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DETERMINATION OF ACCUMULATION AND DISTRIBUTION OF EFFECTIVE ANTIGELMINT DRUGS IN BIOLOGICAL OBJECTS

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Abstract. The distribution and accumulation of anthelmintic drugs in the internal organs of experimental animals were studied. According to the results of the analysis, mebendazole can be stored for 60 days in decaying biological objects and 90 days in canned biological objects. levamisole can be stored for 30 days in decaying biological objects and 60 days in canned biological objects. According to the results, in cases of acute poisoning with mebendazole were found in large quantities in the liver, kidneys, small intestine, colon, blood and urine. Acute levamisole poisoning has been reported in large amounts in the liver, spleen, kidneys, small intestine, colon, blood and urine.

Key words: Mebendazole, levamisole, experimental animal organs, extraction, thin-layer chromatography, UV spectrophotometry.

Mebendazole is an anthelmintic drug that disrupts the microtubular system of intestinal duct cells in helminthes, by destroying their function, slowing down the transport of secretory substances and absorption of nutrients, causing irreversible degeneration of the intestinal tract and death of helminthes. Peak plasma concentrations occur in 0.5-7 hours after oral administration. Mebendazole is poorly bound to plasma proteins. Mebendazole biologically converted to inactive metabolites by decarboxylation. Approximately 10% of the unchanged substance is excreted in the urine within 1-2 days. Most of it is excreted in the feces. Mebendazole is used mainly for mixed infections caused by enterobiasis, trichocephaly, ascariasis, hookworm, strongyloidiasis, teniosis and other helminthes [1,2].

Taking an extra dose of mebendazole by accident can cause the following side effects in humans, which include dizziness, nausea, and abdominal pain. When

Mebendazole is used in high doses for a long time: vomiting, diarrhea, headache, allergic reactions (skin rash, angiodema), "liver" transaminases, hypercreatinemia, leukopenia, anemia, eosinophilia, hair loss, hematuria, even may adversely effect to the fetal development.

Levamisole, a drug containing imizadol derivatives, has a selective effect on helminthes. It is used as a remedy against ascariasis, which occurs in humans. Levamisole paralyzes helminthes, through causing muscle depolarization. In addition, in helminthes Levamisole inhibits fumarate reductase and destroys bioenergetic processes. Paralyzed helminthes are excreted from the human body through the intestines within 24 hours. Levamisole also has an immunomodulatory effect, normalizing cellular immunity. It has a complex effect on the immune system: increases the production of antibodies against various antigens, enhances the effect of T-cells, activates and accelerates the proliferation of T-lymphocytes, increases the function of monocytes, macrophages and neutrophils [1,2].

As noted above, the anthelmintic and immunomodulatory effects of levamisole are widely used, and cases of poisoning due to misuse have been reported in the literature. Improper use of the drug can cause the following side effects on digestive system: salivation, vomiting, abdominal pain, diarrhea; on the cardiovascular system: bradycardia, collapse; on the respiratory system: difficulty breathing, tachypnea; nervous system miosis (narrowing of the pupil), convulsions, depression.

In cases of acute drug poisoning, death from respiratory failure may occur within 1 hour after taking Levamisole. Symptoms of intoxication from Levamisole can be observed for 5 to 15 minutes.

In an organism poisoned by anthelmintic drugs, the toxin spreads to the internal organs, poisoning them and leaving or accumulating in some organs. Based on the information above, when investigating the causes of poisoning, it is important for the forensic chemist to know in which organs the alleged poison is concentrated, and it is advisable to obtain such organs for analysis. This will allow medical personnel to provide emergency medical care to patients in cases of acute

poisoning with anthelmintic drugs, and to clarify the subject for specialists during court proceedings.

Research methods. To determine the storage of mebendazole in biological objects, several samples of biological substance were prepared (50 g), placed in glass jars, and the jars were tightly closed. Until the time of experiment, all objects were stored at room temperature without adding preservatives (95%ethyl alcohol)[10].

Samples were separated according to days of storage 5, 10, 15, 30, 60, 90 and 120, respectively. After 5, 10, 15, 30, 60, 90 and 120 days, the residual amounts of mebendazole in these objects were isolated as follows. To conduct isolations, each biological object were taken and placed in a flask with 0.1 M hydrochloric acid solution for one hour, periodically shaking. The extract was merged from objects. After, the extract was quickly filtered and solid part of the biological object were immersed for a second time into 0.1 hydrochloric acid solution for one hour. First and second extract solutions were combined and centrifuged at 3000 rpm for 10 min. The liquid part was separated, about 20-30 ml of 0.1 hydrochloric acid solution was added to precipitate and left for one hour. The extract is centrifuged and liquid layer were added to the total extract. After extraction processes, total liquid were transferred to a tap funnel and brought to a pH=6.0-7.0 by adding 25% ammonia solution. The Mebendazole residue was extracted with 20 ml chloroform. Chloroform extracts were filtered through a filter paper containing 5.0 g of dehydrated sodium sulphate and was distilled off at room temperature. The dry residual content was dissolved in 5 ml of ethanol. The extracted Mebendazole chromatographed in a laboratory-prepared silica gel storage plate, as an optimal mobile phase a mixture of chloroform-ethyl alcohol-formic acid was chosen in a ratio of 8: 1: 1. Spots of mebendazole on the chromatographic plate were detected and then eluated using 0.1 M hydrochloric acid solution, and the eluate-containing mebendazole was quantified against standard solutions at a wavelength of $\lambda = 286$ nm on a spectrophotometer (8453E Spectroscopy System Agilent Technologies).

According to the results of the analysis, Mebendazole can be stored in biological facilities for 60 days without preservation[3,4,6,7].

To study the effect of ethyl alcohol on the accumulation Mebendazole in biological objects, 50 g of animal viscera (crushed liver) were taken, several samples were prepared, placed in conical flasks. Following, 95% ethyl alcohol was added until biological object were covered. The jars were sealed and left at room temperature. After that, at different times, 95% of the ethyl spirit which were added to the sample as a preservative was removed from the sample at room temperature, and based on the experiment above; Mebendazole was isolated, purified from foreign substances, and analyzed by UV spectrophotometry. The results of the analysis showed that Mebendazole can be stored for 90 days when the biological object is preserved with 95% ethyl alcohol.

Based on the results, it was found that Mebendazole can be stored for 60 days in decaying biological objects and 90 days in canned biological objects.

To determine the storage of Levamisole in biological objects, several samples of biological substance were prepared (50 g), placed in glass jars, and the jars were tightly closed. Until the time of experiment, all objects were stored at room temperature without adding preservatives (95 % ethyl alcohol)[10].

Samples were separated according to days of storage 5, 10, 15, 20, 25, 30 and 60, respectively.

After 5, 10, 15, 20, 25, 30 and 60 days, the residual amounts of Levamisole in these objects were isolated as follows. To conduct isolations, each biological object were taken and placed in a flask with 0.02 M sulfuric acid solution for one hour, periodically shaking. The extract was merged from objects. After, the extract was quickly filtered and solid part of the biological object was immersed for a second time into 0.02 M sulfuric acid solution for one hour. First and second sulfuric acid were combined and centrifuged at 3000 rpm for 10 min. The liquid part was separated; about 20-30 ml of 0.02 M sulfuric acid solution was added to precipitate and left for one hour. The extract is centrifuged and liquid layer were added to the total extract. After extraction processes, total liquid were transferred to a tap funnel

and brought to a pH=3.0-4.0 by adding 25% ammonia solution. The Levamisole residue was extracted three times with 20 ml chloroform. Chloroform extracts were filtered through a filter paper containing 5.0 g of dehydrated sodium sulphate and was distilled off at room temperature. The dry residual content was dissolved in 5 ml of ethanol.

The residue was dissolved in 5 ml of ethyl alcohol and chromatographed in a 4: 2: 1 solvent mixture of chloroform-ethyl alcohol-formic acid on a laboratory-prepared silica gel storage plate. The Levamisole spots on the chromatographic plate were identified and then eluated using a 0.1 M sulfuric acid solution, and the eluate levamisole was quantified on standard spectrophotometer (8453E Spectroscopy System Agilent Technologies) for standard solutions at $\lambda= 210$ nm. According to the results of the analysis, Levamisole can be stored in biological objects for 30 days without [5,6,8,9].

To study the effect of ethyl alcohol on the accumulation Levamisole in biological objects, 50 g the internal organs of the animal (crushed liver) were taken. Several samples were prepared, placed in conical flasks and covered with 95% ethyl alcohol. The jars were sealed and left at room temperature. At different times, 95% of the ethyl spirit was removed from the object by evaporation at room temperature, and based on the above experiment; Levamisole was isolated, purified from foreign substances, and analyzed by UV spectrophotometry. The results of the analysis showed that Levamisole can be stored for 60 days when the biological object is preserved with 95% ethyl alcohol.

Based on the results, it was determined that Levamisole can be stored for 30 days in decaying biological objects and 60 days in canned biological objects.

Ethyl alcohol slows down the process of decomposition, which begins in biological objects, reduces the metabolism and rate of decomposition of toxins in it. In this regard, this information should be taken into account in the analysis of biological objects suspected of containing anthelmintic drugs, which were sent for inspection.

The percentage of anthelmintic drugs isolated from biological objects is calculated and a metrological report is made, based on the following formula:

$$X\% = \frac{D \cdot V \cdot 10 \cdot 100}{E_{1SM}^{1\%} \cdot a \cdot 100}$$

X% - the percentage of the substance under test;
 D - optical density of the detectable substance;
 V - standard solution volume;
 $E_{1SM}^{1\%}$ - standard specific light absorption index;
 a - substance weight (a.t.)

Chromatographic purification and detection of anthelmintic drugs from foreign substances.

Thin-layer chromatography (TLC) is a chromatography technique, is performed in order to detect, separate, identify, and remove co-extracted components in a mixture.

In order to purify anthelmintic drugs from foreign substances, glass plates coated with silica gel, prepared in the laboratory was used. Chromatographic plates "Silufol UV 254" were used for qualitative analysis. Using a capillary tube, a small spot of solution containing the sample was applied to a plate, from the bottom edge. Near another spot from the standard working solutions of mebendazole, levamisole were applied. Plate was dried at room temperature. As an optimal mobile phase mixture of organic solvents: chloroform - ethyl alcohol - formic acid and chloroform - ethyl alcohol - formic acid were chosen in ratio (8: 1: 1) and (4: 2: 1), respectively. When it reached a height of 10 cm near the finish line, the plates were removed and dried at room temperature. UV-254 was used to determine the location of the accumulation of substances on the chromatographic plates. After that plates was sprayed with Dragendorff reagent on the dripping part of the drug from the objects. The stained parts of the substance were identified, the sorbent layers are removed and the analysis was performed[4,5,6]. The results of the analysis are given in Table 1.

Recommended conditions for the analysis of TLC for the studied anthelmintic drugs.

Substances	Solvent system	Identification	Rf indicator	Eluent
Mebendazole	chloroform - ethyl alcohol - formic acid (8:1:1)	UV light; reagent of Dragandorf modified with Munye	0,60	0,1M hydrochloric acid
Levamisole	chloroform - ethyl alcohol - formic acid (4:2:1)	reagent of Dragandorf modified with Munye	0,47	0,1 M Sulfuric acid

In organisms poisoned by toxins, under the influence of various factors, toxins can be released into internal organs and then excreted in different ways. However, some toxins can be accumulated in some tissues. In order to obtain reliable results from analysis, it is imported for the forensic chemist to know in which organ poison is concentrated.

To investigate the distribution of Mebendazole in the internal organs, 620mg/kg of an aqueous solution of Mebendazole was directly injected into stomach of experimental animals (rabbits). Experimental animals died after 12 hours. Their internal organs stomach, colon, small intestine, liver, kidneys, spleen, lungs, brain, muscles to Sections, blood and urine were separated and analyzed using the methods described above.

The results showed that in cases of acute poisoning by Mebendazole, the large concentration of poison accumulated in the liver, kidneys, small intestine, colon, blood and urine. Our study suggest that, in the case of death occurred by Mebendazole poisoning it is better for forensic chemists to use blood, urine, liver, kidneys, small intestine and colon.

To study the distribution of Levamisole in the internal organs, 180 mg/kg of an aqueous solution of Levamisole was directly injected into stomach of an experimental animals(rabbits). Experimental animals died after 12 hours. Their internal organs stomach, colon, small intestine, liver, kidneys, spleen, lungs, brain, muscles to Sections, blood, and urine were separated and analyzed using the methods described above. The results of the experiment showed that in cases of acute poisoning with levamisole were detected in large quantities in the liver, spleen, kidneys, small intestine, colon, blood and urine.

Conclusions. The distribution and accumulation of anthelmintic drugs in the internal organs of experimental animals were studied. According to the results of the analysis, mebendazole can be stored for 60 days in decaying biological objects and 90 days in canned biological objects. levamisole can be stored for 30 days in decaying biological objects and 60 days in canned biological objects. The results showed that in cases of acute poisoning by Mebendazole, the large concentration of poison accumulated in the liver, kidneys, small intestine, colon, blood and urine. Our study suggest that, in the case of death occurred by Levamisole poisoning it is better for forensic chemists to use blood, urine, liver, spleen, kidneys, small intestine and colon.

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