

ANALYSIS OF THE SAFETY AND TOLERANCE OF A NOVEL ORAL SUSPENSION WITH IVERMECTIN IN SHEEP

ANALIZA SIGURANȚEI ȘI TOLERANȚEI UNEI NOI SUSPENSII ORALE CU IVERMECTINĂ LA OVINE

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ABSTRACT | REZUMAT

Ivermectin is a macrocyclic lactone with a wide therapeutic range, being active against many species of parasitic nematodes and arthropods in animals. The mechanism of action of Ivermectin consists of paralysis, death and elimination of these pathogens by interfering with neurotransmitters at the level of GABA receptors and glutamate-sensitive channels of chlorine. The selectivity of the pharmacological action of Ivermectin is mammalian specific, being correlated with the lack of target receptors for glutamate and the limitation of GABA encephalic receptors. In dogs, neurointoxications with Ivermectin are possible, due to stimulation of GABA receptors, with extremely high encephalic concentrations compared to liver and plasma concentrations. The aim of this research was to monitor the health of a sheep batch after oral administration of two suspensions of Ivermectin, in order to evaluate the bioequivalence, safety and tolerance of the proposed test formula for interchanges in sheep antiparasitic therapy. The main clinical and paraclinical health indices were also monitored in a sample of native sheep (n=36), during the bioequivalence testing of two formulas with Ivermectin, using a single-center, randomized, single-dose, two-sequence protocol, two periods and a break of 2 weeks. The study required clinical, hematological (with the Abacus junior Vet automatic analyzer and Dia-Quick Panoptic colored smears) and blood biochemistry (with the VetScan automatic analyzer and Large Animal Profile kits). These were grouped into the following four sets of assessments: initial (for selection of healthy animals), pre-dose (before product administration), post-dose (on the day of product administration) and final. Clinical and paraclinical examinations were also based on the detection, characterization, and possible adverse reactions, in order to evalu-

Ivermectina este o lactonă macrociclică cu spectru terapeutic larg, fiind activă față de numeroase specii de nematode și artropode parazite la animale. Mecanismul de acțiune al Ivermectinei constă în paralizia, moartea și eliminarea acestor patogeni prin interferarea neurotransmițătorilor de la nivelul receptorilor GABA și canalelor glutamat-sensibile ale clorului. Selectivitatea acțiunii farmacologice a Ivermectinei este specifică mamiferelor, fiind corelată cu lipsa receptorilor țintă pentru glutamat și limitarea receptorilor encefalici GABA. La câine sunt posibile neurointoxicații cu Ivermectină, datorate stimulării receptorilor GABA, cu realizarea de concentrații encefalice extrem de crescute față de cele hepatice și plasmatic. Scopul acestei lucrări constă în monitorizarea stării de sănătate la un eșantion de oi după administrarea orală a două suspensii cu Ivermectină, în vederea evaluării bioechivalenței, siguranței și toleranței formulei test propuse pentru interschimb în terapia antiparazitara la ovine. Au fost monitorizate principalii indici clinici și paraclinici ai stării de sănătate la un eșantion de oi indigene (n=36), în cursul testării bioechivalenței a două formule cu Ivermectină, utilizând un protocol de tip unicentric, randomizat, cu doză unică, două secvențe, două perioade și o pauză de 2 săptămâni. Studiul a necesitat efectuarea de examene clinice, hematologice (cu analizorul automat Abacus junior Vet și pe frotiuri colorate Dia-Quick Panoptic) și biochimice sanguine (cu analizorul automat VetScan și kituri Large Animal Profil). Acestea au fost grupate în următoarele patru seturi de evaluări: inițiale (pentru selectarea de animale sănătoase), pre-dosis (înaintea administrării produselor), post-dosis (în ziua administrării produselor) și finale. Examenle clinice și paraclinice au stat și la baza depistării, respectiv caracterizării, eventualelor reacții adverse, în vederea evaluării siguranței și toleranței produselor investigate. Testarea bioechivalenței a necesitat recoltarea de probe sanguine seriate (pe EDTA), pre- și post-dosis (6 mL/10 kg), la diferite intervale de timp (0-480 ore), de la fiecare subiect, în cursul celor două faze ale studiului. Probele sanguine proaspăt recoltate au fost centrifugate, timp de 10 minute la 2000 rpm pentru separarea plasmei, iar din

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ate the safety and tolerance of the investigated products. Bioequivalence testing required the collection of serial blood samples (on EDTA), pre- and post-dose (6 mL/10 kg), at different time intervals (0-480 hours), from each subject, during the two phases of the study. The freshly collected blood samples were centrifuged for 10 minutes at 2000 rpm, and from the obtained plasma, Ivermectin concentrations were determined by using liquid chromatography method coupled with mass spectrometry (LC-MS/MS). Plasma concentration analysis was the basis for determining the pharmacokinetic parameters and assessing the bioequivalence of test/reference (T/R) formulas. By using a non-compartmental model, the primary pharmacokinetic parameters (C_{max} , T_{max} and AUC) have been extrapolated to infinity, and the bioequivalence is confirmed based on the evaluation of 90% confidence intervals (CI) of C_{max} and AUC_{last} . We also used the Tukey and Dun biostatistic tests to calculate the mean, median, standard deviation, standard error, 95% CI and probability index "p". In the clinical and hemato-biochemical examinations, no subjects presented adverse side effects, these developments revealing a good tolerance and therapeutic safety of oral suspensions with Ivermectin in sheep. The maximum plasma concentrations of Ivermectin reached very close levels, the mean values for the T/R ratio being $41.22 (\pm 8.7)/42.18 (\pm 10.46)$ ng/mL. In this respect, the evolution of the average values of the main pharmacokinetic parameters, which for the T/R groups, showed statistically insignificant differences in the case of T_{max} (17.5/18.33 hours) and $T_{1/2}$ (84.29/91.04 hours), indicating absorption and the slow elimination of Ivermectin after digestive administration. The analysis of the values obtained in the calculation of CI 90%, for the T/R ratio, of the C_{max} (0.92-1.04), AUC_{last} (0.86-1.06) and $T_{1/2}$ (0.85-1.05), revealed statistically insignificant variations, with the framing of the average values in the reference interval (0.8-1.25), indicating the bioequivalence of the two suspensions with Ivermectin. In conclusion, the investigation of the two suspensions, proven to be bioequivalent and interchangeable in ovine therapy, outlined a good tolerance and therapeutic safety, their pharmacokinetic profiles indicating a slow absorption rate of Ivermectin and a sufficiently long contact time with gastrointestinal parasites.

Keywords: Ivermectin, oral suspension, tolerance, therapeutic safety, sheep

probele de plasme obținute au fost determinate concentrațiile Ivermectinei, utilizând metoda cromatografiei lichide, cuplate cu spectrometria de masă (LC-MS/MS) dezvoltată în acest scop. Analiza concentrațiilor plasmatice (CP) a stat la baza determinării parametrilor farmacocinetici și evaluării bioechivalenței formulelor test/referință (T/R). Prin utilizarea unui model necompartamental au fost extrapolate la infinit parametrii farmacocinetici primari (C_{max} , T_{max} și AUC), urmând ca bioechivalența să fie confirmată pe baza evaluării intervalelor de încredere (IC) 90% ale C_{max} și AUC_{last} . Am recurs totodată, la testele biostatistice Tukey și Dun pentru calculul mediei, medianei, deviației standard, erorii standard, IC 95% și indicelui de probabilitate „p”. La examenele clinice și hemato-biochimice, nici un subiect nu a prezentat reacții secundare adverse, aceste evoluții relevând o bună toleranță și siguranță terapeutică a suspensiilor orale cu Ivermectină la ovine. Concentrațiile plasmatice ale Ivermectinei au atins niveluri foarte apropiate, valorile medii pentru raportul T/R fiind de $41.22 (\pm 8.7) / 42.18 (\pm 10.46)$ ng/mL, indicând absorbția lentă a Ivermectinei după administrarea pe cale digestivă. În această privință, relevantă s-a dovedit și evoluția valorilor medii ale principalilor parametri farmacocinetici, care pentru grupele T/R, au indicat diferențe nesemnificative statistic în cazul T_{max} (17.5/18.33 ore) și $T_{1/2}$ (84.29 / 91.04 ore). Analiza valorilor obținute la calculul CI 90%, pentru raportul T/R, al C_{max} (0.92-1.04), AUC_{last} (0.86-1.06) și $T_{1/2}$ (0.85-1.05), a relevat variații nesemnificative statistic, cu încadrarea mediilor în intervalul de referință (0.80-1.25), indicând bioechivalența celor două suspensii cu Ivermectină. În concluzie, investigarea celor două suspensii, dovedite bioechivalente și interschimbabile în terapia ovină, a conturat o bună toleranță și siguranță terapeutică, profilele lor farmacocinetice indicând o rată lentă de absorbție a Ivermectinei și un timp de contact suficient de lung cu paraziți gastro-intestinali.

Cuvinte cheie: Ivermectină, suspensie orală, toleranță, siguranță terapeutică, ovine

Oral suspensions with Ivermectin have become in the last decades a real alternative to the use of parenteral products with Ivermectin, which dominates the market. Ivermectin {22,23-dihydroivermectin B1a and 22,23-dihydroivermectin B1b} is an active substance from the macrocyclic lactone family, extremely effective in endo- and ectoparasitic therapy, with a

major impact in controlling the main parasites in sheep and other animal species (6, 7, 11). At very low doses, Ivermectin is particularly active against larvae and adult forms of many species of parasitic nematodes and arthropods, in various species of domestic animals (3, 13, 10, 22). The action mechanism of Ivermectin is to interfere with neurotransmission at

the level of GABA receptors and sensitive glutamate channels of chlorine, which causes flaccid paralysis, death and elimination of parasites (10, 21). In mammals, the selectivity of the pharmacological action of Ivermectin is known, due to the lack of target receptors for glutamate and the limitation of the GABA-type encephalic. Thus, dogs can present neurointoxication with Ivermectin, by stimulating GABA receptors, which results in extremely high encephalic concentrations compared to liver and plasma (16, 19).

The testing of medical-veterinary products benefits from a wide range of European and national legislation, which also includes clear provisions regarding the assessment of bioavailability and bioequivalence, based on the detailed analysis of the pharmacological, hemato-biochemical and clinical parameters (1, 8). We have recalled that bioequivalence studies are performed exclusively on animals of the target species, and they also contribute to the evaluation of therapeutic efficacy and drug tolerance (11, 12, 18). We have also noted that studies of drug bioavailability and bioequivalence represent a current and accessible procedure for testing and authorizing generic pharmaceutical products in the field of veterinary medicine. Basically, these studies consist of comparing the pharmacokinetic profile of the test and reference products, the main purpose being the evaluation of the therapeutic equivalence and the establishment of their possibility of therapeutic interchange (5, 12). We also recall that these studies are relevant for evaluating the quality of newly manufactured products, a major impact having the pharmacokinetic variability and the therapeutic index (5, 9). Studies on the bioavailability and bioequivalence of oral products with Ivermectin in sheep are still extremely sporadic, and these are based on determining the rate of absorption, given by the plasma profiles of this active substance after oral administration. Few data concern the therapeutic efficacy of oral Ivermectin antiparasitic products (15, 20). In view of the therapeutic potential and the benefits of using Ivermectin suspensions in sheep and cattle, we consider the new pharmacokinetic studies to be appropriate in order to optimize treatments and minimize possible adverse effects. The present research can be framed in this context, in which we propose the analysis of the plasma concentration-time profile in order to formulate and evaluate the tolerance of a new generic product containing Ivermectin with oral administration in sheep.

MATERIALS AND METHODS

The main goal of this research was to monitor the health of a sheep batch during the bioequivalence testing of two oral suspensions with Ivermectin, in order to evaluate their tolerance and inter-exchange in

sheep therapy. In this regard, the performed investigations have involved the adoption of a specific protocol for the studies of drug bioequivalence, adapted for test and reference formulas, containing oral suspension with Ivermectin. The implemented protocol was in accordance with the national and European legislation in the field (CVMP, 2018), and finally it was approved by the Bioethics Commission of the UASVM Cluj-Napoca.

Animals. The study was performed on a batch of 36 adult sheep, Țurcană breed, clinically healthy, weighing between 41-54 kg and 1-1.3 years, consisting of 26 females (non-pregnant and non-lactating) and 10 males.

Protocol structure. The protocol implemented for the bioequivalence test was unicentric, randomized, cross-linked, with two sequences, two treatments and a 14-day pause between them, including clinical, hemato-biochemical, pharmacokinetic and statistical analyses. In the investigated animals, the state of health was monitored during the test, by evaluating the main clinical and hemato-biochemical parameters, drug tolerance and possible adverse reactions.

Clinical evaluations included monitoring of rectal temperature, general condition and major functions, respectively detailed examination of the systems and devices. These were performed initially, pre-dose, post-dose and at the end of the test, and the results obtained were centralized in the Pharma Quest grid, following the completion of the study to be included in the individual type I reports.

Hematological tests were performed during the initial screening, in order to select the batch of healthy sheep and during the final examinations, focused on re-evaluating the health status and detecting any adverse reactions. The hematological profile was evaluated with the Abacus junior Vet automatic analyzer and by cytomorphological examinations on colored Dia – Quick Panoptic smears, including the determination of the total number of red blood cells (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean red blood cell constants (MCV, MCH, MCHC), white blood cells (WBC) and leukocyte formula (LY, MI, GR).

The metabolic profile analyses were performed with the VetScan automatic analyzer, using the Large Animal Profile kits, which were used to determine the following blood biochemical parameters: ALP (alkaline phosphatase; U/L), AST (aspartate aminotransferase; U/L), GGT (gamma-glutamyl transferase; U/L), BUN (blood urea nitrogen; mmol /L), TP (total protein; g/L), ALB (albumin; g/L), GLOB (globulin; g/L), Ca (calcium; mmol /L), P (phosphorus; mmol /L), Mg (magnesium; mmol/L). At the same time as the blood samples were gathered, urine samples were also collected from the majority of the subjects in the investigated batch (during the voluntary or provoked mo-

tions, by inducing a passenger apnea). In these cases, the usual parameters (with Uryspec plus rapid tests) were determined, of which we considered the evolution of pH and density to be most relevant.

Pharmacokinetic analyses, the two phases of the bioequivalence study, began by administering the single dose (0.6 mL/kg body weight) of the test product (Trimectin Oral Suspension; Pharma VIM Kft., Hungary) or Reference (Fasimec Duo Oral Suspension; Elanco Animal Health, UK), according to the randomization list. In the days of product administration, the sheep received water *ad libitum*, and their feeding was suspended at least 10 hours before and up to 24 hours after administration. Blood samples (6 mL, on K3 EDTA) were collected from the sheep subjected to testing, by catheterization of the brachial cephalic veins, or the jugular vein puncture. Serial collections during each phase, totaled 30 blood samples, 16 in the first 24 hours and the remaining 14 at intervals of 1, 2 or 3 days. Blood samples were collected at the time intervals stipulated in the implemented protocol, the first one being achieved prior the administration of the test product or reference, and the next 30, after the administration of the test product or reference (Table 1). The blood samples were centrifuged, not later than 60 minutes after their collection, at 5000 rpm, for 10 minutes. After separation, the plasmas were divided into 2 tubes, representing the main sample and the counter-test (necessary in case of consumption, destruction or contamination of the main sample). The tubes were labelled and frozen immediately at -20°C, and thus stored until analysis. Plasma concentrations of Ivermectin were measured by liquid chromatography coupled with mass spectrometry (LC-MS/MS). Developed in-house for the present study, this method has been verified and validated in accordance with the applicable international guidelines (23).

Table 1

The time required for collecting serial blood samples for the determination of Ivermectin plasma concentrations

Day	Time required for collecting serial blood samples (hours; minutes)
1	0.0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22
2	24, 28, 36
3	48
4	72
5	96
6	120
7	144
8	168
10	216
12	264
15	336
18	408
21	480

Statistical analyses, individual data obtained from clinical and hemato-biochemical evaluations were statistically processed, using the usual biostatistics tests (Tukey and Dunn), for calculating the mean, standard deviation, minimum and maximum. The data obtained from the entire batch were introduced in the bioequivalence assessment, as all the 36 subjects completed the study and they were included in the statistical analysis of the pharmacokinetic data. The pharmacokinetic parameters of Ivermectin were determined by applying the non-compartmental pharmacokinetic analysis method. All statistical and pharmacokinetic analyses have complied with the requirements and provisions of the current guidelines on bioavailability and bioequivalence of veterinary products (CVMP, 2018). The statistical analyses of the pharmacokinetic parameters were based on the logarithmic data transformation and the application of the ANOVA system (KINETICA 5, ThermoLabsystems software, USA), the reference interval for 90% CI being estimated between 0.8 and 1.25. Statistical analyses also included the determination of primary (AUC_{last} and C_{max}), secondary (T_{max} and AUC_{tot}) and additional ($T_{1/2}$ and MRT-mean residence time) pharmacokinetic parameters, along with summary statistical parameters (arithmetic, geometric mean, SEM, standard deviation, median). The non-transformed data were simultaneously processed by non-parametric tests, Kruskal-Wallis and Friedman, for T_{max} comparison. The MRT and $T_{1/2}$ values were determined by applying the statistical tests used to calculate the primary parameters, with the determination of 90% CI for the T/R ratio, after the logarithmic transformation of the data. The bioequivalence assessment of the two suspensions with Ivermectin was based on the 90% confidence interval values, calculated for the T/R ratio, in the case of the basic pharmacokinetic parameters (C_{max} , AUC_{last} , $T_{1/2}$). The ANOVA system also allowed the evaluation of the possible statistical meanings of the pharmacokinetic interactions, by analysing the variance of the main pharmacokinetic parameters, using the linear model for the subject - treatment variables.

RESULTS AND DISCUSSIONS

The clinical analyses revealed that the majority of the investigated parameters were within the physiological intervals, with no adverse reactions being reported. There were also some slight deviations of some clinical parameters. In the case of these subjects, it was finally clarified that, the recorded deviations were insignificant in the general context of the physiological oscillations and the medical history of the involved subjects (Table 2). Based on the clinical results that were recorded, we appreciate that the two oral suspensions with Ivermectin presented a good tolerance and safety in the doses used in the study, justifying

Table 2

Statistical values of the main haematological, biochemical blood and urinary parameters recorded at the initial screening and final examination of the tested sheep batch

Values	Screening				Final examination			
	Mean	D.St.	Min	Max	Mean	D.St.	Min	Max
Temp ($^{\circ}\text{C}$)	38.5	0.3	38.1	39.0	38.5	0.3	38.1	39.0
Pulse (/min)	85.0	5.4	70.0	90.0	84.0	5.4	75.0	90.0
Resp. (/min)	21.6	2.1	18.0	26.0	20.7	2.9	16.0	25.0
Haematocrit (%)	30.55	3.05	25.64	41.34	28.90	3.89	21.03	41.34
Haemoglobin (g/dL)	10.5	1.1	8.9	15.1	10.05	1.55	6.80	15.10
Red blood cells (T/L)	10.11	0.85	8.13	12.80	9.56	1.23	6.53	12.80
MCV (fl)	30.22	1.94	25.00	33.00	30.25	2.37	25.00	36.00
MCH (pg)	10.4	0.6	9.0	11.8	10.5	0.7	9.0	12.0
MCHC (g/dL)	34.5	1.5	32.1	38.2	34.7	1.7	31.5	38.7
White blood cells	10.95	0.85	8.07	12.19	11.50	1.77	8.07	17.02
Lymphocytes (%)	65	8	45	75	61	9	43	73
Monocytes (%)	2	2	1	8	3	1	1	6
Neutrophils (%)	33	8	20	49	33	8	22	48
Eosinophils (%)	0	1	0	4	3	2	0	6
Basophils (%)	0	0	0	0	0	0	0	0
Thrombocytes	317	94	196	628	377	106	229	676
ALP (UI/L)	169	38	103	274	182	54	98	349
AST (UI/L)	111	19	55	139	110	25	55	151
GGT (UI/L)	47	7	31	66	46	12	31	90
BUN (mmol/L)	6.3	1.6	2.9	8.8	4.5	1.6	1.0	8.0
TP (g/L)	74	4	67	82	74	8	44	85
ALB (g/L)	43	3	32	48	38	6	25	49
GLOB (g/L)	32	5	24	49	34	6	19	49
Ca (mmol/L)	2.49	0.15	2.05	2.73	2.42	0.25	1.50	2.73
P (mmol/L)	2.53	0.41	1.99	3.67	2.33	0.42	1.45	3.23
Mg (mmol/L)	1.00	0.07	0.86	1.13	1.10	0.23	0.53	1.79
Urine pH	6.5	0.4	6.0	7.0	6.5	0.4	6.0	7.0
Urine specific gravity	1024	11	1010	1045	1024	10	1010	1045

fying the assessment of bioequivalence in order to exchange them in veterinary practice.

The analysis of the haematological profile showed insignificant oscillations of the total number of erythrocytes (6.53-12.8 T/L), haematocrit (21.03-41.34%) and haemoglobin concentration (6.53-12.80 g/dL), noting the absence of negative effects of Ivermectin products, or of the collection of serial blood samples, on the volemia, haematopoiesis and erythrocyte functions in the tested subjects (Table 2). The evolution of leukograms revealed several cases of leucocytosis at the end of the study (with a maximum of 17.02 G/L), which was, however, devoid of pathological significance, probably due to the animal's adjustment to the experimental conditions (Table 2). The evolution of the total number of platelets also indicated oscillations, lacking pathological significance, the microlesions generated by the frequency of venous punctures, explaining the mild cases of thrombocytosis that appeared at the end of the testing. The overall

evolution of the individual and average values of the erythrocyte, leukocyte and thrombocyte indices, suggested the lack of negative influences of the tested pharmaceutical formulas, the collection of blood samples or the stress generated on the haematological profile in sheep.

The analysis of the metabolic profile indices revealed important oscillations of the enzymatic levels, consisting of the increase of the average values of ALP, at the beginning (169.19 U/L) and at the end (181.86 U/L) of the testing, reaching the maximum of 274.00 U/L. The AST values were within the physiological intervals, except for one case, that reached the maximum levels in both tests (139, respectively 151 U/L) (Table 2). We mention that elevated AST values can be found in cardiomyopathies, myodystrophies or muscle trauma. We have also observed a slight increase of GGT values (46-47 U/L) and such evolutions could be correlated with the presence of a mild parasitic infestation (Ognean et al., 2019).

Table 3

Values of some pharmacokinetic and statistical parameters
used in the evaluation of tolerance and bioequivalence of oral suspensions with Ivermectin

Parameter	Test product		Product Reference		90% CI	Geo. mean
	Mean	D.St.	Mean	D.St.	Ref. (0.8 -1.25)	Ratio T/R
C_{max} (ng/mL)	41.22	8.6637	42.182	10.462	0.92099 -	0.981343
T_{max} (h)	17.500	4.0107	18.333	5.6061	-	-
AUC_{last}	3804.3	1295.3	3926.9	1310.5	0.8626 - 1.0636	0.957828
AUC_{tot}	4001.0	1338.7	4172.6	1389.2	-	-
T_{1/2} (h)	84.295	27.945	91.045	27.042	0.85619 -	0.949778
MRT (h)	118.36	30.72	124.97	29.278	-	-

* significant difference for $p < 0.05$; MRT – mean residence time

The analysis of the hepatic profile indices suggested physiological values, with slight deviations of the albuminemia (38-43 g/L), representing possible cases of pseudoalbuminemia. The initial and final analysis of the ionogram values revealed the evolution within the physiological limits of calcium (2.49, respectively 2.42 mmol/L), phosphatemia (2.53, respectively 2.33 mmol/L) and magnesium (1.0, respectively 1.1 mmol/L). Regarding the evolution of the physical parameters of the urine, it is worth mentioning that the individual and average values of pH and density are within the physiological intervals (Table 2). The results of the blood biochemical analyses outlined the major impact of the metabolic profile indices in the monitoring of the health status of the animals used in the testing of the safety, tolerance and bioequivalence of drugs (17, 20).

Pharmacokinetic analyses indicated that the plasma concentrations of Ivermectin were maintained at high relative average values within 480 hours post-administration, which was detected even at the end of the testing. These developments of plasma concentrations attest the late elimination of Ivermectin from the body, being able to be correlated with a good plasma availability and implicitly, a good level of bioavailability of this active substance. The detailed analysis of the pharmacokinetic data showed that, between the individual values of the initial and final plasma concentrations of Ivermectin, there were no differences of more than 20% compared to the average, in the case of 84% of the samples. The C_{max} evolution presented high average values of Ivermectin and their framing in very close intervals, namely 41.2 ± 8.7 µg/mL for the Test product and 42.2 ± 10.5 µg/mL for the Reference product. However, we assigned the IC 90% of the T/R ratio for C_{max} in the range 0.92-1.05 in the case of Ivermectin (Table 3). A good safety and a good therapeutic effect were also associated with the grouped evolution of the T_{1/2} values (84.29-91.04 hours), respectively

the values of T_{max} (17.50 - 18.33 hours) of Ivermectin. However, we attributed the evolution of the 90% CI values of the AUC in the case of the T/R ratio, which indicated intervals of 0.86-1.06 for Ivermectin (Table 3).

The similar pharmacokinetic profile of the two formulas described in this research is also reflected by the close evolution of the MRT values, which were within the range 118.36-124.97 hours for Ivermectin (14). Other research in the field sustain comprehensive concerns regarding the pharmacokinetics of Ivermectin as well as combined products containing Ivermectin (2, 4, 15, 20). The overall analysis of pharmacokinetic investigations suggests the correlation of the higher level of bioavailability with the increased therapeutic potential of oral administration of Ivermectin in helminthiasis and other parasites in animals. In order to make a relevant analysis of the results obtained in this study, we have compared them with those reported by Lifschitz et al. (2009), who observed a delay in the Ivermectin elimination, considering that it presents a plasma availability, 3 times higher than other active substances, such as Triclabendazole. The evolution of the pharmacokinetic profiles of the tested formulas revealed the correlation of a slow absorption rate with the long-term persistence of Ivermectin in the gastrointestinal system of sheep. We consider that these pharmacokinetic characteristics ensure a high level of therapeutic efficacy of oral products with Ivermectin, which remain sufficiently long in contact with adult forms of parasitic nematodes, while also retaining sufficiently active plasma concentrations.

The overall evolution of the plasma concentrations values attested that the bioequivalence testing met the standards of the current legislation regarding the validation of the bioanalytical methods, because the percentage difference between the initial and final values was within $\pm 20\%$ compared to the average values, for more than 67 % of the investigated samples. The bioequivalence of the two products was

fully confirmed by the framing of the 90% CI values of the T/R ratio in the reference range for validating the drugs bioequivalence, between 80-125%, for all primary pharmacokinetic parameters. According to the provisions of the current legislation in the field, if during the course of the bioequivalence studies of the veterinary medical products, the concentration in the predose is less than or equal to 5% of the C_{max} value of the tested subject, its values can be included without changes in all measurements and pharmacokinetic calculations (24).

CONCLUSIONS

The overall evolution of the clinical and hemato-biochemical parameters in the investigated animals revealed a good tolerance and therapeutic safety of two oral suspensions with Ivermectin, supporting their use in therapy in sheep and other animal species. The testing of the two suspensions with Ivermectin confirmed the bioequivalence of the new developed product with the one existing on the market, their primary pharmacokinetic parameters recording values of 90% confidence interval within the reference limits (0.8-1.25), for the Test / Reference report. The obtained pharmacokinetic profiles of the tested suspensions were characterized by slow absorption rates, correlated with the persistence of a long duration of Ivermectin in the gastrointestinal system of sheep. We consider that such physio-pharmacological profiles ensure good therapeutic efficacy of oral suspensions with Ivermectin, due to their sufficiently long contact time with adult forms of parasitic nematodes and the high level of plasma concentrations.

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