Fexinidazole: Evaluation of Pharmacokinetics Following Single IV and Oral Administration to Beagle Dog

Product Name:	Fexinidazole
Study Number:	0320-2007
Study Director	
Status:	Final

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1. STUDY CONDUCT

The study, sponsored by Drugs for Neglected Diseases *initiative* (DND*i*), was performed within Accelera, Nerviano Medical Sciences, Italy according to the internal Standard Operating Procedures as a non-GLP regulated study.

2. OBJECTIVE

The objective of this study was to evaluate the pharmacokinetics of Fexinidazole and its sulphone and sulphoxide metabolites and the absolute bioavailability of Fexinidazole after single IV 1 mg/kg and oral 10 mg/kg doses of the compound to male Beagle dogs.

3. ABBREVIATIONS

The following abbreviations are used in this document:

$AUC_{0\text{-}t(last)}$	Area under the plasma concentration *vs.* time curve up to finite time
$AUC_{0\text{-}\infty}$	Area under the plasma concentration *vs.* time curve up to infinite time
$C_{0.083}$	Concentration at 5 minutes after IV dosing
C_{max}	Maximal plasma concentration
$\%EXT_{t(last)\text{-}\infty}$	Fraction of $AUC_{0\text{-}\infty}$ accounted for by extrapolation
CV	Coefficient of variation of the mean
F	Absolute bioavailability
h	Hours
ID	Animal identification code
IV	Intravenous
LC	Liquid chromatography
LLOQ	Lower limit of quantification
MS	Mass-spectrometry
Norm	Normalized value
R^2	Correlation coefficient
SD	Standard deviation of the mean
SOP	Standard operating procedure
STD	Standard sample
$t_{1/2,z}$	Terminal half-life
t_{max}	Time to peak plasma concentration
t_{last}	Time of the last detectable concentration
ULOQ	Upper limit of quantification

4. METHODS

4.1. Study Design

The study was conducted according to the study protocol [1].
Fexinidazole was given as IV and oral administrations to male Beagle dogs according to the following scheme

Dog ID	Route	Dose (mg/kg)	Formulation
1979, 2197, 2205	IV	1	70 % PEG 400 in 5 % dextrose solution
	Oral	10	5 % Tween 80 and 0.5% Methocel suspension

IV dose was administered *via* the radial vein, the oral dose was administered by gastric gavage. A washout period of 12 days was allowed between the IV (Day 1) and oral (Day 12) doses.

4.2. Sample Collection and Handling

Blood was withdrawn from jugular vein and collected in heparinized plastic tubes kept on an ice/water bath, then centrifuged at 1200g for 10 minutes at 4°C. Three aliquots of the separated plasma (about 0.2 mL) was stored at -80°C until analysis for Fexinidazole and its sulphone and sulphoxide metabolites. Blood was taken at pre-dose and 0.083 (5 minutes), 0.25, 0.5, 1, 2, 4, 6 and 10 h after IV dosing; at pre-dose and 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h after oral dosing.

4.3. Bioanalytical Method

Plasma concentrations of Fexinidazole and its sulphone and sulphoxide metabolites were determined by LC-MS-MS method following plasma protein precipitation. The lower limit of quantification of the bioanalytical method was 5 ng/mL for all analytes. Bioanalytical data were stored in Watson LIMS (v. 6.4.0.04, Thermo Fisher Scientific, Waltham, MA, USA). Details of the bioanalytical method used are reported in Appendix 2 and analytical performances of calibration standards are reported in Appendix 3.

4.4. Pharmacokinetic Calculations

Pharmacokinetic evaluations of Fexinidazole and sulphone and sulphoxide metabolites were carried out using non-compartmental approach with the aid of the Watson package (v. 6.4.0.04, Thermo Fisher Scientific, Waltham, MA, USA).
For the calculations, the pre-dose concentrations of Fexinidazole were set equal to $C_{0.083}$ after IV administration, whilst the pre-dose concentrations of Fexinidazole after oral administration and the pre-dose concentrations of the metabolites after both doses were set equal to zero.

After IV and oral dosing, C_{max} ($C_{0.083}$ for Fexinidazole after IV dosing) and t_{max} of each compound were read from raw data as the coordinates of the highest measured concentration. After both doses, for each compound, the area under plasma concentration *vs.* time curve to finite time, $AUC_{0\text{-}t(last)}$, was determined by the linear trapezoidal rule up to the last detectable concentration. The half-life of the terminal phase, $t_{1/2,z}$, was determined by linear regression analysis of the natural-log concentration *vs.* time curve, where $t_{1/2,z}$ = ln(2)/slope of the regression line. The area under the concentration vs. time curve up to infinite time, $AUC_{0\text{-}\infty}$, was determined as

$$AUC0 - \infty = AUC0 - t(last) + \frac{Ct(last) \cdot t_{1/2,z}}{\ln(2)}$$

The fraction of $AUC_{0\text{-}\infty}$ accounted for by the extrapolated area under the curve was calculated as follows:

$$\%EXTt(last) - \infty = 100 \cdot \frac{AUCt(last) - \infty}{AUC0 - \infty}$$

After IV dosing, plasma clearance and volume of distribution at steady state of Fexinidazole were calculated as follows:
$CL = Dose/AUC_{0\text{-}\infty}$
$V_{ss} = CL \cdot MRT$, where MRT is the mean residence time.

Absolute bioavailability of Fexinidazole was calculated from the ratio of individual oral to IV dose-normalized $AUC_{0\text{-}t(last)}$ and $AUC_{0\text{-}\infty}$ values.

Metabolite to parent ratio was calculated based on C_{max} and $AUC_{0\text{-}t(last)}$ values.

After both doses, C_{max} and AUC values of each compound were also normalized to a 1 mg/kg dose level.

Descriptive statistics (mean ± SD, %CV) were reported for plasma concentrations and pharmacokinetic parameters sorted by compound and route of dosing.

Plasma concentrations and pharmacokinetic parameters of each compound were reported to three significant figures.

5. RESULTS

5.1.1. Clinical and Physical Examinations

5.1.1.1. Clinical Signs

Individual clinical signs are reported in Appendix 4.

No significant treatment related clinical signs were observed after intravenous and oral treatments with Fexinidazole. No meaningful clinical signs were recorded during wash out period.

5.1.1.2. Body Weight

Body weights are included in Appendix 5.

No treatment-related changes were observed during the study. Normal fluctuations of the body weight were recorded.

5.1.2. Pharmacokinetic analysis

Mean ±SD systemic exposure parameters of Fexinidazole and its metabolites after IV and oral administrations of Fexinidazole are reported in Table 1. Individual and mean pharmacokinetic parameters of Fexinidazole and metabolites after IV and oral administrations are reported in Tables 2 - 5. Individual and mean plasma concentrations of the compounds are plotted in Figures 1 - 6 and reported in Tables 1A1 - 4A1 of Appendix 1.

5.1.2.1. IV dosing

After dosing, mean ±SD $C_{0.083}$ value of Fexinidazole was 320 ±136 ng/mL. The plasma concentrations of the compound declined poly-exponentially with mean terminal half-life of about half an hour (Figure 5). Mean ±SD systemic clearance, 273 ±121 mL/min/kg, was higher than dog hepatic blood flow (41 mL/min/kg, [2]), indicating high rate of elimination of the compound from the systemic circulation. Mean ±SD volume of distribution at steady state, 3260 ±131 mL/kg, was five-six times higher than dog total body water (about 600 mL/kg [3]), indicating extensive tissue distribution.

Sulphone detectable pre-dose concentration was measured in the dog ID 2197. This value, 9.25 ng/mL, close to the LLOQ of the analytical method, was likely due to a contamination and it was excluded from the calculations.
After dosing, detectable concentrations of the sulphone metabolite were measured at the first sampling time (5 minutes post dosing). The concentrations of the compound achieved the maximal concentration, on average 681 ng/mL, 4 - 6 h post dosing, and showed a plateau profile up to the last sampling time (10 h post dosing, Figure 5). This behaviour prevented an unbiased estimate of the pharmacokinetics of the compound. Mean $AUC_{0\text{-}t(last)}$ was 5270 ng·h/mL. The metabolite to parent $AUC_{0\text{-}t(last)}$ ratio, on average 85, indicated that Fexinidazole was extensively metabolised to its sulphone metabolite.

The plasma profile of the sulphoxide metabolite was different from that of the sulphone metabolite (Figure 5). Sulphoxide maximal concentration, on average 763 ng/mL, was rapidly achieved, within 15 minutes post dosing; then the concentrations of the metabolite declined mono-exponentially with mean half-life of about 1 h. Mean $AUC_{0\text{-}t(last)}$ value was 1480 ng·h/mL. The metabolite to parent $AUC_{0\text{-}t(last)}$ ratio was, on average, 25, indicating that Fexinidazole was extensively metabolised to its sulphoxide metabolite.

5.1.2.2. Oral dosing

After dosing, mean ±SD maximal plasma concentration of Fexinidazole, 24.2 ±5.9 ng/mL, was achieved within 1 h post dosing. The concentrations of the compound were detectable up to 4 h post dosing. Mean ±SD $AUC_{0\text{-}t(last)}$ was 57.2 ±13.4 ng·h/mL. In the dog ID 2205, the plasma profile did not allow an unbiased estimate of the half-life. Mean (n=2) apparent terminal half-life of the compound was 2 h, longer than that after IV dosing, suggesting that the absorption processes affected the disposition of the compound. Mean ±SD (n=2) $AUC_{0\text{-}\infty}$ was 85.3 ±1.27 ng·h/mL. Absolute bioavailability, based on $AUC_{0\text{-}t(last)}$ and $AUC_{0\text{-}\infty}$ values, was 10 and 14 %, respectively.

After dosing, the maximal concentration of the sulphone metabolite, on average 3260 ng/mL, was achieved 6 - 8 h post dosing. After the achievement of the maximal concentration, the concentrations of the metabolite slowly declined up to the end of the experiment (24 h post dosing). $AUC_{0\text{-}t(last)}$ value was, on average, 44700 ng·h/mL. The metabolite to parent $AUC_{0\text{-}t(last)}$ ratio was very high, on average 810.

The plasma profile of the sulphoxide metabolite was different from that of the sulphone metabolite (Figure 6). Sulphoxide maximal concentration, on average 1903 ng/mL, was achieved 1 h post dosing, then the concentrations of the metabolite declined poly-exponentially with mean terminal half-life of 6 h, longer than that after IV dosing. Mean $AUC_{0\text{-}t(last)}$ value was 8560 ng·h/mL. Metabolite to parent $AUC_{0\text{-}t(last)}$ ratio was, on average, 158.

6. CONCLUSIONS

After both doses, the coefficient of variation of the mean parameters of Fexinidazole and its metabolites was lower than 50 %.
Fexinidazole was endowed with high volume of distribution and plasma clearance. After oral dosing, the half-life of Fexinidazole was longer than that after IV dosing, suggesting that the absorption processes affected the disposition of Fexinidazole. The oral bioavailability of Fexinidazole was 10 %. After both doses, Fexinidazole was extensively metabolized to the sulphone and sulphoxide derivatives. For both metabolites, the metabolite to parent ratio was higher after oral than after IV dosing, suggesting an extensive first pass effect metabolism.

7. CONTRIBUTORS

8. ARCHIVING

The protocol, raw data, pharmacokinetic analysis and final report were archived within Accelera Archive, Nerviano Medical Sciences, Italy, according the Unit Standard Operating Procedures.

9. REFERENCES

1. Fexinidazole: Evaluation of Pharmacokinetics Following Single IV and Oral Administration to Beagle dogs. Nerviano Medical Sciences Study Protocol no. 0320-2007-P, October 22, 2007.

2. Boxenbaum, H. Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: Extrapolation of data to benzodiazepines and phenytoin. J. Pharmacokin. Biopharm. 8: 165-176, 1980.

3. Spector, W. S. Handbook of Biological Data. Philadelphia, W.B. Saunders Co., 1956.

TABLES AND FIGURES

Table 1. Summary table of mean ±SD systemic exposure values of Fexinidazole and its metabolites after single IV 1 mg/kg and oral 10 mg/kg doses of Fexinidazole in male Beagle dogs.

Dose (mg/kg)	Compound	C_{max} (ng/mL)	t_{last} (h)	$AUC_{0\text{-}t(last)}$ (ng·h/mL)	$AUC_{0\text{-}\infty}$ (ng·h/mL)	F% $AUC_{0\text{-}t(last)}$
1	Fexinidazole	320 ±136	1	66.9 ±31.8	70.6 ±33.8	
	Sulfone	681 ±162	10	5270 ±1230	NA	
	Sulfoxide	763 ±45.3	6	1480 ±197	1520 ±208	
10	Fexinidazole	24.2 ±5.86	4	57.2 ±13.4	85.3 ±1.27	9.60 ±4.32
	Sulfone	3260 ±762	24	44700 ±3810	NA	
	Sulfoxide	1903 ±186	24	8560 ±767	8780 ±895	

NA: not assessable

Table 2. Individual and mean (±SD, %CV) pharmacokinetic parameters of Fexinidazole after single IV 1 mg/kg dose of the compound in male Beagle dogs.

Parameter (Units)	Dog ID			Mean	SD	%CV
	ID 1979	ID 2197	ID 2205			
$C_{0.083}$ (ng/mL)	468	200	293	320	136	43
t_{last} (h)	2	0.5	1	1.17	0.764	65
$AUC_{0\text{-}t(last)}$ (ng·h/mL)	102	40.1	58.6	66.9	31.8	48
Regression Range (h)	0.25 - 2	0.083 - 0.5	0.25 - 1	N/A	N/A	N/A
$t_{1/2,z}$ (h)	0.704	0.111	0.403	0.406	0.297	73
$AUC_{0\text{-}\infty}$ (ng·h/mL)	108	42.2	61.7	70.6	33.8	48
$\%EXT_{t(last)\text{-}\infty}$	5.15	4.87	5.08	5.03	0.146	3
CL (mL/min/kg)	154	395	270	273	121	44
V_{ss} (mL/kg)	3380	3120	3280	3260	131	4
$C_{0.083}$, norm [(1)]	468	200	293	320	136	43
$AUC_{0\text{-}t(last)}$, norm [(1)]	102	40.1	58.6	66.9	31.8	48
$AUC_{0\text{-}\infty}$, norm [(1)]	108	42.2	61.7	70.6	33.8	48

N/A: not applicable

[(1)] $C_{0.083}$ (ng/mL) and AUC (ng·h/mL) values normalized to 1 mg/kg dose.

Table 3. Individual and mean (±SD, %CV) pharmacokinetic parameters of sulphone and sulphoxide metabolites of Fexinidazole after single IV 1 mg/kg dose of Fexinidazole in male Beagle dogs.

Sulphone						
Parameter (Units)	Dog ID			Mean	SD	%CV
	ID 1979	ID 2197	ID 2205			
C_{max} (ng/mL)	763	494	785	681	162	24
t_{max} (h)	6	4	4	4.67	1.15	25
t_{last} (h)	10	10	10	10	0	0
$AUC_{0\text{-}t(last)}$ (ng·h/mL)	6040	3850	5920	5270	1230	23
C_{max}, norm [1]	763	494	785	681	162	24
$AUC_{0\text{-}t(last)}$, norm [1]	6040	3850	5920	5270	1230	23
[2]	1.63	2.47	2.68	2.26	0.555	25
[3]	59.2	96.0	101	85.4	22.8	27
Sulphoxide						
Parameter (Units)	Dog ID			Mean	SD	%CV
	ID 1979	ID 2197	ID 2205			
C_{max} (ng/mL)	721	811	757	763	45.3	6
t_{max} (h)	0.25	0.083	0.25	0.194	0.0964	50
t_{last} (h)	6	6	6	6	0	0
$AUC_{0\text{-}t(last)}$ (ng·h/mL)	1710	1380	1360	1480	197	13
Regression Range (h)	0.25 - 6	0.083 - 6	0.25 - 6	N/A	N/A	N/A
$t_{1/2,z}$ (h)	1.28	1.12	1.08	1.16	0.106	9
$AUC_{0\text{-}\infty}$ (ng·h/mL)	1760	1410	1390	1520	208	14
C_{max}, norm [1]	721	811	757	763	45.3	6
$AUC_{0\text{-}t(last)}$, norm [1]	1710	1380	1360	1480	197	13
$AUC_{0\text{-}\infty}$, norm [1]	1760	1410	1390	1520	208	14
[2]	1.54	4.06	2.58	2.73	1.26	46
[3]	16.8	34.4	23.2	24.8	8.93	36

N/A: not applicable

[1] C_{max} (ng/mL) and AUC (ng·h/mL) values normalized to 1 mg/kg dose.

[2] C_{max}, metabolite / C_{max}, parent

[3] $AUC_{0\text{-}t(last)}$, metabolite / $AUC_{0\text{-}t(last)}$, parent

Table 4. Individual and mean (±SD, %CV) pharmacokinetic parameters of Fexinidazole after single oral 10 mg/kg dose of the compound in male Beagle dogs.

Parameter (Units)	Dog ID			Mean	SD	%CV
	ID 1979	ID 2197	ID 2205			
C_{max} (ng/mL)	28.8	26.2	17.6	24.2	5.86	24
t_{max} (h)	0.5	0.5	1	0.667	0.289	43
t_{last} (h)	4	4	4	4	0	0
$AUC_{0\text{-}t(last)}$ (ng·h/mL)	69.9	58.5	43.2	57.2	13.4	23
Regression Range (h)	0.5 - 4	0.5 - 4	(1)	N/A	N/A	N/A
$t_{1/2,z}$ (h)	1.62	2.24		1.93	0.438	23
$AUC_{0\text{-}\infty}$ (ng·h/mL)	84.4	86.2		85.3	1.27	2
$\%EXT_{t(last)\text{-}\infty}$	17.2	32.2		24.7	10.6	43
C_{max}, norm (2)	2.88	2.62	1.76	2.42	0.586	24
$AUC_{0\text{-}t(last)}$, norm (2)	6.99	5.85	4.32	5.72	1.34	23
$AUC_{0\text{-}\infty}$, norm (2)	8.44	8.62		8.53	0.127	2
F% $AUC_{0\text{-}t(last)}$	6.85	14.6	7.37	9.60	4.32	45
F% $AUC_{0\text{-}\infty}$	7.81	20.4		14.1	8.92	63

N/A: not applicable

(1) The plasma profile did not allow an unbiased estimate of the half-life

(2) C_{max} (ng/mL) and AUC (ng·h/mL) values normalized to 1 mg/kg dose.

Table 5. Individual and mean (±SD, %CV) pharmacokinetic parameters of sulphone and sulphoxide metabolites of Fexinidazole after single oral 10 mg/kg dose of Fexinidazole in male Beagle dogs.

Sulphone						
Parameter (Units)	Dog ID			Mean	SD	%CV
	ID 1979	ID 2197	ID 2205			
C_{max} (ng/mL)	2820	4140	2820	3260	762	23
t_{max} (h)	6	6	8	6.67	1.15	17
t_{last} (h)	24	24	24	24	0	0
$AUC_{0\text{-}t(last)}$ (ng·h/mL)	42600	49100	42400	44700	3810	9
C_{max}, norm [1]	282	414	282	326	76.2	23
$AUC_{0\text{-}t(last)}$, norm [1]	4260	4910	4240	4470	381	9
[2]	97.9	158	160	139	35.4	25
[3]	609	839	981	810	188	23
Sulphoxide						
Parameter (Units)	Dog ID			Mean	SD	%CV
	ID 1979	ID 2197	ID 2205			
C_{max} (ng/mL)	2030	1990	1690	1903	186	10
t_{max} (h)	1	0.5	2	1.17	0.764	66
t_{last} (h)	24	24	24	24	0	0
$AUC_{0\text{-}t(last)}$ (ng·h/mL)	7760	8620	9290	8560	767	9
Regression Range (h)	6 - 24	6 - 24	6 - 24	N/A	N/A	N/A
$t_{1/2,z}$ (h)	4.83	6.3	6.03	5.72	0.782	14
$AUC_{0\text{-}\infty}$ (ng·h/mL)	7830	8890	9610	8780	895	10
C_{max}, norm [1]	203	199	169	190	18.6	10
$AUC_{0\text{-}t(last)}$, norm [1]	776	862	929	856	76.7	9
$AUC_{0\text{-}\infty}$, norm [1]	783	889	961	878	89.5	10
[2]	70.5	76.0	96.0	80.8	13.4	17
[3]	111	147	215	158	52.8	33

N/A: not applicable

[1] C_{max} (ng/mL) and AUC (ng·h/mL) values normalized to 1 mg/kg dose.

[2] C_{max}, metabolite / C_{max}, parent

[3] $AUC_{0\text{-}t(last)}$, metabolite / $AUC_{0\text{-}t(last)}$, parent

Figure 1. Individual plasma concentrations (ng/mL) of Fexinidazole after single IV 1 mg/kg dose of the compound in male Beagle dogs.

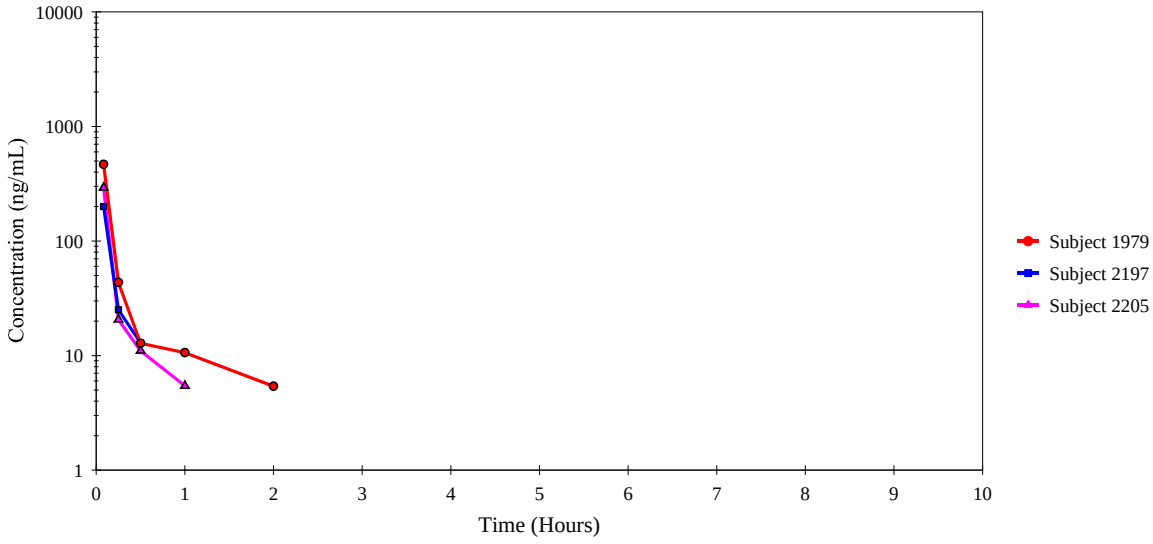


Figure 2. Individual plasma concentrations (ng/mL) of sulphone (upper panel) and sulphoxide (lower panel) metabolites of Fexinidazole after single IV 1 mg/kg dose of Fexinidazole in male Beagle dogs.

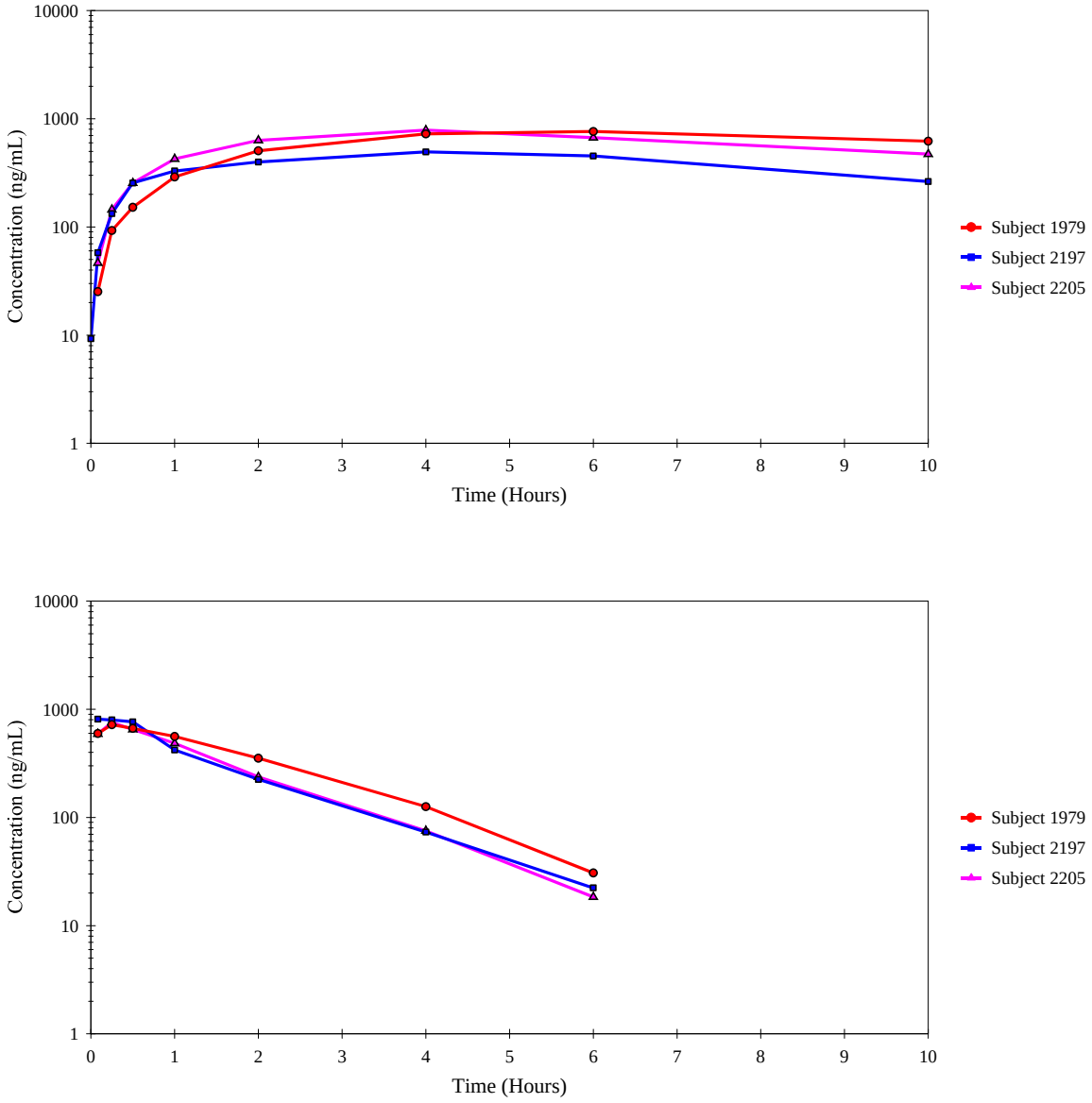


Figure 3. Individual plasma concentrations (ng/mL) of Fexinidazole after single oral 10 mg/kg dose of the compound in male Beagle dogs.

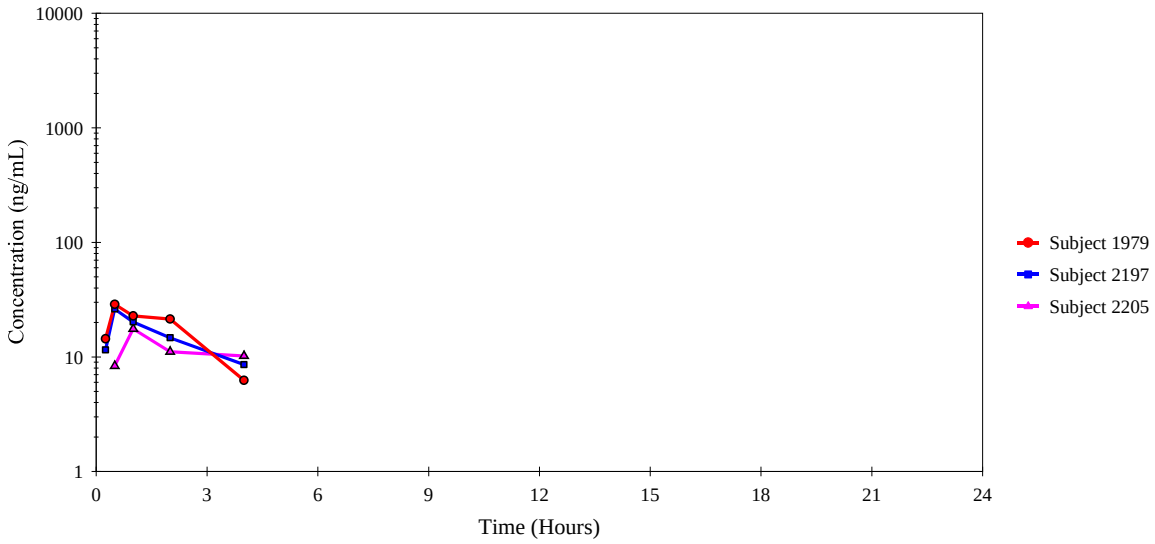


Figure 4. Individual plasma concentrations (ng/mL) of sulphone (upper panel) and sulphoxide (lower panel) metabolites of Fexinidazole after single oral 10 mg/kg dose of Fexinidazole in male Beagle dogs.

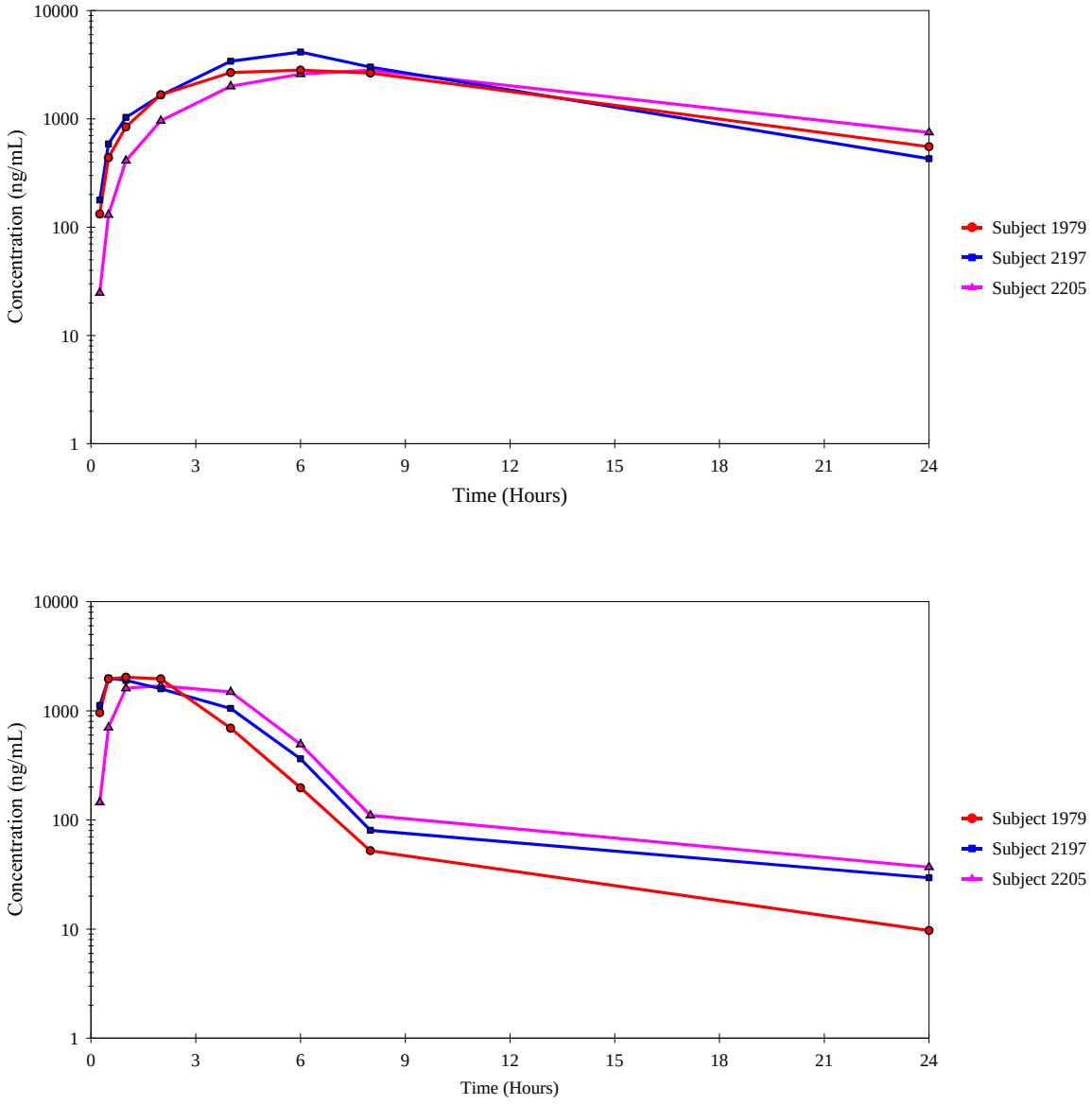


Figure 5. Mean (+SD) plasma concentrations (ng/mL) of Fexinidazole and its sulphone and sulphoxide metabolites after single IV 1 mg/kg dose of Fexinidazole in male Beagle dogs.

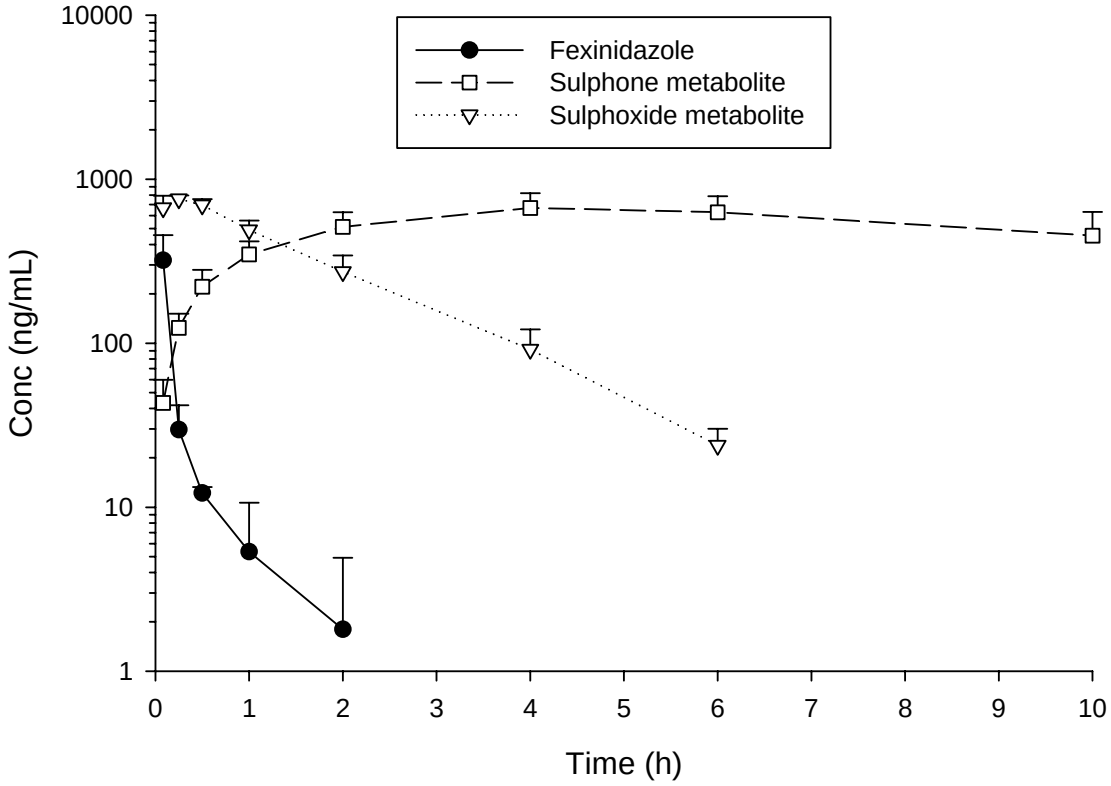
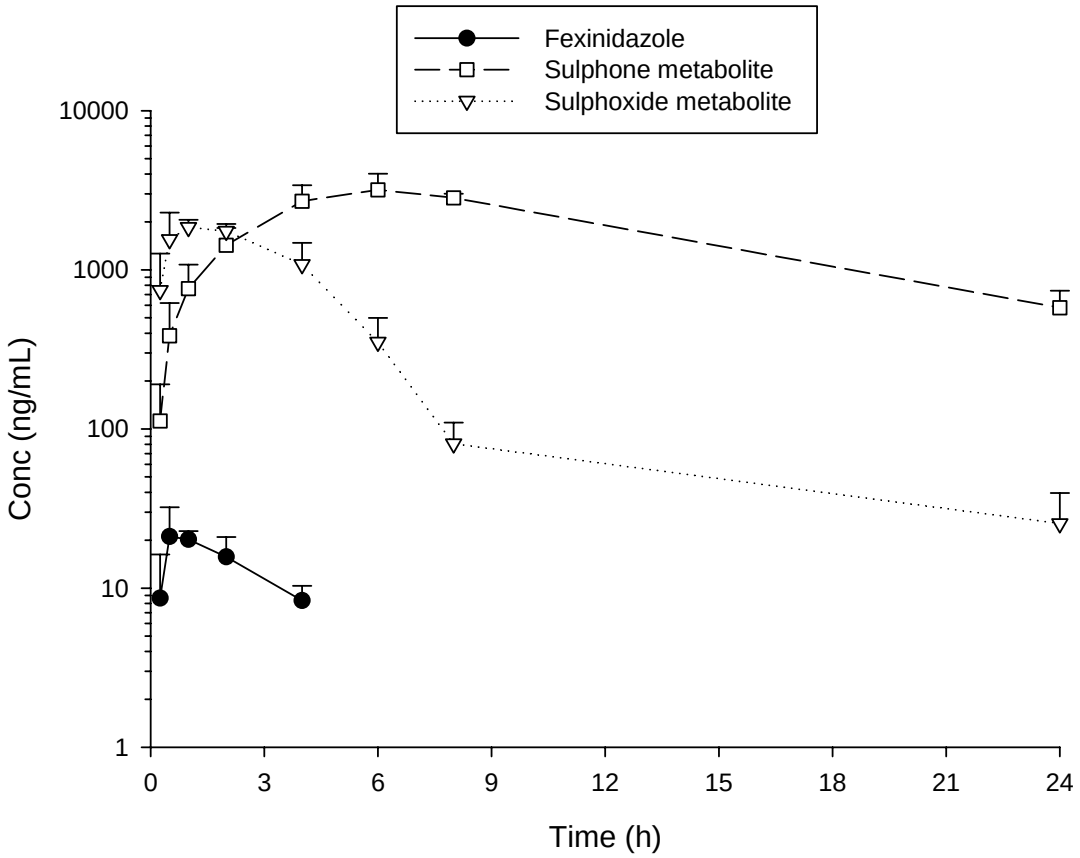


Figure 6. Mean (+SD) plasma concentrations (ng/mL) of Fexinidazole and its sulphone and sulphoxide metabolites after single oral 10 mg/kg dose of Fexinidazole in male Beagle dogs.



APPENDICES

Appendix 1. Individual plasma concentrations

Table 1A1. Individual and mean (±SD, %CV) plasma concentrations (ng/mL) of Fexinidazole after single IV 1 mg/kg dose of the compound in male Beagle dogs.

Time (h)	Dog ID			Mean	SD	%CV
	1979	2197	2205			
Pre-dose	<5.00	<5.00	<5.00	N/A	N/A	N/A
0.083	468	200	293	320	136	43
0.25	43.5	25.1	20.6	29.7	12.1	41
0.5	12.8	12.9	11	12.2	1.07	9
1	10.6	<5.00	5.45	5.35	5.3	99
2	5.4	<5.00	<5.00	1.8*	3.12	173
4	<5.00	<5.00	<5.00	N/A	N/A	N/A
6	<5.00	<5.00	<5.00	N/A	N/A	N/A
10	<5.00	<5.00	<5.00	N/A	N/A	N/A

Estimates of mean based on approximation that values below LLOQ are equal to zero. For values marked *, more than half of the individual levels were below LLOQ; descriptive statistics was reported even if strongly biased.

N/A: not applicable.

Table 2A1. Individual and mean (±SD, %CV) plasma concentrations (ng/mL) of sulphone and sulphoxide metabolites of Fexinidazole after single IV 1 mg/kg dose of Fexinidazole in male Beagle dogs.

Sulphone						
Time (h)	Dog ID			Mean	SD	%CV
	1979	2197	2205			
Pre-dose	<5.00	9.25[(1)]	<5.00	N/A	N/A	N/A
0.083	25.2	57.8	46.5	43.2	16.6	38
0.25	92.7	133	145	124	27.4	22
0.5	152	255	255	221	59.5	27
1	290	329	426	348	70	20
2	506	399	632	512	117	23
4	725	494	785	668	154	23
6	763	453	670	629	159	25
10	621	263	472	452	180	40
Sulphoxide						
Time (h)	Dog ID			Mean	SD	%CV
	1979	2197	2205			
Pre-dose	<5.00	<5.00	<5.00	N/A	N/A	N/A
0.083	597	811	593	667	125	19
0.25	721	797	757	758	38	5
0.5	666	766	651	694	62.5	9
1	561	421	485	489	70.1	14
2	353	225	237	272	70.7	26
4	126	73.3	75.3	91.5	29.9	33
6	30.7	22.3	18.4	23.8	6.29	26
10	<5.00	<5.00	<5.00	N/A	N/A	N/A

[(1)] Value was likely due to contamination

N/A: not applicable.

Table 3A1. Individual and mean (±SD, %CV) plasma concentrations (ng/mL) of Fexinidazole after single oral 10 mg/kg dose of the compound in male Beagle dogs.

Time (h)	Dog ID			Mean	SD	%CV
	1979	2197	2205			
Pre-dose	<5.00	<5.00	<5.00	N/A	N/A	N/A
0.25	14.4	11.5	<5.00	8.63	7.62	88
0.5	28.8	26.2	8.34	21.1	11.1	53
1	22.8	20.2	17.6	20.2	2.6	13
2	21.4	14.7	11.1	15.7	5.23	33
4	6.25	8.57	10.2	8.34	1.99	24
6	<5.00	<5.00	<5.00	N/A	N/A	N/A
8	<5.00	<5.00	<5.00	N/A	N/A	N/A
24	<5.00	<5.00	<5.00	N/A	N/A	N/A

Estimates of mean based on approximation that values below LLOQ are equal to zero.

N/A: not applicable.

Table 4A1. Individual and mean (±SD, %CV) plasma concentrations (ng/mL) of sulphone and sulphoxide metabolites of Fexinidazole after single oral 10 mg/kg dose of Fexinidazole in male Beagle dogs.

Sulphone						
Time (h)	Dog ID			Mean	SD	%CV
	1979	2197	2205			
Pre-dose	<5.00	<5.00	<5.00	N/A	N/A	N/A
0.25	132	178	24.7	112	78.7	70
0.5	438	587	130	385	233	61
1	843	1030	411	761	317	42
2	1670	1650	961	1430	404	28
4	2680	3410	2000	2700	705	26
6	2820	4140	2590	3180	836	26
8	2650	3010	2820	2830	180	6
24	553	429	750	577	162	28
Sulphoxide						
Time (h)	Dog ID			Mean	SD	%CV
	1979	2197	2205			
Pre-dose	<5.00	<5.00	<5.00	N/A	N/A	N/A
0.25	961	1120	145	742	523	71
0.5	1960	1990	703	1550	735	47
1	2030	1900	1610	1850	215	12
2	1960	1590	1690	1750	191	11
4	693	1050	1490	1080	399	37
6	197	363	493	351	148	42
8	52.2	80.2	110	80.8	28.9	36
24	9.68	29.5	36.9	25.4	14.1	56
N/A: not applicable.						

Appendix 2. Bioanalytical method

Fexinidazole, Fexinidazole Sulphoxide (M1) and Fexinidazole Sulphone (M2) (as free base) were assayed using an LC-MS-MS method. Bioanalytical data are stored in Watson LIMS under Watson study 0320-2007 in the 348-Fexinidazole project.

Plasma Sample Preparation
Standards were prepared using dog plasma. Plasma proteins were precipitated by adding 200 μL of methanol to 25 μL of plasma in a 96 well plate. After capping and vortex mixing, the plate was centrifuged for 10 minutes at 4000 rpm at 6°C. An aliquot of 100 μL of supernatant was transferred in a 96 well plate and mixed with 100 μL of 10 mM ammonium formate pH 3.5 injected onto the LC-MS-MS system.

LC-MS/MS conditions

HPLC system: Hewlett Packard 1100 series
Mobile phase: Channel A: Ammonium Formate (10 mM pH 3.5)
Channel B: Methanol

Elution mode: Gradient
Elution conditions:

Time (min)	0.0	2.10	2.30	5.00	5.20	6.00
% A	65	65	40	40	65	65
% B	35	35	60	60	35	35

Total Run Time: 6.0 minutes
Flow rate: 1.0 mL/min
Approximate retention time: Fexinidazole: about 4.7 min.
M1: about 2.00 min
M2: about 2.15 min
Column oven temp.: 40 °C
Analytical column: Chromolith RP-18 50 * 4.6 mm (Merck)
Autosampler type: Perkin Elmer PE 200
Injection volume: 10 μL
Autosampler temperature: RT

MS instrument: Perkin Elmer SCIEX API 4000
Ionisation: TURBO ION SPRAY in positive ion mode
MRM transitions:

Fexinidazole:	m/z	280.2	m/z	140.2
M1	m/z	296.2	m/z	140.2
M2	m/z	312.2	m/z	140.2

Resolution Q1: Unit
Q3: Unit
LLOQ: 5.00 ng/mL

ULOQ: 5000 ng/mL

Batch No. of standard: N/A

Software used

Acquisition and processing: Analyst 1.4

Import data from Analyst file: 0320-2007-Run1.rdb

Data file in Analyst: 0320-2007\Run1.wiff

Appendix 3. Analytical performance

Table 1A3. Analytical Performance: Back-Calculated Concentrations (ng/mL) of Fexinidazole Calibration Standard in Dog Plasma for Study Protocol 0320-2007.

Assay Date	Analytical Run Number	STD.1 5.00 ng/mL	STD.2 10.0 ng/mL	STD.3 50.0 ng/mL	STD.4 100 ng/mL	STD.5 500 ng/mL	STD.6 1000 ng/mL	STD.7 4000 ng/mL	STD.8 5000 ng/mL
07-Nov-2007	1	4.59	9.01	46	96.6	534	1000	4140	5280
		5.45	11.1	*41.4	88.4	481	953	4340	5250
Mean		5.02	10.1	46	92.5	508	977	4240	5270
SD		0.608	1.48		5.8	37.5	33.2	141	21.2
%CV		12.1	14.7		6.3	7.4	3.4	3.3	0.4
%Bias		0.4	1	-8	-7.5	1.6	-2.3	6	5.4
n		2	2	1	2	2	2	2	2

* Reason Deactivated : Accuracy more than 15%.

Table 2A3. Calibration Curve Parameters for Fexinidazole Calibration Standards in Dog Plasma for Study Protocol 0320-2007.

Run Date	Curve Number	Slope	Intercept	R^2	LLOQ ng/mL	ULOQ ng/mL	Regression Footnote(s)
07-Nov-2007	1	2056.37	-1724.58	0.9926	5	5000	1
Mean		2056.37	-1724.58	0.9926			
n		1	1	1			

Regression Footnote(s):
1) Resp. = Slope * Conc. + Intercept

Table 3A3. Analytical Performance: Back-Calculated Concentrations (ng/mL) of Fexinidazole Sulphoxide Calibration Standard in Dog Plasma for Study Protocol 0320-2007.

Assay Date	Analytical Run Number	STD.1 5.00 ng/mL	STD.2 10.0 ng/mL	STD.3 50.0 ng/mL	STD.4 100 ng/mL	STD.5 500 ng/mL	STD.6 1000 ng/mL	STD.7 4000 ng/mL	STD.8 5000 ng/mL
07-Nov-2007	1	4.49	8.81	43.4	95.3	556	1040	4030	5080
		5.88	*13.6	*40.3	*82.4	491	967	4120	5350
Mean		5.19	8.81	43.4	95.3	524	1000	4080	5220
SD		0.983				46	51.6	63.6	191
%CV		18.9				8.8	5.2	1.6	3.7
%Bias		3.8	-11.9	-13.2	-4.7	4.8	0	2	4.4
n		2	1	1	1	2	2	2	2

* Reason Deactivated : Accuracy more than 15%.

Table 4A3. Calibration Curve Parameters for Fexinidazole Sulphoxide Calibration Standards in Dog Plasma for Study Protocol 0320-2007.

Run Date	Curve Number	Slope	Intercept	R^2	LLOQ ng/mL	ULOQ ng/mL	Regression Footnote(s)
07-Nov-2007	1	1616.65	-2015.67	0.9903	5	5000	1
Mean		1616.65	-2015.67	0.9903			
n		1	1	1			

Regression Footnote(s):
1) Resp. = Slope * Conc. + Intercept

Table 5A3. Analytical Performance: Back-Calculated Concentrations (ng/mL) of Fexinidazole Sulphone Calibration Standard in Dog Plasma for Study Protocol 0320-2007.

Assay Date	Analytical Run Number	STD.1 5.00 ng/mL	STD.2 10.0 ng/mL	STD.3 50.0 ng/mL	STD.4 100 ng/mL	STD.5 500 ng/mL	STD.6 1000 ng/mL	STD.7 4000 ng/mL	STD.8 5000 ng/mL
07-Nov-2007	1	4.59	8.86	44.2	99.5	549	1020	4200	5320
		5.79	*13.3	*40.8	85.1	480	951	4270	5510
Mean		5.19	8.86	44.2	92.3	515	986	4240	5420
SD		0.849			10.2	48.8	48.8	49.5	134
%CV		16.4			11.1	9.5	4.9	1.2	2.5
%Bias		3.8	-11.4	-11.6	-7.7	3	-1.4	6	8.4
n		2	1	1	2	2	2	2	2

* Reason Deactivated : Accuracy more than 15%.

Table 6A3. Calibration Curve Parameters for Fexinidazole Sulphone Calibration Standards in Dog Plasma or Study Protocol 0320-2007.

Run Date	Curve Number	Slope	Intercept	R^2	LLOQ ng/mL	ULOQ ng/mL	Regression Footnote(s)
07-Nov-2007	1	2414.79	-2648.69	0.9893	5	5000	1
Mean		2414.79	-2648.69	0.9893			
SD							
%CV							
n		1	1	1			

Regression Footnote(s):
1) Resp. = Slope * Conc. + Intercept

Appendix 4. Clinical signs

Individual Animal Clinical Signs for Study Protocol 0320-2007.			
Animal ID	Clinical Signs	Day Present	Study Day(s) Noted
1979	Normal/No significant signs	21	Pretest phase 1-10
			Test Period 1-7, 10-11
			Test Period (2) 1-2
	Diarrhea, Slight	2	Test Period 8-9
2197	Normal/No significant signs	23	Pretest phase 1-10
			Test Period 1-11
			Test Period (2) 1-2
2205	Normal/No significant signs	23	Pretest phase 1-10
			Test Period 1-11
			Test Period (2) 1-2
Test Period: IV Dose Test Period (2): Oral Dose			

Appendix 5. Body Weight

Dog ID	Body weight (kg)		
	Pre-test	IV (Day 1)	Oral (Day 12)
1979	9.76	9.60	9.70
2197	8.54	8.42	8.79
2205	7.52	7.33	7.71
Mean	8.60	8.45	8.74
SD	1.12	1.14	0.997