

Determination of the timing of slaughter of sheep after the use of the drug MONIZEN® forte

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Abstract. The article is devoted to the results of determining the residual amounts of active ingredients of the medicinal product for veterinary use - MONIZEN® forte - in the organs and tissues of sheep. MONIZEN® forte is a solution for oral and parenteral use, the active ingredients include praziquantel and ivermectin. MONIZEN® forte is used for prophylactic and therapeutic purposes in small ruminants with cestodoses, nematodes of the lungs and gastrointestinal tract, trematodoses, estrosis, psoroptosis, chorioptosis, sarcoptosis, ixodidosis, sifunculatosis, and also melicophagoses, arachinoses, and biliary tract. Carrying out this study is mandatory for the introduction of the drug into wide industrial practice. As a result of the studies, the terms of slaughter of small ruminants after the use of the drug Monizen® forte were established.

It is advisable to slaughter sheep for meat 35 days after the last use of the drug.

1 Introduction

Livestock is an important sector of the country's national economy, which is a source of production such products as wool, meat, milk. Sheep breeding is well developed in Russia in Dagestan, Kalmykia, Stavropol Territory, Astrakhan Region, Karachay-Cherkessia. For 2019, the total number of sheep and goats in Russia is up to 22.5 million heads. For the high-quality maintenance of sheep, it is important not only to maintain certain conditions for keeping and feeding animals, but also to carry out timely therapeutic and prophylactic measures, including the treatment of sheep from endoparasites and ectoparasites, which cause significant mortality of young animals, a decrease in body weight and quality of animal hair. The company LLC "NVC Agrovetzashchita" together with VNIIVSGE, has developed the antiparasitic drug MONIZEN® forte. MONIZEN® forte is a solution for oral and injection use, the active ingredients include ivermectin 5 mg / ml and praziquantel 60 mg / ml.

After parenteral and oral administration of the drug, praziquantel and ivermectin are well absorbed, enter the systemic circulation and are distributed in the organs and tissues of animals and poultry, providing a long-term antiparasitic effect. MONIZEN® forte is used for the treatment and prevention of nematodes, cestodes, trematodes, gadfly larvae, sarcoptoid, gamazoid and ixodid ticks, bloodsuckers, malophagous (lice-eating) cattle and small ruminants. Deworming of animals is carried out before stalling and in the spring before

pasture, as well as at other times according to indications; treatment against gadfly invasions is carried out after a period of activity of gadflies; treatment for psoroptosis, chorioptosis, sarcoptic mange, ixodidosis, sifunculatosis, melophagosis, bovikolez, arachnoentomoses - according to indications.

The main targets of ivermectin action are glutamate-sensitive chlorine channels and gamma-aminobutyric acid receptors. Paralysis and death of parasites occurs due to a change in the process of penetration of chlorine ions, which disrupts the conduction of impulses. [1, 2]. In most animal species, ivermectin has a terminal half-life. It is metabolized in the liver by oxidative processes, excreted mainly in the feces. Less than 5% of the drug (in the form of the parent substance or metabolites) is excreted in the urine [3, 4].

Praziquantel refers to a synthetic derivative of pyrazinisoquinoline. Praziquantel acts on impaired glucose transport and microtubular function of flatworms, which causes impaired muscle innervation, paralysis and death of helminths. [5]. In low concentrations in vitro, the drug disrupts the function of the parasite's suckers and stimulates its mobility. At high in vitro concentrations, praziquantel enhances the contractility of helminth strobila. Praziquantel destroys trematodes of the genus *Shistosoma* and others by increasing the flow of calcium ions into the cells of the parasite. Praziquantel is metabolized in the liver by CYP3A enzymes to metabolites, the activity of which is not known. It is excreted mainly in the urine [3, 5- 7].

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In order to implement the introduction of the drug into wide industrial practice, a complex of preclinical studies and a study of its effectiveness on target species of animals were carried out [8]. So the work on testing the drug MONIZEN® forte for parasitic diseases of sheep was carried out on the farm on 460 lambs weighing 25-30 kg with a high infection with nematodes, cestodes and larvae of *Oestrus ovis*.

The results of coprological studies showed that the drug MONIZEN® forte, used in group treatment in a mixture with concentrated feed, showed 100% efficiency in moniesiasis, dictyocaulosis and sheep estrosis, and against nematodes of the gastrointestinal tract, it showed IE = 96.7%, EE = 95%. During the observation of side effects and complications the drug is in a therapeutic dose [9].

In this work, we highlight the results of the determination of the residual amounts of active substances in the organs and tissues of sheep and the establishment of the waiting period.

2 Materials and Methods

To study the dynamics of elimination of residual amounts of ivermectin and praziquantel, 12 sheep were injected with the drug MONIZEN® forte twice (with an interval of 14 days), subcutaneously at a therapeutic dose of 1 ml / 15 kg of animal weight. In 30 and 35 days after the last injection of the drug, the animals were slaughtered and samples of organs and tissues (muscles, liver, heart, lungs, spleen, internal fat) were taken for further analysis. For the work, the analytical standards of active substances and the preparation were used.

Ivermectin Analytical Standard (22, 23-Dihydroavermectin B1, MK-933), FLUKA, CAS Number 70288-86-7, analytical standard (Fig. 1).

Praziquantel analytical standard (2-(Cyclohexylcarbonyl)-1,2,3,6,7-11b-hexahydro-4H-pyrazino[2,1-*a*isoquinolin-4-one), 98,8%, VETRALAL, CAS Number 55268-74-1 (Fig. 2).

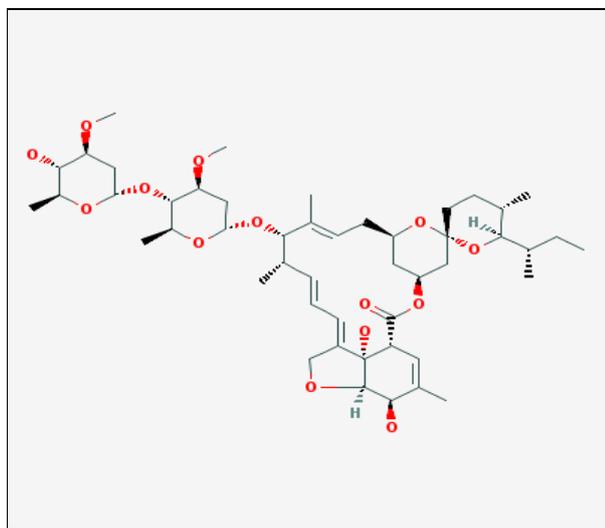


Fig. 1. Ivermectin Analytical Standard

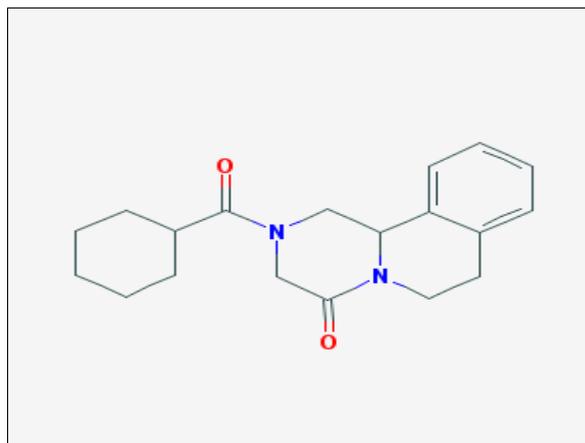


Fig. 2. Praziquantel analytical standard

To carry out the research, the following steps were carried out: preparation of solutions of a standard sample of ivermectin for plotting a calibration graph, preparation of “blank” samples of organs and tissues with introduced solutions of a standard sample of ivermectin for plotting a calibration graph, preparation of solutions of a standard sample of praziquantel for plotting a calibration graph, preparation of “blank »- samples of organs and tissues with applied solutions of a standard sample of praziquantel for plotting a calibration graph, preparation of samples of organs and tissues for analysis of ivermectin, preparation of samples of organs and tissues for analysis of praziquantel.

The preparation of solutions of a standard sample of ivermectin for plotting a calibration graph was carried out as follows. Stock solution: on an analytical balance, about 0.1 g (exact weighed) of a standard sample of ivermectin was weighed on an analytical balance, the sample was transferred into a 100 ml volumetric flask, 50 ml of the mobile phase was added, thoroughly mixed until complete dissolution, after which the volume the solution was brought to the mark with the mobile phase [10]. The resulting solution had a concentration of 1000 µg ivermectin / ml. Then, calibration solutions of standard samples with ivermectin concentrations of 200, 100, 50, and 10 ng / ml were prepared from the stock solution by the mobile phase dilution method.

Then, “blank” samples of organs and tissues were prepared with introduced solutions of a standard sample of ivermectin to build a calibration graph.

The preparation of solutions of the standard sample of praziquantel for the construction of the calibration graph was done according to the following procedure. Stock solution: on an analytical balance, about 0.1 g (exact weighed) of a standard sample of praziquantel was weighed on an analytical balance, the weighed portion was transferred into a 100 ml volumetric flask, 50 ml of the mobile phase was added, thoroughly mixed until complete dissolution, after which the volume the solution was brought to the mark with the mobile phase [10]. The resulting solution had a concentration of 1000 µg praziquantel / ml. Then, calibration solutions of standard samples with praziquantel concentrations of 1000, 500, 100, and 50 ng / ml were prepared from the stock solution.

After that, “blank” samples of organs and tissues were prepared with introduced solutions of a standard sample of praziquantel to build a calibration graph.

In 4 polypropylene tubes, 1 ml of solutions of a standard sample of praziquantel with concentrations of 1000, 500, 100 and 50 ng / ml were transferred, the solutions were evaporated to dryness, after which 1 g of previously homogenized samples of organs and tissues weighing 1 g, obtained from animals, were added to the tubes of control groups. 0.10 ml of 0.2 N sodium hydroxide and 0.5 ml of 0.025 M potassium phosphate buffer (pH = 5.8) were added to the samples, after which the samples were shaken on a shaker for 10 minutes.

The HPLC-FLD method was used to determine ivermectin in organs and tissues [10]. The method for the determination of ivermectin in organs and tissues is based on homogenization of organ and tissue samples, selective extraction from homogenates with ethyl acetate, derivatization, isocratic elution using a Luna C18 (2) chromatographic column (100 Å, 5 µm) and fluorescence detection at λ_{ex} = 365 nm, λ_{em} = 480 nm. To determine praziquantel in organs and tissues, the HPLC-UV method was used. The method for the determination of praziquantel in organs and tissues is based on homogenization of organ and tissue samples in the presence of sodium hydroxide solution and potassium phosphate buffer, extraction from samples using SPE cartridges, and isocratic elution using a Supelcosil LC-18-DB chromatographic column (5 µm) and ultraviolet detection at λ = 215 nm. To carry out a quantitative analysis of the obtained extracts (determination of analyte concentrations), we used the procedure for calibrating chromatographic data (external standard method). Based on the deviation of the calibration curves of the biosubstrates, the detection limits (LoD) and the limits of quantitation (LoQ) are established. Determination of (LoD) and (LoQ) was carried out in accordance with [11, 12] according to the formulas:

$$LoD = 3 * SD_b * k^{-1}$$

$$LoQ = 10 * SD_b * k^{-1},$$

SD_b – standard deviation of the free coefficient b;

k – correlation coefficient (slope).

Metrological certification of methods was carried out in accordance with [11-13]. For the experiments, we used extracts of biosubstrates (calibration samples) with praziquantel concentrations of 50, 100, 500, and 1000 ng / ml (ng / g) and ivermectin 10, 50, 100, 200 ng / ml (ng / g).

3 Results

Analysis of biosubstrates from animals was timed. The analysis of each time point was carried out starting from "zero". A mobile phase analysis was performed between each time point. Based on the deviation of the calibration curves of the biosubstrates, the detection limits (LoD) and the limits of quantitation (LoQ) are established.

Calibration results for ivermectin and praziquantel are shown in figs. 3, 4.

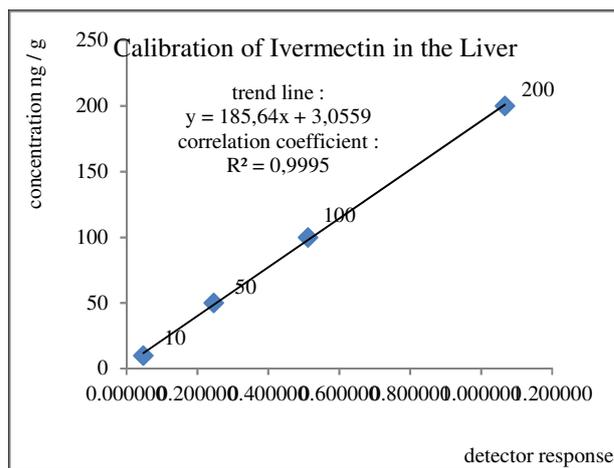


Fig. 3. Calibration of Ivermectin in the Liver. Function type - linear: $y=185,64x+3,0559$ ($R^2=0,9995$).

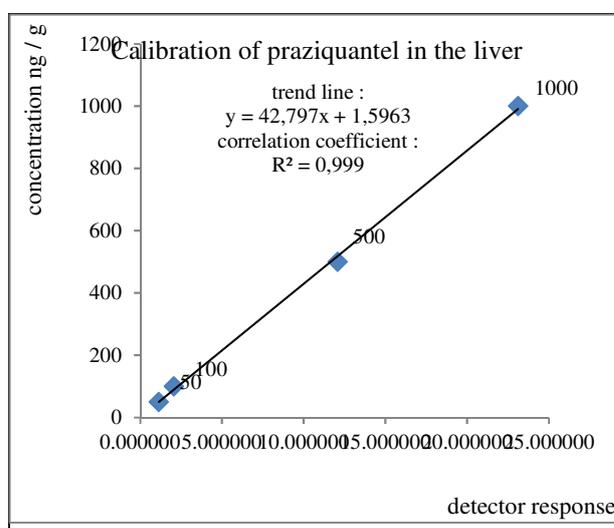


Fig. 4. Calibration of praziquantel in the liver. Function type - linear: $y=42,797x+1,5963$ ($R^2=0,9990$).

The LoQ and LoD values of ivermectin and praziquantel in biosubstrates are shown in Table 1.

It was found that after application of the drug, no residual amounts of praziquantel were found in any of the organs / tissues (concentrations below LoD). Residual amounts of ivermectin were recorded in the liver (9-10 ng / g), in internal fat (10-15 ng / g), as well as in muscle tissue (15-18 ng / g) 30 days after the drug was administered.

Table 1. Limits of Quantification (LoQ) and Limits of Detection for LoD in serum, milk, liver, muscle, lung, heart, spleen, and omental fat samples.

Biosubstrate	Ivermectin		Praziquantel	
	LoD (ng / ml, ng / g)	LoQ (ng / ml, ng / g)	LoD (ng / ml, ng / g)	LoQ (ng / ml, ng / g)
Liver	2,7	9,0	13,5	44,5
Muscles	2,8	9,2	13,5	44,4
Lungs	2,8	9,1	13,4	44,1
Heart	2,7	9,0	13,2	43,6
Spleen	2,9	9,7	14,5	47,7
Internal fat	3,0	10,0	14,5	47,7

When examining organs and tissues of sheep 35 days after the administration of MONISEN® forte, no residual amounts of ivermectin and praziquantel were found in any of the organs / tissues (concentrations below LoD). The metrological characteristics of the methods used for the determination of ivermectin and praziquantel in biosubstrates are presented in Tables 2 and 3.

It should be noted that in accordance with the "Hygienic Standards for the Content of Pesticide Active Substances in Environmental Objects, Food Raw Materials, Food Products" on the territory of the Russian

Federation (and the countries of the Eurasian Economic Union), there are approved maximum permissible levels (MRL) of residual amounts of ivermectin in animal products: for sheep liver - no more than 0.015 mg / kg (15 ng / g); for adipose tissue - no more than 0.02 mg / kg (20 ng / g).

There are no MRLs for praziquantel residues. Thus, based on the results obtained, it can be concluded that it is advisable to slaughter sheep for meat no earlier than 35 days after the last use of the drug.

Table 2. Linearity and parameters of the method for the determination of ivermectin.

Biosubstrate	Detection limit LoD (ng / ml, ng / g)	Limit of quantification LoQ (ng / ml, ng / g)	Concentration response equation	Linearity range (ng / ml, ng / g)
Liver	2,7	9,0	$y=185,64x+3,0559$ ($R^2=0,9995$)	10-200
Muscles	2,8	9,2	$y=193,37x+2,4008$ ($R^2=0,9990$)	10-200
Lungs	2,8	9,1	$y=187,62x+3,2467$ ($R^2=0,9995$)	10-200
Heart	2,7	9,0	$y=201,11x-0,8406$ ($R^2=0,9990$)	10-200
Spleen	2,9	9,7	$y=205,95x+1,9214$ ($R^2=0,9983$)	10-200
Glandular fat	3,0	10,0	$y=213,91x+1,1363$ ($R^2=0,9989$)	10-200

Table 3. Linearity and parameters of the method for determining praziquantel.

Biosubstrate	Detection limit LoD (ng / ml, ng / g)	Limit of quantification LoQ (ng / ml, ng / g)	Concentration response equation	Linearity range (ng / ml, ng / g)
Liver	13,5	44,5	$y=42,797x+1,5963$ $R^2=0,9990$	50-1000
Muscles	13,5	44,4	$y=45,151x-4,6191$ $R^2=0,9996$	50-1000
Lungs	13,4	44,1	$y=41,85x+10,664$ $R^2=0,9991$	50-1000
Heart	13,2	43,6	$y=41,041x+10,731$ $R^2=0,9993$	50-1000
Spleen	14,5	47,7	$y=46,614x+1,1337$ $R^2=0,9999$	50-1000
Glandular fat	14,5	47,7	$y=49,33x-8,6906$ $R^2=0,9992$	50-1000

4 Conclusion

As a result of the research, the terms of slaughter of small ruminants were established after the use of the drug MONISEN® forte. It is advisable to slaughter sheep for meat no earlier than 35 days after the last use of the drug. These restrictions are included in the instructions for use of the medicinal product.

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