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Pharmacokinetics and bioequivalence of parenterally administered doramectin in cattle

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Plasma concentrations of doramectin in 40 cattle dosed by subcutaneous (sc) or intramuscular (i.m.) injection (200 μg/kg) were compared to assess the bioequivalence of the two routes of administration. Peak concentration (C_{max}), and areas under the concentration curve ($AUC_{0-\infty}$) were determined from plasma concentrations. Animals treated by the sc route showed a mean $AUC_{0-\infty}$ of 457 ± 66 ng·day/mL (± SD) and a mean C_{max} of 27.8 ± 7.9 ng/mL. Results from the i.m. treatment group showed a mean $AUC_{0-\infty}$ of 475 ± 82 ng·day/mL and a mean C_{max} of 33.1 ± 9.0 ng/mL. Absorption constants (k_a) determined by modelling were 0.542 ± 0.336 day^{-1} after sc administration and 0.710 ± 0.357 day^{-1} after i.m. administration. The 90% confidence limits on the difference between mean $AUC_{0-\infty}$ values for the sc and i.m. groups fell within 20% of the mean value for the subcutaneous group. C_{max} was somewhat greater for the i.m. route. The 90% confidence limits on the difference in mean $\ln(T_{max}+1)$ also fell within 20% of the mean sc value. Based on this analysis, bioequivalence of the sc and i.m. formulation has been established.

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INTRODUCTION

Doramectin (Fig. 1) is a fermentation-derived antiparasitic agent having broad spectrum endo- and ectoparasitic activity in animals. It was selected as the best of a series of novel avermectins for use as a therapeutic agent for veterinary medicine.

When given parenterally in cattle, doramectin exhibits potent therapeutic and prophylactic activity against both nematodes and arthropods that is consistent with the pharmacokinetic profile of doramectin utilizing an experimental aqueous micelle formulation (Goudie *et al.*, 1993). However, studies with ivermectin, the first member of the avermectin class of compounds to be commercialized, have shown that the pharmacokinetics and hence efficacy of this compound can be substantially modified by formulation and route of administration (Lo & Williams, 1983). Other investigators have shown that the anthelmintic efficacy of ivermectin in cattle is associated with prolonged plasma concentrations (Campbell & Benz, 1984; Bogan & McKellar, 1988). It was anticipated doramectin would be sensitive to these factors in a similar manner.

Following a formulation development and screening programme which is reported elsewhere (Wicks *et al.*, 1993), a parenteral formulation (Dectomax™ Pfizer Inc,.) was identified. The formulation optimized the pharmacokinetic profile producing the desired combination of therapeutic and prophylactic efficacy at the projected dosage of 200 μg/kg doramectin. Bioequivalence between subcutaneous and intramuscular (i.m.) sites was also sought, because it would provide flexibility for the user, and its use would not be compromised by inadvertent administration. In this developmental programme, the anthelmintic efficacy of doramectin was established in a dose–response study in which cattle were given an experimental infection of *Cooperia oncophora* followed by subcutaneous or intramuscular administration of an oil-based formulation (Weatherley *et al.*, 1993). The effects achieved by doramectin against *C. oncophora* were comparable for the subcutaneous and i.m. routes. The persistent efficacy of doramectin in preventing infection in cattle was ascribed to the pharmacokinetics as well as potency of the compound. Thus, the study reported here was designed to compare the pharmacokinetic profiles of doramectin in cattle following administration by the i.m. or subcutaneous routes, and to establish bioequivalence for the two routes of administration according to U.S. regulatory guidelines (Anon., 1990). To confidently accomplish this goal improvements in the reported methodology for the analysis of avermectins were also necessary.

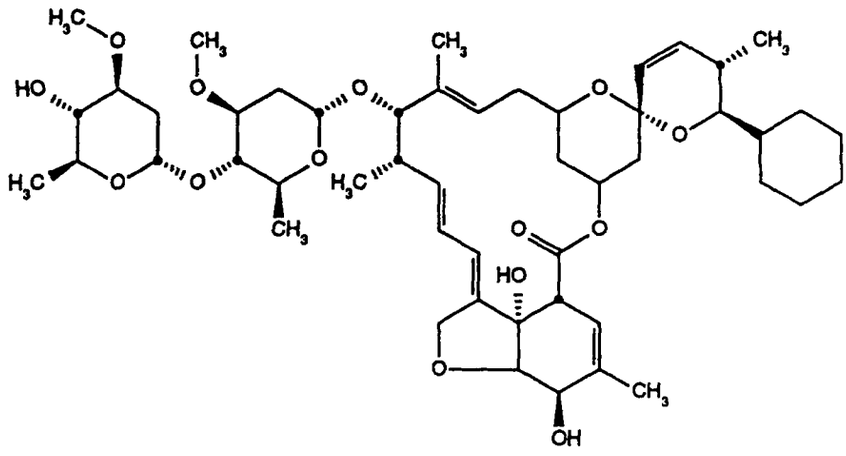

Fig. 1. Structure of doramectin

MATERIALS AND METHODS

Kinetics study

Forty crossbred cattle (20 males and 20 females) weighing between 183 and 278 kg were individually identified and randomized into two doramectin-treatment groups (T1 and T2) on the basis of sex and body weight. On the day of dosing (day 0) individual animals were weighed, and duplicate 10 mL samples of blood were collected from the jugular vein of each calf into evacuated glass tubes containing sodium heparin (Venoject, Terumo Medical, Elkton, MD, USA). Immediately thereafter, calves in group T1 received an injection of doramectin at a dose of 200 μg/kg by the intramuscular route into the semimembranosus muscle, and calves in group T2 received the same dose into the subcutaneous tissue of the neck. Blood samples were taken from all calves in both doramectin-treated groups by direct venipuncture of the external jugular vein at 12 h after treatment, and then at 1, 2, 3, 4, 5, 6, 10, 15, 20, 25 and 30 days post-treatment. Within 30 min of collection the blood was centrifuged at 2500 rpm for 10 min, the plasma removed and stored at −5°C until analysed.

Doramectin assay

Analysis of plasma samples for unchanged doramectin was conducted by modification of the method of de Montigny *et al.* (1990) and Sommer *et al.* (1992). For preparation of the standard curve, doramectin-naive cattle plasma samples (2.0 mL) were fortified with doramectin (100 μL of 10, 20, 50, 100, 200, 500 and 1000 ng mL^{-1}) and with the internal standard UK-71647 (the 22,23 dihydro, 23 hydroxy analog of doramectin) (100 μL of 200 ng mL^{-1}) each in methanol. Plasma samples (2.0 mL) from treated animals were also fortified with (100 μL of 200 ng mL^{-1} of the internal standard in methanol. Plasma proteins were precipitated by the addition of 5 mL of methanol. After mixing thoroughly, the solutions were centrifuged 2000 *g* for 3 min, and 4 mL of water was added to the supernatant. After mixing again and centrifugation (2000 *g* for 3 min), the supernatant was applied to a C2 Bond Elut (Analytichem International, part no. 1211–3026) cartridge preconditioned with methanol (1 mL) and (60:40) methanol-water (3 mL). The applied sample was slowly drawn through the cartridge using a Vac Elut SPS24 vacuum manifold (Analytichem International) under low vacuum. The column was washed with water (1 mL) and then (60:40) methanol-water (3 mL) under vacuum. The analytes were eluted with the addition of methanol (0.5 mL). This eluate was evaporated to dryness under a gentle stream of dry nitrogen at elevated temperature (60°C) in a water bath. The dried residue was dissolved in (1:1) triethylamine-acetonitrile (100 μL and the derivatization initiated by the addition of (1:2) trifluoroacetic anhydride-acetonitrile (150 μL). (The reagents used for derivatization were kept under nitrogen whilst being used; triethylamine 99+%, Aldrich or Kodak; Trifluoroacetic anhydride, 99+%, Aldrich.) After completion of the reaction (< 30s) the samples were concentrated to approximately 75 μL under nitrogen in a water bath (50°C) and 2.0 M ammonia in methanol added (250 μL). The samples were concentrated to approximately 75μL under nitrogen in a water bath (50°C), cooled, diluted with acetonitrile (150μL), and centrifuged at 2000 *g* for 3 min. To avoid photoisomerization of the derivatives under ambient light conditions (Sklavounos *et al.*, 1994), solutions were stored in amber glassware during HPLC analysis. An aliquot of the supernatant was injected directly into the chromatograph. Chromatography was performed on a 5 μ Zorbax RX C18 analytical column (4.6 × 150 mm) preceded by a 5 μ C18 guard column (Supleco) with fluorescence detection (ABI model 980) at an excitation wavelength of 360 nm and an emission wavelength of 470 nm. The mobile phase consisted of (45:25:30) acetonitrile-tetrahydrofuran-water pumped (LDC model CM4000) at a flow rate of 1.4 mL min^{-1}. Under these conditions, the fluorescent analogues of doramectin and internal standard (UK- 71647) eluted with average retention times of 12 and 6.8 min, respectively. Peak heights were measured with a computing integrator (Spectra Physics model 4200). A standard curve was constructed from the weighted (1/x least squares linear regression analysis of HPLC peak height ratios of doramectin/UK-71647 (dependent variable) and nominal concentrations of fortified plasma samples (independent variable).

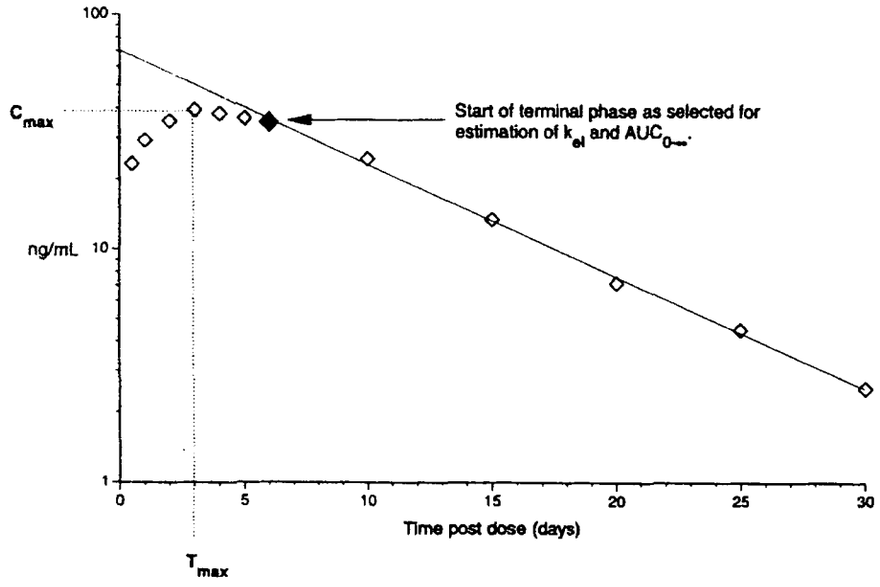

Fig. 2. An example of doramectin plasma concentration-time profile following sc injection of 0.2 mg/kg in cattle showing the start of the terminal log-linear elimination phase (◆). sc 513 (◇).

Calculation of pharmacokinetic parameters

The area under the plasma concentration–time curve from time zero to the last time t with a measurable concentration (C_p) was estimated using linear trapezoidal approximation. The area from time t to infinity was estimated as C_p/K_{el}, where K_{el} represents the terminal elimination rate constant. The total area under the curve was estimated as the sum of the two, i.e. $AUC_{(0-\infty)} = AUC_{(0-t)} + AUC_{(t-\infty)}$. K_{el} was estimated using least squares regression analysis of the concentration–time data obtained during the terminal log-linear elimination phase (Fig. 2). The mean elimination half-life ($t_{1/2}$) was calculated as $0.693/k_{el}$, utilizing the mean k_{el} for each treatment group. The maximum plasma concentration (C_{max}) was estimated directly from the experimental data, with T_{max} defined as the time of the first occurrence of C_{max}. The absorption rate constant (k_a) was determined after fitting individual plasma data, using a weighted, non-linear least squares regression program (PCNONLIN®, Statistical Consultants Inc., Lexington, KY, USA), to a one-compartment model with first-order absorption (k_a) and elimination (K_{el}). The harmonic mean absorption half-life ($t_{1/2a}$) was $0.693/\text{mean } k_a$. A least squares weighting 1/y (1/conc) was used. Initial estimates for k_a and k_{el} were obtained by Hartley's modification of Gauss–Newton least squares regression and final results were obtained by the Nelder–Mead method (these two methods are part of the PCNONLIN® program). Initial estimates for the volume of distribution, V, used by the models were obtained by the program from the absorption and elimination rate constant estimates (Gabrielsson & Weiner, 1994).

Statistical analysis

Following preliminary statistical validation to check for outliers, normality and equality of variances, $AUC_{(0-\infty)}$ and T_{max} were analysed using analysis of variance of a one-period parallel group design. To help normalize the data, the T_{max} values were transformed using $\ln(T_{max}+1)$, prior to the analysis. Since $AUC_{(0-\infty)}$ is the primary measure used to assess bioequivalence, the two routes can be considered statistically bioequivalent (Anon, 1990) if the upper and lower 90% confidence limits for the difference between the mean AUCs of the two routes both lie within 20% of the mean AUC of the reference (i.e. subcutaneous) route.

A similar calculation was made for the secondary measure of bioequivalence ($\ln(T_{max}+1)$). However, the strong non-normality of the $\ln(T_{max}+1)$ values reduce their statistical validity.

Analysis of C_{max} was not performed because it is considered clinically irrelevant for doramectin. The use of C_{max} as a measure of bioequivalence for veterinary drugs has been described as non-pivotal (Martinez & Riviere, 1994).

RESULTS

The derivatization method of de Montigny *et al.* (1990) and Sommer *et al.* (1992) was modified to ensure long-term chemical stability of the fluorophores formed in the procedure. This enabled us to discount the variation between samples with respect to the time of derivatization and wait time prior to HPLC analysis, also noted by Rabel *et al.* (1991). Substitution of the stronger base catalyst, triethylamine, for N-methylimidazole in acetylation with trifluoroacetic anhydride, followed by elimination of trifluoroacetyl groups with methanolic ammonia provided stable derivatives of doramectin and the internal standard, UK-71,647 (Fig. 3) with suitable retention times (Fig. 4).

The lower limit of quantitation for this method was 0.5 ng/mL and the upper limit was 50 ng/mL. The deviation of calculated drug concentrations from nominal was + 4.0% at 0.5 ng/mL and –2.2% at 50 ng/mL. The standard deviations among five replicates at 0.5 and 50 ng/mL were 0.017 and 0.899, respectively. Mean concentrations with per cent relative standard deviations of 0.93 (± 5.7%), 9.5 (± 2.3%), and 39.0 (± 3.4%) were found in replicate analysis (n = 5) of plasma fortified at 1.0, 10.0 and 40.0 ng/mL, respectively.

Mean plasma concentrations of doramectin following subcuta-

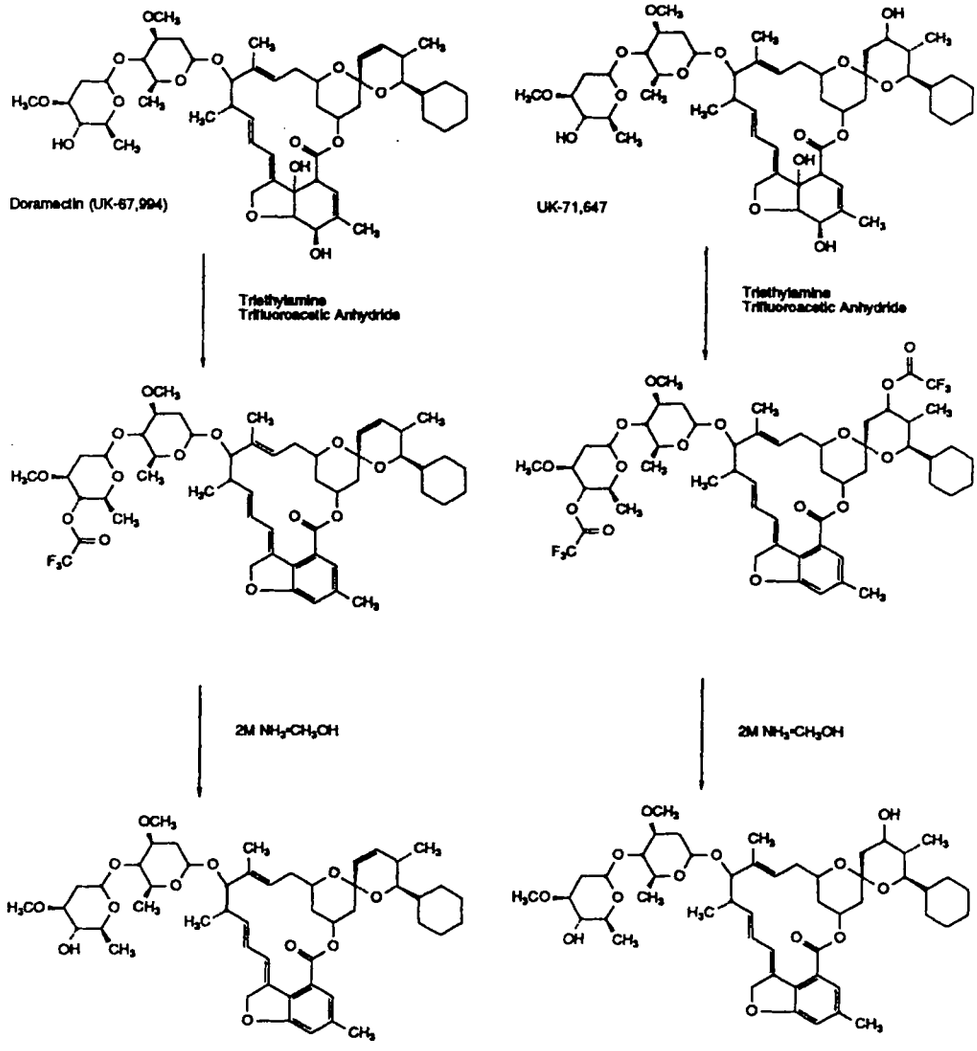

Fig. 3. Conversion of doramectin and the internal standard, UK-71,647, to fluorophores.

neous and intramuscular administration to 20 cattle are given in Table 1. All plasma concentrations were within the validated range of the assay. Mean concentrations in plasma for each route of administration are depicted in Fig. 5. The major pharmacokinetic parameters related to these routes of administration are recorded in Tables 2 & 3. Except for one animal with a T_{max} on day 15, peak plasma concentrations were observed between 3 and 6 days, with a mean C_{max} of 33.1 ng/mL for i.m. dosed cattle. Subcutaneous (sc) treated cattle showed more variation. Two animals showed peak concentrations occurring on day 15, whereas peak concentrations occurred for the other 18 animals between 2 and 10 days with a C_{max} of 27.8 ng/mL. However, mean *AUCs* for the two routes were similar and the 90% confidence limits for the difference between the two means was well within the 20% bounds for demonstrating bioequivalence (Table 4). A similar conclusion was obtained for $\ln(T_{max}+1)$, but this must be tempered by the non-normality of the data values.

Absorption and elimination rate constants were also estimated by modelling the plasma concentration vs. time data. The results

Table 1. Mean plasma concentrations for doramectin after administration of a single dose of 0.2 mg/kg to cattle ($n = 20$)

Time post-dose (days)	sc mean doramectin concentration ng/mL ± SD	i.m. mean doramectin concentration ng/mL ± SD
0	0	0
0.5	10.0 ± 6.1	15.3 ± 6.0
1	16.0 ± 8.0	21.2 ± 8.5
2	21.9 ± 10.3	27.4 ± 8.9
3	24.1 ± 9.7	31.1 ± 9.9
4	25.0 ± 8.9	31.0 ± 8.0
5	25.4 ± 7.8	30.1 ± 6.7
6	24.8 ± 6.6	28.3 ± 5.7
10	20.0 ± 3.5	20.7 ± 4.3
15	13.7 ± 2.9	13.1 ± 3.7
20	8.7 ± 2.6	8.3 ± 3.1
25	5.3 ± 2.0	4.6 ± 2.1
30	3.3 ± 1.5	2.7 ± 1.4

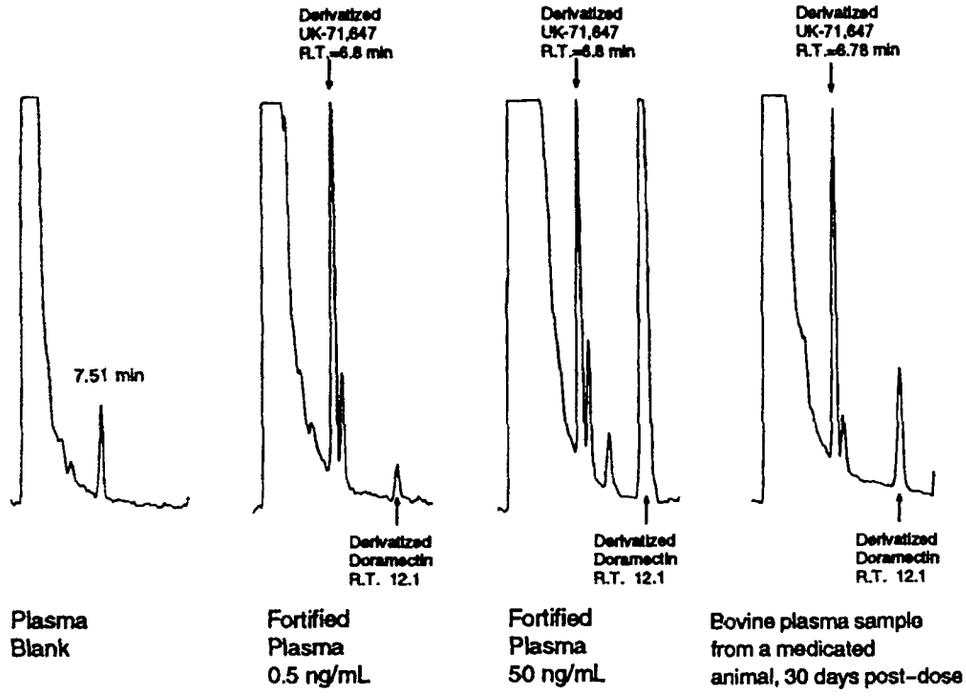

Fig. 4. Chromatograms of doramectin and internal standard, UK-71,647, in bovine plasma.

Animal no.	$AUC_{0-\infty}$ ng·day/mL	C_{max} ng/mL	T_{max} (days)	k_{el} day^{-1}	Half-life (days)
487	569	40.5	4	0.13915	5.0
488	430	35.4	5	0.12715	5.5
489	235	22.6	4	0.14476	4.8
497	445	26.6	4	0.08466	8.2
498	534	43.8	4	0.11036	6.3
499	531	34.0	4	0.10139	6.8
500	401	32.3	3	0.11274	6.2
501	409	34.8	5	0.13613	5.1
505	409	38.5	3	0.12383	5.6
506	583	26.8	5	0.08074	8.6
509	507	18.5	15	0.07498	9.2
510	515	33.1	4	0.09459	7.3
512	419	28.8	5	0.09858	7.0
520	423	23.6	5	0.07236	9.6
521	469	35.5	6	0.13177	5.3
525	572	56.0	3	0.10659	6.5
526	535	47.1	3	0.12576	5.5
527	472	25.8	3	0.06946	10.0
529	531	28.6	6	0.08481	8.2
531	505	30.2	3	0.10259	6.8
Mean	475	33.1		0.10612	6.5*
Standard Deviation	82	9.0		0.02360	

*Harmonic mean.

Table 2. Plasma pharmacokinetic parameters for doramectin after administration of a single dose of 0.2 mg/kg to cattle ($n = 20$) by the intramuscular route

Table 3. Plasma pharmacokinetic parameters for doramectin after administration of a single dose of 0.2 mg/kg to cattle ($n = 20$) by the subcutaneous route

Animal no.	$AUC_{0-\infty}$ ng·day/mL	C_{max} ng/mL	T_{max} (days)	k_{el} day^{-1}	Half-life (days)
484	520	35.1	5	0.11344	6.1
486	445	27.8	4	0.07990	8.7
490	510	36.2	3	0.10670	6.5
491	408	22.4	5	0.09581	7.0
492	557	43.0	2	0.10859	6.4
495	511	15.4	10	0.03556	19.0
496	401	23.8	6	0.09419	7.4
503	358	19.4	6	0.10147	6.8
504	442	20.2	15	0.09218	8.0
507	535	33.6	3	0.07598	9.1
508	389	22.4	4	0.07503	9.2
511	468	37.4	4	0.11939	5.8
513	466	30.7	4	0.08462	8.2
514	359	25.2	6	0.11504	6.0
515	454	32.4	5	0.11683	5.9
518	395	30.6	2	0.11065	6.0
522	405	20.9	6	0.07760	9.0
523	421	18.7	10	0.06815	10.0
532	568	21.3	15	0.07639	9.0
533	535	39.4	3	0.11100	6.2
Mean	457	27.8		0.09293	7.5*
Standard Deviation	66	7.9		0.02128	

*Harmonic mean.

Table 4. Analysis of variance, least square means and their standard errors, significance levels and interval estimates of pharmacokinetic response variables, $AUC_{(0-\infty)}$ and $\ln(T_{max}+1)$

			Pharmacokinetic response variables				
			$AUC_{(0-\infty)}$		$\ln(T_{max}+1)$		
Source of variation	Degrees of freedom	MS	F	$\hat{\alpha}$	MS	F	$\hat{\alpha}$
Route of administration	1	3010	0.55	0.4645	0.182	1.1	0.3008
Residual	38	5515			0.165		
Route		n	mean	standard error	n	mean	standard error
Intramuscular		20	474.7	16.6	20	1.68	0.091
Subcutaneous		20	457.4	16.6	20	1.815	0.091
Difference (i.m.-sc)			17.3			–0.135	
90% confidence limits for the difference			–22.2 to 56.9 (–4.9% to + 12.5%)		–0.351 to +0.082 (–19.4% to +4.5%)		

are shown in Tables 5 & 6. Data from four animals (no. 509 from the i.m. group and nos. 484, 504 and 514 of the sc group) were analysed using a model which assumed equal absorption and elimination rates. After sc administration, the mean absorption constant (k_a) was 0.542 ± 0.336 day^{-1} with a harmonic mean absorption half-life ($t_{1/2a}$) of 1.3 days. Analysis of data obtained from the i.m. route resulted in a mean k_a ± SD of 0.710 ± 0.357 day^{-1}, with a corresponding harmonic mean $t_{1/2a}$ of 0.098 days. Because of the large variation in the k_a's the difference between the means of the two routes could not be assessed statistically by the methods used here with a meaningful result. The mean elimination rate constants determined in the modelling exercise were identical for both routes, $k_{el} = 0.105$ day^{-1}, $t_{1/2} = 6.6$ days.

DISCUSSION

The persistence of doramectin in the plasma of cattle dosed parenterally by subcutaneous or intramuscular administration of the oil-based formulation was borne out in this study. Variability is evident in the data as illustrated in Tables 2, 3, 5 and 6. Following subcutaneous administration the disposition of doramectin was

Animal no.	Absorption constant k_a	Absorption half-life (days)	Elimination constants k_{el}	Elimination half life (days)
487	0.359	1.9	0.118	5.9
488	0.283	2.5	0.179	3.9
489	0.513	1.4	0.167	4.2
497	0.448	1.5	0.0869	8.0
498	0.914	0.76	0.104	6.6
499	1.05	0.66	0.0788	8.8
500	0.944	0.73	0.102	6.8
501	0.457	1.5	0.143	4.9
505	1.18	0.59	0.121	5.7
506	0.527	1.3	0.0567	12
509	0.111	6.3	0.111	6.3
510	1.43	0.48	0.0726	9.5
512	0.537	1.29	0.0967	7.2
520	1.00	0.69	0.0585	12
521	0.754	0.92	0.105	6.6
525	0.965	0.72	0.117	5.9
526	0.717	0.97	0.127	5.4
527	1.06	0.66	0.0644	11
529	0.178	3.9	0.119	5.8
531	0.767	0.90	0.0665	10
Mean ± SD	0.71 ± 0.36	0.98*	0.105 ± 0.03	6.6*

*Harmonic mean.

Table 5. Absorption and elimination constants determined by fitting a PCNONLIN® model to doramectin plasma concentration–time data and the corresponding absorption and elimination half lives obtained from the i.m. route of administration

Animal no.	Absorption constant k_a	Absorption half life (days)	Elimination constant k_{el}	Elimination half life (days)
484	0.169	4.1	0.169	4.1
486	0.695	1.0	0.0783	8.9
490	0.926	0.75	0.0906	7.6
491	0.649	1.1	0.0700	9.9
492	0.926	0.75	0.0973	7.1
495	0.0454	15	0.279	2.5
496	0.323	2.1	0.0984	7.0
503	0.235	2.9	0.0979	7.1
504	0.0917	7.6	0.0917	7.6
507	0.852	0.81	0.0740	9.4
508	0.695	1.0	0.0679	10
511	0.553	1.3	0.111	6.2
513	0.873	0.79	0.0816	8.5
514	0.159	4.4	0.159	4.4
515	0.392	1.8	0.128	5.4
518	0.812	0.85	0.107	6.5
522	1.06	0.65	0.0576	12
523	0.186	3.7	0.0909	7.6
532	0.276	2.5	0.0468	15
533	0.927	0.75	0.0943	7.3
Mean ± SD	0.542 ± 0.34	1.3*	0.105 ± 0.05	6.6*

*harmonic mean.

Table 6. Absorption and elimination constants determined by fitting a PCNONLIN® model to doramectin plasma concentration–time data and the corresponding absorption and elimination half lives obtained from the sc route of administration

characterized by a broad maxima of concentrations from day 3 to day 6 with a mean C_{max} of 27.8 ng/mL. Thereafter, plasma concentrations declined to a mean concentration of 3.3 ng/mL, 30 days after dosing (Fig. 5) with a terminal half-life of approximately 7.5 days. When given by i.m. administration, the mean maximum was reached on day 4, with a mean C_{max} of 33.1 ng/mL, declining with a terminal half-life of 6.5 days to a mean concentration of 2.7 ng/mL on day 30. Compared with results of subcutaneous administration, animals in the intramuscular group had slightly higher AUCs, and C_{max}'s. Nevertheless, comparison of the AUCs revealed

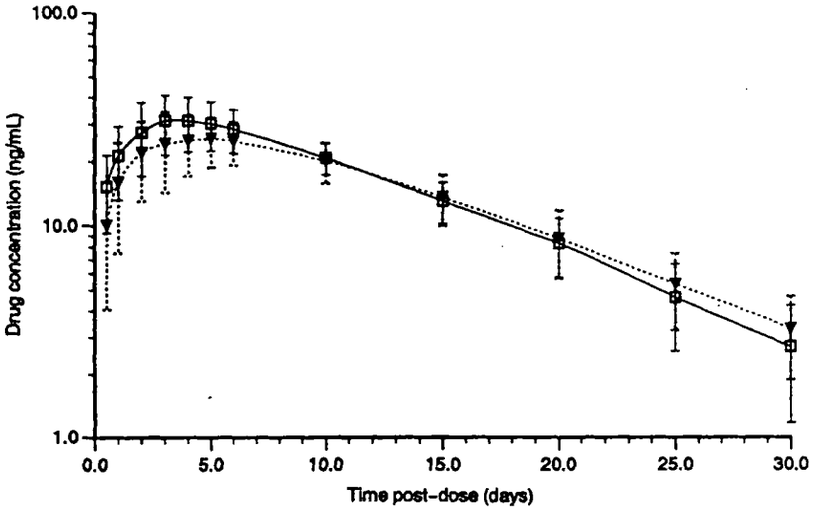

Fig. 5. Mean concentration of doramectin in bovine plasma after i.m. or sc administration of a single dose of 0.2 mg/mg.

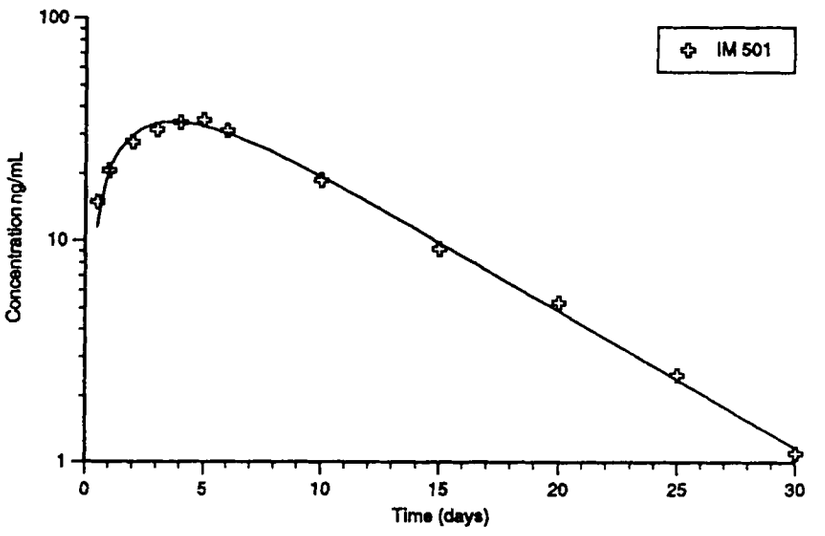

Fig. 6. Plasma profile from an individual animal showing a modelled fit which suggested 'flip-flop' kinetics. (η) i.m.; (.) sc.

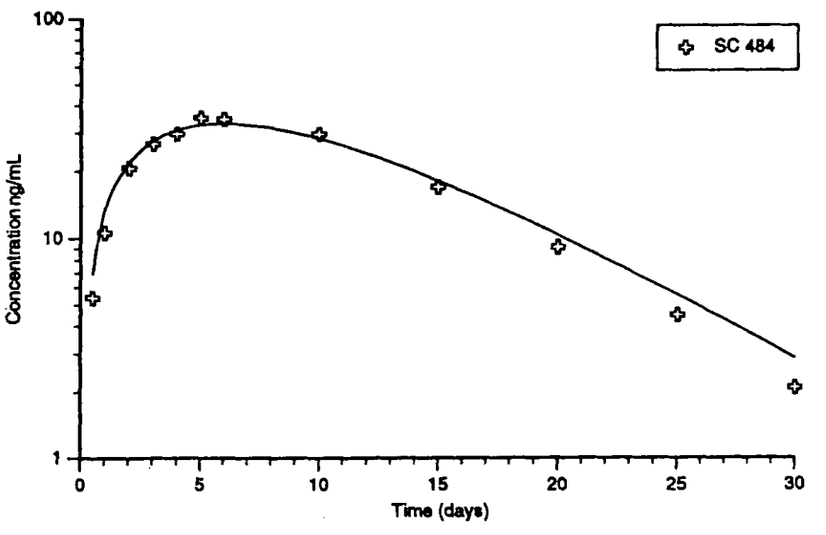

Fig. 7. Plasma profile showing a modelled fit with $k_a = k_{el}$.

that the two routes of administration are statistically bioequivalent.

To gain further insight into the absorption process we performed a modelling exercise as described earlier. Two of the models provided by PCNONLIN® were utilized in the analysis of the data summarized here. They are described as one compartment with first-order input, first-order output and no time lag (exemplified in Fig. 6, most of the data were treated by this model) and one compartment with equal first-order input, first-order output and no time lag (exemplified in Fig. 7, used in the analysis of data from animal number 509 from the i.m. group and 484, 504, and 514 of the sc group). For comparative purposes both the absorption and elimination rate constants obtained in this modelling exercise are shown in Tables 5 and 6. The mean absorption rate constant was slightly greater for the i.m. route suggesting faster absorption (as also indicated by the mean C_{max} and T_{max} for the i.m. route) by this route. However, the difference in mean absorption constants cannot be meaningfully assessed because of inter-animal variability. Because of the large variability in k_a the study has very low power in being able to detect a difference between the sc and i.m. routes by analysis of the modelled absorption constants. The observed variability (standard deviation) in absorption constants was nearly identical (0.36 vs. 0.34) for both routes of administration. The mean elimination rate constants determined in this exercise were identical (0.105 day^{-1}) for both i.m. and sc treated animals, with the results from the sc group being slightly more variable.

The two routes of administration appear bioequivalent, i.e. doramectin is absorbed with equal bioavailability in terms of extent and rate of absorption as measured by *AUC* and T_{max}, respectively. Since the only difference between the two groups was the route of administration, the similarities in absorption and elimination are probably due to differences in blood flow to the injection site or a difference in surface area at the site of administration. It would be expected that blood flow to an i.m. injection site would be greater than that to a sc site. But, since a sc injection tends to take the shape of the space into which it is administered, the dose increases the amount of surface area with which it is in contact. By increasing its surface area, the sc route compensates for decreased blood flow by increasing its area of absorption (Hirano *et al.*, 1982).

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REFERENCES

Anon (1990) *Bioequivalence Guideline.* Center for Veterinary Medicine, Food and Drug Administration. Rockville, MD. USA.

Bogan, J.A. & McKellar, Q.A. (1988) The pharmacodynamics of ivermectin in sheep and cattle. *Journal of Veterinary Pharmacology and Therapeutics,* 11, 260–268.

Campbell, W.C. & Benz, G.W. (1984) Ivermectin: A review of efficacy and safety. *Journal of Veterinary Pharmacology and Therapeutics,* 7, 1–16.

D Montigny, P., Shim, J.-S. K. & Pivnichny, J.V. (1990) Liquid chromatographic determination of ivermectin in animal plasma with trifluoroacetic anhydride and N-methylimidazole as the derivatization reagent. *Journal of Pharmaceutical and Biomedical Analysis,* 8, 507–511.

Gabrielsson, J. & Weiner, D. *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications.* Swedish Pharmaceutical Press: Stockholm, Sweden, 1994.

Goudie, A.C., Evans, N.A., Gration, K.A.F., Bishop, B.F., Gibson, S.P., Holdom, K.S., Kaye, B., Wicks, S.R., Lewis, D., Weatherley, A.J., Herbert, A. & Seymour, D.J. (1993) Doramectin - A potent novel endectocide. *Veterinary Parasitology,* 49, 5–15.

Hirano, K., Ichihashi, T. & Yamada, H. (1982) Studies on the absorption of practically water-insoluble drugs following injection V: subcutaneous absorption in rats from solutions in water immiscible oils. *Journal of Pharmaceutical Sciences,* 71, 495–500.

Lo, P.-K.A. & Williams, J.B. (1983) Solubilization of ivermectin in water. U.S. Patent 4,389,397. *Chemical Abstracts,* 99, 76906q.

Martinez, M.N. & Riviere, J.E. (1994) Review of the 1993 Veterinary Drug Bioequivalence Workshop. *Journal of Veterinary Pharmacology and Therapeutics,* 17, 85–119.

Rabel, S.R., Stobaugh, J.F. Bostick, J.M. & Heinig, R. (1991) Determination of ivermectin in plasma using narrow bore HPLC with automated precolumn derivatization and laser induced fluorescence detection. *Pharmaceutical Research,* 8, S-24.

Sklavounos, C., Demers, N., Lukaszewicz, J. & Nowakowski, M. (1994) Photoisomerization of Aromatic Doramectin Derivatives. *Journal of Agricultural and Food Chemistry,* 42, 1228–1231.

Sommer, C., Steffansen, B., Overgaard Neilsen, B., Grønvold, J., Van Jensen, K.-M., Brøchner Jespersen, J., Springborg, J. & Nansen, P. (1992) Ivermectin excreted in cattle dung after subcutaneous injection or pour-on treatment: concentrations and impact on dung fauna. *Bulletin of Entomological Research,* 82, 257–264.

Weatherley, A.J., Hong, C., Harris, T.J., Smith, D.G., & Hammet, N.C. (1993) Persistent efficacy of doramectin against experimental nematode infections in calves. *Veterinary Parasitology,* 49, 45–50.

Wicks, S.R., Kaye, B., Weatherley, A.J., Lewis, D., Davison, E., Gibson, S.P. & Smith, D.G. (1993) Effects of formulation on the pharmacokinetics and efficacy of doramectin. *Veterinary Parasitology,* 49, 17–26.