ISSN: 1220-3173; E-ISSN: 2457-7618

ANALYSIS AND BIOEQUIVALENCE EVALUATION OF TWO ORAL ANTHELMINTIC FORMULAS WITH MILBEMYCIN OXIME AND PRAZIQUANTEL IN HEALTHY DOGS

ANALIZA FARMACOCINETICĂ ȘI EVALUAREA BIOECHIVALENȚEI A DOUĂ FORMULE ANTIHELMINTICE ORALE CU MILBEMICIN OXIMĂ ȘI PRAZIQUANTEL LA CÂINI SĂNĂTOȘI

Diana Denisa TODORAN[1)], Octavia TAMAS-KRUMPE[1)], S. COMAN[3)], Melania CRIȘAN[1),*)], D. NECULA[1),2)], Daria FENEȘAN[1)], Andreea MÎRZA[1)], L. OGNEAN[1)]

ABSTRACT | REZUMAT

Along with the increased accessibility to pharmacokinetic and statistical evaluations, the use of bioequivalence tests for obtaining and authorising generic veterinary medicinal products has also increased. This is also the purpose of the present study, intended for the pharmacokinetic and statistical analysis of the bioavailability and bioequivalence of a new generic product, with Milbemycin oxime and Praziquantel for dogs, to increase the opportunity for interchange in anthelmintic therapeutics. The organisation of the study can be summarised in the testing of two products with milbemycin oxime and praziquantel on an eligible sample of healthy adult dogs (n=22), following a unicentric, randomised, cross-over, two-sequence, two-treatment and 30-day wash-out study design. Determination of the plasma concentrations of the two active molecules was performed by two rapid, selective high-performance liquid chromatography coupled with mass spectrometry (LC-MS/MS) methods. According to the implemented protocol, the statistical analysis of the obtained data corroborated a series of descriptive parameters (mean, standard deviation, range) for the sample of subjects (age, weight), with relevant pharmacokinetic parameters for the tested active substances (C_{max}, AUC_{last}, AUC_{tot}), with additional parameters (% extrapolated AUC, t_{half}, MRT) and drug safety (adverse events, clinical and laboratory screening and follow-up examinations). The testing results revealed the 90% confidence intervals of the primary pharmacokinetic parameters (C_{max},$AUC_{last}$and AUC tot) within the allowed limits (0.8-1.25%) for the Test/Reference ratio, confirming the bioequivalence of the tested products and the opportunity for their therapeutic exchange. The analysis of the pharmacokinetic profiles revealed a slower rate of absorption of milbemycin oxime than praziquantel, proving their sufficiently long persistence in the gastrointestinal tract of the dog, especially milbemycin oxime. The high plasma concentrations and long duration of contact with the adult forms of parasitic worms ensure the effectiveness of the active molecules tested in the prevention and treatment of mixed helminthiasis, including heartworm disease.

Keywords: generic product, pharmacokinetics, bioequivalence

Odată cu creșterea accesibilității la evaluări farmacocinetice și statistice a crescut și utilizarea testelor de bioechivalență ca procedură de obținere și în autorizare a pieței a produselor generice medical veterinare. Acesta este și scopul studiului de față, destinat analizei cinetice și statistice a biodisponibilității și bioechivalenței unui nou produs generic, cu Milbemicin oximă și Praziquantel pentru câine, în vederea creșterii oportunităților de interschimbare în terapeutica antihelmintică. Organizarea studiului se poate sinteza la testarea a două produse (test și referință) cu milbemicin oximă și praziquantel, pe un eșantion eligibil de câini adulți sănătoși (n=22). Designul studiului este unul de tip unicentric, randomizat, încrucișat, în două secvențe, cu două tratamente și 30 de zile de repaus. Determinarea concentrațiilor plasmatice ale celor două molecule active a fost efectuată prin două metode rapide, selective de cromatografie lichidă de înaltă performanță cuplată cu spectrometrie de masă (LC-MS/MS). Conform protocolului implementat, analiza statistică a datelor obținute a coroborat o serie de parametri descriptivi (medie, deviație standard, interval) pentru eșantionul de subiecți (vârstă, greutate), cu parametri farmacocinetici relevanți pentru substanțele active testate (C_{max}, AUC_{las}t, AUC_{tot}), respectiv cu parametri adiționali (% extrapolated AUC, t_{half}, MRT) și siguranța medicamentoasă (reacțiile adverse, screeningul clinic și testele de laborator). Rezultatele testării au relevat încadrarea intervalelor de încredere 90% ale parametrilor cinetici primari (Cmax, AUClast și AUCtot) în limitele admise (0,8-1,25%) pentru raportul Test/Referință, confirmând bioechivalența produselor testate și oportunitatea interschimbării lor terapeutice. Evoluția de ansamblu a parametrilor statistici și farmacocinetici principali, secundari și adiționali a confirmat caracteristicile farmacocinetice apropiate ale celor două formule farmaceutice antihelmintice. Analiza profilelor farmacocinetice a relevat o rată mai lentă de absorbție a milbemicin oximei decât a praziquantelului, dovedind persistența suficient de lungă a acestora în tubul digestiv al câinelui, mai ales a milbemicin oximei. Concentrațiile plasmatice ridicate și duratele lungi de contact cu formele adulte ale viermilor paraziți, asigură o bună eficacitate a moleculelor active testate în prevenția și tratamentul helmintozelor mixte, inclusiv în dirofilarioză.

Cuvinte cheie: produs generic, farmacocinetică, bioechivalență

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
2) Country Centre of Mountain Economy "CE-MONT", INCE, Romanian Academy, Vatra Dornei, Romania
3) CSV Hoghiz, Brasov, Romania
*) *Corresponding author: melaniacrisan@gmail.com*

Drug bioequivalence testing allows the introduction of a generic product on the market, similar to the innovative one. Thus, a new competitive product is formulated, which does not have to go through the entire testing protocol that the innovative one went through. Bioequivalence studies allow a pharmacokinetic and pharmacodynamic comparison of active molecules and some resulting metabolites (11). The difference in bioavailability between the two products (test and reference) must fall within the average reference ranges, between 80 and 125% (4), as it did in the present study. Veterinary medical therapeutics is dominated by generic products, which are more accessible and cheaper than the innovative ones, whose increased cost is given by the new active substances and the extent of the preclinical and clinical pharmaceutical studies necessary to obtain and approve them (15, 18). European and national legislation in the field of testing medical-veterinary products includes express provisions regarding the pharmacological and clinical standards used in the evaluation of medicinal bioavailability and bioequivalence (6,8, 10). Currently, bioequivalence testing is performed exclusively on animals from the targeted species, which ensures a good assessment of equivalence and therapeutic efficacy, respectively tolerance and adverse reactions (2, 5, 10). Bioequivalence studies are an accessible and rapid procedure for testing and approval of pharmaceutical products. These consist in comparing the profile of the Test-Reference products in order to evaluate their therapeutic equivalence and the possibility of their interchangeability in therapeutic practice (3, 5). Currently, bioequivalence testing also provides results for the evaluation of the quality of the manufacturing process, along with those related to pharmacokinetic variability, therapeutic efficiency and other characteristics of the investigated products. The active substances of the formulas assessed in this study are represented by Milbemycin oxime (MO) and Praziquantel (PZ). Milbemycin oxime is part of the group of macrocyclic lactones (LM), which includes two well-known subfamilies: Avermectins and Milbemycins (9). MO is a 16-atom anthelmintic active substance, particularly efficient in the therapy of helminthiasis caused by numerous species of nematodes (1). According to the literature, its effectiveness has been proven against the adults of some species of lung and heart helminths, as well as against the larval forms of some heart and arthropod helminths, and against some species of intestinal nematodes (7). Praziquantel is an acylated pyrazine-isoquinoline derivative that is highly active against parasitic worms like cestodes and trematodes. The characteristic of the active molecule of PZ is the vermicidal action against parasites from the class of trematodes and cestodes (16). The association between MO and PZ molecules has recently been introduced to the market, especially in canine and feline therapeutics for the treatment and prevention of *Dirofilaria immitis* infestations. Thus, the molecular synergism of these antiparasitics gave significant clinical results - following the multimodal expansion of the therapeutic effect, analysed separately, they have a limited therapeutic spectrum (14). The purpose of this study can be summarised in the pharmacokinetic and statistical analysis of the bioavailability and bioequivalence of two anthelmintic products, milbemycin oxime, and praziquantel, to estimate their interchangeability in therapy by implementing a physio-pharmacological protocol on dogs as a target species.

MATERIALS AND METHODS

The animals

The study was carried out with the approval of the USAMV Cluj-Napoca Bioethics Commission and by the current norms of well-being and good practice (Directive 86/609/CEE; EMEA/CVMP/133/00-FINAL), respectively, with the requirements of the Guidelines for the conduct of bioequivalence studies for veterinary medicinal products (EMA/CVMP/016/00-Rev.2) (10). Testing was performed on a batch of clinically healthy adult Siberian Husky dogs (n = 22) (20 males and 2 females) with a mean age of 3.4 years (2.0-5.0 years) and an average weight of 22.2 kg (±1.8). Before the study was initiated, the subjects' eligibility was evaluated according to the inclusion criteria and the physiological ranges for clinical, haematological, and blood biochemical parameters. The selected subjects were maintained in generous pens and fed with good-quality granulated food, with free access to drinking water. From the total sample of 22 eligible dogs, 20 were included into the bioequivalence test, and 2 remained as reserves and were maintained under similar conditions.

Study design

The realisation of the study required the implementation of a single-centre, randomised, crossover design with two sequences and two treatments, separated by a 30-day break. This protocol has been documented and verified in advance for adaptation to the specificity of the HS race, as well as to the particularities of the investigation of dual-active anthelmintic products (12, 13). The study design respected the inclusion-exclusion criteria and the randomisation list in the composition of the sample and the distribution of subjects for the administration of the test and reference products.

The products introduced in the bioequivalence test

There were represented by two oral anthelmintic formulations for dogs, milbemycin oxime and praziquantel. These represented the test product (Milbenin 12.5 mg/125 mg chewable tablets for dogs A.U.V., of the company Vim Spectrum SRL, specialised in the development of generic drugs) and the reference product (Milbemax rágótabletta kutyáknak A.U.V., tablets, brand Elanco France S.A.S).

The blood sample collection procedures

A specific sampling method for each investigation

set was used. The first included the usual collection of samples on EDTA and lithium heparin for haematological, respectively blood biochemical tests, used in the assessment of the health status at the selection of eligible subjects (before the administration of the products), at the initiation of testing (pre-dose, initial screening) and its completion (final screening). In the second set of investigations, we followed the conduct specific to drug bioavailability and bioequivalence studies, consisting of taking serial blood samples, on ED TA. For this purpose, we performed preliminary tests and decided to adopt the external phlebocentesis procedure (of the cephalic-brachial, saphenous, or even jugular veins) with holder-type devices.

The collection of serial blood

Samples followed the adapted protocol, according to the randomisation list for each subject, returning as many as 31 blood samples during each phase, before and after the administration of the test and reference products (pre-dose and post-dose). Thus, for the entire duration of the testing (72 days),a total of 62 samples were taken to determine plasma concentrations at the following time intervals: pre-dose (0.0) and post-dose (0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 28, 36, 48, 72, 96, 120, 144, 168, 216, 264, 336, 408 and 480 hours).

The clinical and paraclinical analyses for the evaluation of health state

Blood test were carried out in the case of the first set of investigations and consisted of the determination of the main clinical and paraclinical indices (haemato-biochemical) of the selected animals selected which were included in the testing batch (initial screening), respectively their evolution at the end of testing (final screen).

Determination of plasma concentrations

Blood samples from the second set of investigations were processed for plasma separation by centrifugation for 10 minutes at 2000 RPM, and then they were frozen at -18°C and transported to an accredited laboratory for pharmacokinetic analyses. These consisted of determining the plasma concentrations of Milbemycin oxime and Praziquantel, through two methods of liquid chromatography, coupled with mass spectrometry (LC-MS/MS), which were previously verified and validated in the Clinical and Analytical Research Centre laboratories.

Pharmacokinetic and statistical analysis

In the evaluation of bioequivalence, the data obtained from the subjects from the initial sample (n= 22), which remained unchanged at the end of the testing, were entered, and they were included in the statistical analysis of the pharmacokinetic data.The pharmacokinetic parameters of milbemycin oxime and praziquantel were determined by applying the non-compartmental pharmacokinetic analysis method.We note that all pharmacokinetic and statistical analyses complied with the requirements set forth in the current guidelines on bioavailability and bioequivalence of veterinary medicinal products (Blood Level Bioequivalence Study, EMA/CMVP/VICH/751935/2013). The statistical analysis of the pharmacokinetic parameters was performed by the method of logarithmic data transformation and by accessing the ANOVA system (KINETICA 5 software, ThermoLabsystems). According to the guidelines regarding medicinal bioavailability and bioequivalence, the reference interval for the 90% CI was estimated between 0.8 and 1.25. Statistical analyses included the determination of the main primary (AUC_{0-t} and C_{max}), secondary (t_{max} and AUC_{0-inf}), and additional (% extrapolated AUC; $t_{1/2}$ and MRT) parameters, as well as summary descriptive statistics parameters (arithmetic mean, harmonic mean, geometric mean, SEM, standard deviation, median, range). Untransformed data were also processed simultaneously by non-parametric Kruskal-Wallis and Friedman tests, which allow comparison of Tmax. Finally, the level of bioequivalence for Cmax and AUC last was assessed by calculating the 90% confidence interval for the mean Test/Reference (T/R) ratio for these two primary pharmacokinetic parameters. By applying statistical tests, Latin-square ANOVA with determination of 90% CI of the ratio of means T/R after data log-transformation and descriptive statistics, MRT and $t_{1/2}$ values were also calculated. Through the ANOVA system, the possible statistical significance of the pharmacokinetic interactions was also evaluated, analysing the variance of the main pharmacokinetic parameters through the general linear procedures model, in which the subject and the treatment represented the variables.

RESULTS AND DISCUSSIONS

Pharmacokinetic dominance analysis

The quantification of data on the evolution of plasma concentrations (CP) required a prior calibration of the samples to validate the method and verify the eligibility of the system for the type of samples analysed. Indicators regarding selectivity, calibration curve, blank control samples, matrix and recovery effect, analyte stability in solution and plasma, dilution integrity, and long-term testing were also established. The samples were tested without knowing their origin as a test or reference, which ensured the veracity of the study. The calibration range varied depending on the active molecule, being 0.7-140 ng/ml for MO A_3, 4.27-853 ng/ml for MO A_4, and 2-2000 ug/ml for PZ. The evolution of the average values of the plasma concentrations of MO and PZ during the two phases of the bioequivalence test (0-480 h) is shown in Fig. 1, and for comparison, a detailed evolution of the average values of the plasma concentrations in the interval 0-100 h post-administration (Fig. 2). According to the pharmacokinetic peculiarities and the data presented in the summary of the characteristics of the reference product, plasma concentrations for PZ were detected only in the interval 0 - 96 hours.

Analysis of statistical parameters in correlation with the relevance of individual and average data

The evolutions of the average values of the plasma concentrations achieved by MO and PZ in correlation with the distribution of time curves after the administration of the test and reference products are relevantly illustrated in Figures 1 and 2. From the t_{max} analysis for CP of MO A3, it appears that the maximum value was reached at 2 hours from the administration of the dosage in the case of the tested product and 3 hours after the administration of the reference product, also revealing CP levels even after 480 hours in the case of both products. During the t_{max} analysis of CP performed by PZ, the maximum value was reached in the case of the test formula one hour after the administration of the dose, and in the case of the reference product at 1.5 hours. CP maintenance was detected in the case of the test product up to 36 hours and in the case of the reference one up to 26 hours. Based on the analysis of each subject under his control, it can be considered that the C_{max} values for the test and reference products were similar in terms of the variation of the plasma values of the active molecules, and these products could be considered switchable and interchangeable. The comparative analysis of the values obtained when determining t_{max}, through the non-parametric Kruskal-Wallis and Friedman tests, revealed both in the case of MO and PZ, the lack of statistically significant differences between the average values of this parameter for the Test and Reference groups. The

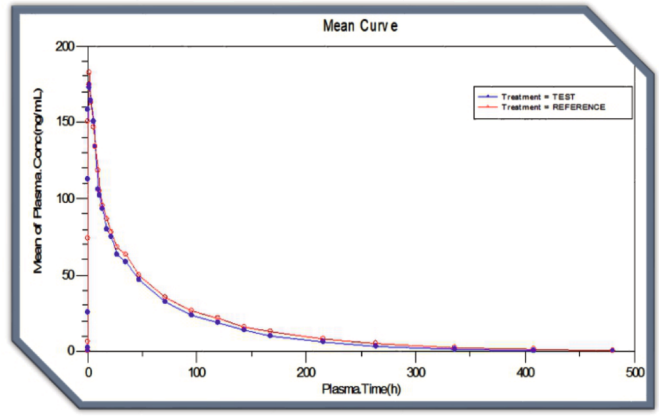

Fig. 1. Distribution values (mean and standard deviation) of plasma concentrations of Milbemycin oxime (MO) molecules recorded in the test product (Milbenin 12.5 mg/125 mg chewable tablets for dogs A.U.V.) and the reference one

mean values of t_{max} for the test and reference groups were 3.3182 and 3.3636 h for MO and 2.0909 and 2.0455 h for PZ, respectively. Evaluation of additional statistical parameters ($t_{1/2}$and MRT) did not contribute to the assessment of bioequivalence but provided additional data for the pharmacokinetic characterisation of MO and PZ. Table 2 shows the descriptive statistical values obtained from the analysis of the additional parameters. It is also mentioned that the values recorded in the statistical analysis of the Latin square ANOVA test, based on the log-transformed data of $t_{1/2}$

Table 1

Distribution of statistical values of primary, secondary and additional pharmacokinetic parameters established for Milbemycin oxime and Praziquantel in the sample of tested dogs

Parameter	Test product			Product Reference		
	Mean	Geo Mean	St.Dev.	Mean	Geo Mean	St.Dev.
Milbemycin oxime						
C_{max} (µg/mL)	217.34	202.64	96.696	212.68	204.75	60.462
T_{max} (h)	3.3182	2.6431	2.3832	3.3636	2.8828	2.4358
AUC_{fin} (µg/mL/h)	7316	7022.8	2394.4	8169.6	7801.9	2585.6
AUC_{tot} (µg/mL/h)	7812.7	7525.5	2434.3	8737	8345.4	2758.7
$T_{1/2}$ (h)	62.777	58.876	23.526	66.662	60.334	31.548
MRT (h)	79.437	73.972	31.543	86.142	78.233	40.425
Praziquantel						
C_{max} (µg/mL)	274.64	258.75	92.967	304.09	273.94	136.78
T_{max} (h)	2.0909	1.6477	1.7904	2.0455	1.6341	2.0581
AUC_{fin} (µg/mL/h)	1037.1	911.1	635.31	1136	938.94	806.95
AUC_{tot} (µg/mL/h)	1056	930.21	637.13	1149.9	952.65	810.27
$T_{1/2}$ (h)	3.1657	2.817	1.8738	2.8109	2.6182	1.0963
MRT (h)	4.7478	4.2767	2.4741	4.5563	4.215	2.0775

and MRT, did not reveal significant differences for the treatment variable. The $t_{1/2}$ values after the administration of the test and reference products were 62.777 h and 66.662 h, respectively, for MO and 3.1657 h and 2.8109 h, respectively, for PZ, and the MRT values were 79.437 h and 86.142 h for MO and, respectively, 4.7478 h and 4.5563 h for PZ.

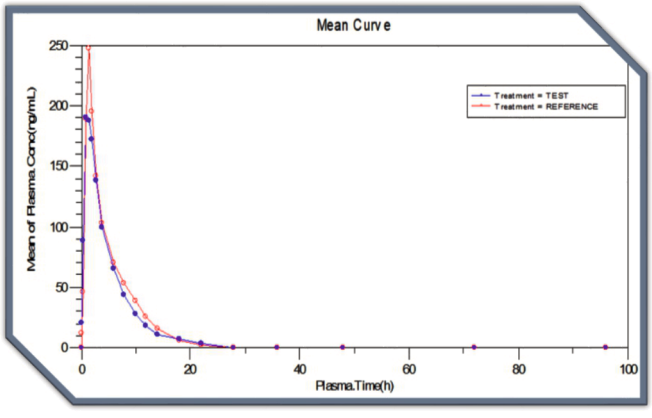

Fig. 2. Distribution values (mean and standard deviation) of the plasma concentrations of praziquantel molecules (PZ) recorded in the test product (Milbenin 12.5 mg/125 mg chewable tablets for dogs A.U.V.) and the reference one

The relevance of the data obtained when calculating the pharmacokinetic parameters of MO (A3 + A_4) and PZ for the test and reference products was ensured by: bioequivalence assessment based on the analysis of the primary pharmacokinetic variables (C_{max}, AUC_{last}); pharmacokinetic and statistical data evaluations by quantifications with Kinetica software ver. 5.0; evaluation of the influences of the period (of the subject involved in the sequence), as well as the variables C_{max}, AUC_{last}, and AUC_{tot}, by analysis of variance (ANOVA, treatment design with Latin square 2); establishing of the effects of the period using AUC_{last} and AUC_{tot} for MO (p= 0.0009422 respectively p= 0.00112), but not in the case of PZ, for which they were identified only in the first 96 hours; recording conclusive results in the analysis of C_{max}, AUC_{last} and AUC_{tot} through the Latin square ANOVA system, for MO (p=0.0318, 1.674e-005 and 9.397e-006) and for PZ (p=0.01212,p=0.0009492,p=0.0009804); the relevance of the tested drug formulations was also confirmed by the conclusiveness of AUC_{last} and AUC_{tot} values for MO (p=0.01944 and 0.01594), but not for PZ.

According to the data presented in Table 2, the final results of the statistical analyses revealed the 90% confidence intervals for the three primary pharmacokinetic parameters (C_{max}, AUC_{last}, and AUC_{tot}) within the allowed limits (0.8-1.25%) in the case of the Test report/Reference, confirming the bioequivalence of the tested products and, at the same time, the opportunity for their therapeutic interchange (Guide EMA/CVMP /016/00-Rev.2, 2011).

The level of bioequivalence achieved in our study can be relevantly explained by analysing the results obtained in the context of those reported by other researchers in the field (2). Thus, the bioequivalence testing on the 20 complete data sets and the evolutionary analysis of CP revealed a high degree of similarity between the primary pharmacokinetic parameters of the two active molecules, determined in the case of the test and reference products.This level corresponds to the current legislation in the field, according to which, if during the drug bioequivalence test, the concentration in the overdose is less than or equal to 5% of the C_{max} value of the tested subject, the obtained values can be included in all measurements and pharmacokinetic calculations without being modified (17). Similar data also reveals the analysis of the results obtained by us with Kruskal-Wallis and Friedman tests, which did not indicate statistically significant differences between the C_{max} and AUC values recorded for the test and reference products. On the other hand, if different distributions and important variations of the

Table 2

Values of 90% confidence intervals of primary, secondary, and additional pharmacokinetic parameters for the Test/Reference ratio of MO and PZ in the sample of tested dogs

Substance	Reference 90% CI	Geo mean ratio T/R	Reference 90% CI
	C_{max}		
Milbemycin oxime	0.87479 - 1.1197	0.989693	0.8-1.25
Praziquantel	0.80184 - 1.1126	0.944543	0.8-1.25
	AUC_{last}		
Milbemycin oxime	0.8381 - 0.96676	0.900135	0.8-1.25
Praziquantel	0.80655 - 1.1674	0.970344	0.8-1.25
	AUC_{tot}		
Milbemycin oxime	0.8381 - 0.96676	0.900135	0.8-1.25
Praziquantel	0.80655 - 1.1674	0.970344	0.8-1.25

statistical data appear in the bioequivalence studies, it is necessary to use new statistical methods (especially non-parametric), as provided by the EMA international guidelines (17). Conversely, if the plasma levels of the test formula were higher than the reference product, they would be uncertain, and the number of subjects in the tested sample should be increased. In the case of testing with highly variable values, we can accept medicinal bioequivalence if the number of subjects is maximum and by the ethics commission's requirements, respectively, if the coefficient of variation is over 30% and if the geometric mean ratio is between 0.8 and 1.25. Finally, based on the comparative analysis of the obtained data with the provisions of the legislation in the field, we can conclude that all the conditions for establishing the bioequivalence of the two products under study have been met, without the need to supplement the number of animals.

CONCLUSIONS

The results of testing thef two anthelmintic products, milbemycin oxime and praziquantel, revealed that the 90% confidence intervals of the primary pharmacokinetic parameters (C_{max}, AUC_{last}, and AUC_{tot}) were within the allowed limits (0.8-1.25%) for the Test report/Reference, confirming the bioequivalence of the tested products and the appropriateness of their therapeutic interchange. The overall evolution of the main, secondary, and additional statistical and pharmacokinetic parameters confirmed the close pharmacokinetic characteristics of the two anthelmintic pharmaceutical formulations. The analysis of the pharmacokinetic profiles revealed a slower rate of absorption of milbemycin oxime than praziquantel, proving their sufficiently long persistence in the digestive tract of the dog, especially milbemycin oxime. The increased level of plasma concentrations and duration of contact with the adult forms of parasitic helminths confers a good therapeutic efficiency to the tested active molecules, including *Dirofilaria immitis*.

Acknowledgements

We would like to especially thank the Vim Spectrum Mureș Commercial Company, the main sponsor of the project (VIM-MILBE-12.20) which financed the present study.

REFERENCES

1. *Adhikari S., Rustum A.M.,* (2022), Structural elucidation of major degradation products of milbemycin oxime drug substance using LC-MS and NMR. Journal of Pharmaceutical and Biomedical Analysis, 217:114862
2. *Arion A., Fernández-Varón E., Cárceles C.M., Gagyi L., Ognean L., (2018),* Pharmacokinetics of praziquantel and pyrantel pamoate combination following oral administration in cats. Journal of feline medicine and surgery, 20(10):900-904
3. *Chow S., Liu J.,* (2009). Design and Analysis of Bioavailability and Bioequivalence Studies, 3rd edition, (Ed.) Chapman & Hall/CRC Press, Boca Raton, FL, USA, 21-24, 60-64
4. *Chow S.C.,* (2014), Bioavailability and bioequivalence in drug development. Wiley Interdisciplinary Reviews: Computational Statistics, 6(4):304-312
5. *Claxton R., Cook J., Endrenyi L., Lucas A., Martinez M.N., Sutton S.C.,* (2012), Estimating product bioequivalence for highly variable veterinary drugs. Journal of veterinary pharmacology and therapeutics, 35:11-16
6. *Cristina R.M., V. Chiurciu,* (2010), Elements of pharmacovigilance and toxicology in veterinary medicine (in Romanian), (Ed.) Brumar, Bucharest, Romania, 45-60
7. *Cvejic D., Schneider C., Fourie J., de Vos C., Bonneau S., Bernachon N., Hellmann K.,* (2016), Efficacy of a single dose of milbemycin oxime /praziquantel combination tablets, Milpro®, against adult *Echinococcus multilocularis* in dogs and both adult and immature *E. multilocularis* in young cats. Parasitology research, 115:1195-1202
8. *European Union,* (2010), Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes Text with EEA relevance. OJ L, 276:33-79
9. *Muñoz-Muñoz L., Shoen C., Sweet G., Vitoria A., Bull T.J., Cynamon M., Thompson C.J., Ramón-García S.,* (2021), Repurposing avermectins and milbemycins against mycobacteroides abscessus and other nontuberculous mycobacteria. Antibiotics, 10(4):381
10. *Navarro C., Séguy L., Vila M., Birckel P.,* (2016), Bioequivalence study between two formulations of ciclosporin A (Cyclavance® oral solution and Atopica® soft capsules) following a single oral administration to dogs. BMC Veterinary Research, 12 (1):1-6
11. *Ognean L., Cernea C., Trinca S., Cernea M., Moldovan M.,* (2010), Testing the in vitro equivalence of two acetylcysteine based products. Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii, Arad, 20(1):75-80
12. *Todoran D.D., Lațiu C., Feneșan D.M., Tamas Krumpe O., Todoran C., Iozon I., Necula D., Ognean L.,* (2022), Influence of serial blood sampling on total blood volume and complete blood count in Siberian Husky dogs during bioequivalence testing of an anthelmintic, 62(2):53-58
13. *Todoran D.D., Crișan M., Tamas-Krumpe O., Feneșan D., Necula D., Ognean L.,* (2024), Analysis of an eligible protocol for bioequivalence testing of two Canine anthelmintics products with two active molecules. Bulletin of the University of Agricultural Sciences & Veterinary Medicine Cluj-Napoca. Veterinary Medicine, 81(1):42-50
14. *Toranmal S.S., Buchade R.S., Tandale S.D., Wagh V.H., Chaure P.P.,* (2019), Development and validation of stability indicating hplc method for simultaneous estimation of milbemycin oxime and

praziquantel from bulk and marketed formulation. Journal of Pharmaceutical Sciences and Research, 11(9):3108-3115
15. *Vlase L., Popa A., Neag M., Muntean D., Leucuţa S.E.,* (2011), Pharmacokinetic interaction between zolpidem and ciprofloxacin in healthy volunteers. European journal of drug metabolism and pharmacokinetics, 35:83-87
16. *Walther F.M., Fisara P., Allan M.J., Roepke R.K., Nuernberger M.C.,* (2014), Safety of concurrent treatment of dogs with fluralaner (Bravecto™) and milbemycin oxime-praziquantel. Parasites & vectors, 7:1-3
17. *The European Agency for the Evaluation of Medicinal Products* (2019). VICH GL52 Bioequivalence: Blood Level Bioequivalence Study, EMA/CMVP/VICH/751935/2013-Corr, Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/international-cooperation-harmonisation-technical-requirements-registration-veterinary-medicinal_en-3.pdf (Accessed: 14 September, 2024)
18. *Yu A., Sun D., Li B.V., Yu L.X.,* (2014), Bioequivalence history, In: FDA Bioequivalence standards,(Ed.) Springer, New York, NY, USA, 1-27.