sample of the test solution, ml;

Results of the Study and their Discussion

As a result of research, a method for extracting antihelmintic drugs from biological fluids has been developed. To identify the extracted substances, the extracts were purified using the thin layer chromatography method. The elution of each substance was carried out using the selected eluant (hydrochloric and sulfuric acid). This made it possible to obtain a more purified extract, as well as to separate the desired substance from various mixtures. The proposed method of TLC analysis is recommended for purification of extracts obtained from biological fluids. The methods of analysis presented in the literature by the authors were used as a method of protein precipitation in the detection of drugs in human blood plasma. This will render the instrument unusable, as protein precipitation leads to rapid fouling of the column, resulting in damage to the chromatograph. The main goal of this work was to develop a technique for the isolation and analysis of some antihelmintic drugs from biological fluids. For this, chromatographic methods for the analysis of standard solutions of albendazole, mebendazole, and levamisole were initially carried out. The retention time of albendazole was 8.2 minutes, mebendazole 3.3 minutes, levamisole 3.9 minutes. On the basis of the obtained chromatograms, the identification and quantitative determination of the studied substances isolated from blood and urine were carried out. At the same time, using the proposed method, 56.46% of albendazole, 65.42% of mebendazole and 67.79% of levamisole were isolated from the blood. When isolated from urine, results were obtained for albendazole 66.48%, mebendazole 72.56% and levamisole 74.43%. The results obtained prove the reproducibility of this technique.

Conclusions

Based on the studies carried out, methods for isolating albendazole, mebendazole, and levamisole from biological fluids have been developed. TLC conditions for purification of obtained extracts of each preparation are proposed. HPLC analysis was carried out using standard solutions of antihelmintic drugs. Under these conditions, the identification and quantitative determination of albendazole, mebendazole and levamisole isolated from blood and urine was carried out. The data obtained showed the reliability of the developed methodology.

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