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ORIGINAL RESEARCH

Validated capillary electrophoretic method for the analysis of ivermectin in plasma after intragastric administration in pigs and horses

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ABSTRACT: A capillary electrophoretic (CE) method has been developed for the determination of ivermectin (CAS 70288-86-7), a new generation drug with antiparasitic activity, in pig and horse plasma. The method was statistically validated for its linearity, accuracy, precision and selectivity. The linear range was from 1 to 30 ng mL^{-1} with correlation coefficients greater than 0.999. The limit of detection was 0.3 ng mL^{-1}, while the quantitative limit was 1 ng mL^{-1}, using a 0.5 mL sample size. The validated procedure was used to determination of pharmacokinetic parameters of ivermectin after ingestion of 0.1 mg for pigs and 0.2 mg dose per kg body weight for horses, respectively. Studies were performed on a group of eight pigs and six horses. There were no significant differences between pigs and horses in any of the estimated pharmacokinetic parameters. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: determination of ivermectin in animal plasma; capillary electrophoresis (CE); pharmacokinetic studies; Ivomec premix; Eqvalan

INTRODUCTION

In the mid-1980s, ivermectin was introduced as probably the most broad-spectrum anti-parasite medication ever. Ivermectin is a derivative of the natural avermectin, including abamectin, produced during fermentation of *Streptomyces avermitilis*. This drug is a semi-synthetic mixture of two homologues containing at least 80% 22,23-dihydroavermectin B_{1A} (H_2B_{1A}) and not more than 20% 22,23-dihydroavermectin B_{1B} (H_2B_{1B}). Ivermectin is an unique and potent compound for controlling endo- and ectoparasites (Campbell *et al.*, 1983) and presently registered for use in beef cattle, pigs, sheep, horses and reindeer (Tway *et al.*, 1981; Chiu *et al.*, 1990; Nordlander *et al.*, 1990; Markus and Sherma, 1992). Furthermore, in more recently published procedures, determination of ivermectin has been reported in Atlantic salmon (Kennedy *et al.*, 1993; Rupp *et al.*, 1998), goat (Alvinerie M. *et al.*, 1993; Scarano *et al.*, 1998) and llama (Jarvinen *et al.*, 2002) tissues.

It is effective against most common intestinal worms, except tapeworms, most mites and some lice. Likewise, it is effective against larval heartworms, the 'microfilariae' that circulate in the blood, but not against adult heartworms that live in the heart and pulmonary arteries. The most common uses in small animal practice for ivermectin would include: monthly prevention of heartworm infection, treatment of especially difficult ear mite situations; clearing heartworm larvae in active heartworm infection; or treatment of sarcoptic, notoedric or demodectic mange. It should be noted that doses of ivermectin used for prevention and treatment of heartworm disease are approximately 50 times lower than doses used for other parasites. With the exception of rare serious reactions such as severe systemic postural hypotension, ivermectin is generally well tolerated in animals and humans. The drug has the clear advantages of ease of administration and better tolerability compared with other agents previously used to treat onchocerciasis. Thus, ivermectin is suitable for inclusion in mass treatment programmes and is the best therapeutic option presently available to combat onchocerciasis. As such it provides hope for many thousands of people at risk of becoming blind and represents a major contribution to tropical medicine (Goa *et al.*, 1991).

Several analytical methods have been developed for the quantitative determination of ivermectin in biological samples. Most of them used liquid chromatography (LC), with spectrophotometric or fluorometric detection, to analyse and to quantify ivermectin in bovine serum (Oehler and Miller, 1989), cattle tissue (Reuvers *et al.*, 1993), milk (Alvinerie *et al.*, 1987; Chiou *et al.*,

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Abbreviations used: MRT, mean residence time.

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1987; Kijak, 1992; Cervenik *et al.*, 2002) or human plasma (Krishna and Klotz, 1993). Moreover, ivermectin has been determined by LC combined with electrospray ionization mass spectrometry (Croubels *et al.*, 2002) and radioimmunoassay (Baraka *et al.*, 1996).

Likewise, many HPLC separation methods have been reported in the literature, employed fluorescence (Markus *et al.*, 1992; Reising, 1992; Craven *et al.*, 2002) or laser inducted fluorescence (LIF) detectors (Rabel *et al.*, 1993), which are more commonly used and give lower detection limits. However, application of HPLC technique requires multi-step sample preparation procedures. Moreover, more sensitive fluorescence detection requires additionally application of derivatization procedure using trifluoroacetic anhydride and *N*-methylimidazole (Chiu *et al.*, 1985; de Monttigny *et al.*, 1990; Schenk *et al.*, 1992; Degroodt *et al.*, 1994; Wiśniewska-Dmytrow *et al.*, 2001).

Tissue distribution of ivermectin residues in cattle and sheep has been studied (Tway *et al.*, 1981; Markus *et al.*, 1992) and it was shown that the major simple component in the edible tissue of the animals was the unaltered drug. The drug tends to be concentrated in the liver and fat, with minimal residues being detected in muscle and kidneys (Fischer *et al.*, 1993). In this respect, the liver is the tissue in which the drug is most persistent, residues being detected over a period of 28 days post-administration. Traditional methods for the isolation of ivermectin from tissues can include multiple homogenization and centrifugation steps, followed by evaporation of large volumes of solvent and further cleanup by hexane/acetonitrile and acetonitrile with water–hexane solvent partition steps (Tway *et al.*, 1981; Markus *et al.*, 1992).

Bioavailability and pharmacokinetic studies based upon plasma analyses have been performed by Tway *et al.* (1981), Prichard *et al.* (1985) and Lo *et al.* (1985) and results indicated that ivermectin was very slowly eliminated with a terminal half-life in the range of 5–7 days. Ivermectin was detected in plasma between 12 h and 35 days post administration of drug (Lifschitz *et al.*, 1999). Furthermore, many other analytical methods have been described to be used in pharmacokinetic studies of ivermectin in cattle (Wilkinson *et al.*, 1985; Toutain *et al.*, 1997; Gayrard *et al.*, 1999; Lifschitz *et al.*, 1999a), pigs (Scott and McKellar, 1992; Lifschitz *et al.*, 1999b; Craven *et al.*, 2002), horses (Baggot, 1992; Perez *et al.*, 1999, 2002; Gokbulut *et al.*, 2001), sheep (Ali and Hennessy, 1996; Cervenik *et al.*, 2002), dogs (Lo *et al.*, 1985) and rabbits (Jackson and Chesterman, 1989). Unfortunately, the presented methods are complicated; time-consuming multiple extraction and cleanup involve the use of procedures which are impracticable for routine application.

As far as we are aware, no high performance capillary electrophoretic (HPCE) method for determination of ivermectin in biological samples has been published. Considering this possibility, we devised an analytical HPCE method for determination of ivermectin in animal plasma, with the speed, precision, specificity and sensitivity required for pharmacokinetic studies in small plasma samples, involving only simple deproteinization. The manual sample preparation steps are eliminated and the time required for processing the sample is greatly shortened.

EXPERIMENTAL

Reagents. Ivermectin and all solvents (methanol, acetonitrile) were of analytical grade from Merck (Darmstadt, Germany). Sodium tetraborate decahydrate (pH 9.26; 10 mM), sodium dihydrogenphosphate (pH 5.73; 10 mM) and boric acid (pH 6.94; 10 mM) buffer solutions were prepared according to standard method, using triple distilled water. Pharmaceutical Enterprise (Polfa, Poland) supplied Imipramine hydrochloride used as internal standard. The pH meter was calibrated with standard buffer solutions purchased from POCh (Gliwice, Poland).

Stock solution. A stock standard solution was prepared in methanol–sodium tetraborate decahydrate buffer (0.01 M; 25:75, v/v) and contained 1 mg mL^{-1} of ivermectin. Working standard solutions were prepared by dilution of the stock standard solution with triple distilled water to concentration 100 µg mL^{-1}, 1 µg mL^{-1} and 100 ng mL^{-1}.

An internal standard stock solution was prepared in triple distilled water and contained 1 mg mL^{-1} of imipramine hydrochloride. A working standard solution was made by dilution of the stock standard solution of imipramine hydrochloride with triple distilled water to 100 µg mL^{-1}. They were stored in the dark under refrigeration to avoid possible decomposition.

Subjects and sample preparation. The pharmacokinetic studies were performed on a group of eight pigs and six horses of both sex. The animals before the experiment were sexed, marked with numbers and the individual weight of each was determined. Their initial weight was 55–82 kg for pigs and 580–608 kg for horses, respectively. Prior to experimental use, drug-free feed and water were provided *ad libitum*. A single dose of 0.1 mg kg^{-1} body weight of ivermectin granulate (Ivomec premix) was administered intragastrically in each pig; each horse was treated with an oral paste formulation (Eqvalan) at the manufacturer's recommended therapeutic a single dose 0.2 mg kg^{-1} body weight.

Blood samples were withdrawn into glass test tubes prior to dose and again at 2 h 30 min and 7, 12, 24, 48, 72 and 96 h after drug ingestion, in pigs and horses simultaneously. The samples were centrifuged and plasma harvested was stored at –20°C until assay.

The plasma samples (0.5 mL) were spiked with imipramine hydrochloride (internal standard) and deproteinized with a mixture of 1 mL acetonitrile and 1 mL methanol. Then the samples were shaken on a rotary mixer for 10 min to complete the process deproteinization and centrifuged at 8000 ***g***. The methanol–acetonitrile phase was transferred to a clean

test tube and evaporated to dryness in a water bath at 55°C. Finally, the residue was suspended in 0.5 mL of buffer solution composed with 25% (v/v) methanol and 75% (v/v) 2 mM sodium tetraborate decahydrate and centrifuged at 8000 ***g***. The supernatant was stored at –20°C until the time of analysis.

Apparatus. Experiments were carried out on a Beckman P/ACE 2100 System instrument, equipped with an autosampler, automatic injector. The system comprised a to 30 kV high-voltage built—in power supply, a selectable fixed-wavelength UV detector, and the Gold software for system controlling and data handling. All capillaries used were obtained from Beckman and had an internal diameter of 50 μm. The temperature was controlled using a fluorocarbon-based cooling fluid.

All experiments were performed at 25°C, using an uncoated silica capillary 47 cm (separation distance 40 cm) × 50 μm i.d. The voltage was maintained at 22 kV. The resultant electropherograms were monitored at 254 nm, with a fixed-wavelength detector. Analytes were introduced into the capillary at anode via a 7 s, 3.45 kPa argon pressure injection, whereas the detector was set on the cathode end of the capillary. In order to equilibrate the capillary and minimize hysteresis effects, the capillary was regenerated between each run by treatment with 0.1 M hydrochloric acid (0.5 min), then with regeneration solution (1 M sodium hydroxide; 1.5 min) and finally with triple distilled water (1.5 min). Each day the system was first purged with regeneration solution for 5 min followed by triple distilled water for 10 min and the working buffer solution for next 10 min.

Validation of analytical method. The concentrations used were based on the range expected during pharmacokinetic investigations. Calibration curves were established by plotting peak-height ratio (ivermectin/IS) vs ivermectin concentration (ng mL^{-1}). For the within-day precision and inaccuracy (bias), pools of plasma were spiked with ivermectin working standard to obtain concentrations of 1, 2, 4, 8, 12, 16, 20 and 30 ng mL^{-1} and with a fixed concentration of internal standard (20 μg mL^{-1}). Between-day validation was calculated for three concentrations (2, 12, 20 ng mL^{-1}) corresponding to quality control samples of the method. The samples were prepared before experiment, stored in the same conditions as samples taken from animals, and were assessed over the calibration curve. The limit of quantification (LOQ) was 1 ng mL^{-1}. Specificity of the assay was determined on the basis of different plasma samples. The stability of the investigated compound, stored in three concentrations (2, 12 and 20 ng mL^{-1}), after each of three freeze–thaw cycles during 2 months, has been also controlled.

RESULTS AND DISCUSSION

Previously reported methods for the determination of ivermectin in animal plasma or tissues were non-specific, based on LC with UV detection. Some of the LC techniques based on fluorescent ivermectin derivatives and fluorescence detection were sensitive and specific, but generally included complicated extraction and time-consuming cleanup procedures. The proposed electrophoretic method here used a simple extraction of the sample with methanol–acetonitrile, which precipitated the proteins from biological matrix simultaneously. Ivermectin was quantitatively extractable with organic solvents from plasma and animal tissues.

Likewise, the correspondingly low concentration of ivermectin in plasma necessitates a sensitive analytical methodology capable of detecting low (ng mL^{-1}) concentrations of drug. Capillary electrophoresis is a recent addition to the arsenal of techniques available to the analytical laboratory, and its range of UV detection is sufficient for quantitatively determination of ivermectin in animal plasma.

Composition of running buffer

Because ivermectin is highly lipophilic, a running buffer containing a high proportion of organic component is required. Methanol was found to be a necessary component in the background electrolyte. The addition of methanol to the running electrolyte increased solubility of ivermectin in buffer solution. Furthermore, the influence of different proportion of methanol, from 5 to 50% v/v on the electrophoretic separation was studied. In order to predict the optimum pH range for the separation of ivermectin, it is necessary to identify the pH values at which the differences between the mobilities of the studies substances are greatest. The best results were obtained using a buffer solution composed with 25% (v/v) methanol and 75% (v/v) sodium tetraborate decahydrate solution (10 mM, pH 9.26), sodium dihydrogenphosphate (10 mM, pH 5.73) and boric acid (10 mM, pH 6.94) buffer solutions.

Linearity, recovery, selectivity, precision, inaccuracy and sensitivy

Using the standard solutions of ivermectin and imipramine hydrochloride, samples of blank control plasma (0.5 mL) were spiked with both compounds at concentrations ranged from 1 to 30 ng mL^{-1} for ivermectin and with fixed concentration of internal standard (20 μg mL^{-1}). All the samples were prepared and analysed using the same procedures, which were described in the Experimental section. A calibration curve based on the peak-height ratios of ivermectin to imipramine hydrochloride was constructed using eight different concentrations of ivermectin analysed six times for each concentration. The data were subjected to linear regression analysis in order to achieve the appropriate calibration factors.

The linearity of the method was confirmed with precision and inaccuracy below 10% over the range 1–30 ng mL^{-1} of ivermectin. A straight line passing through the origin was obtained. The regression line,

Table 1. Precision and inaccuracy within days.

	Amount added							
Ivermectin (ng mL^{-1})	1	2	4	8	12	16	20	30
Imipramine hydrochloride (μg mL^{-1})	20	20	20	20	20	20	20	20
Series	Back-calculated concentrations (ng mL^{-1})							
A	1.0	1.83	4.09	7.91	12.26	16.13	20.30	29.09
B	0.91	2.00	4.17	8.35	12.65	16.30	20.78	30.04
C	0.83	1.83	4.00	7.87	11.87	16.52	19.74	29.74
D	0.96	1.91	3.74	7.39	11.78	16.61	19.30	29.61
E	0.78	1.96	3.61	8.26	11.17	15.39	20.09	29.22
F	0.96	1.91	4.13	8.70	12.78	16.78	20.22	28.74
Average	0.91	1.91	3.96	8.08	12.09	16.29	20.0	29.41
SD	0.08	0.07	0.23	0.5	0.6	0.5	0.5	0.48
RSD (%)	9.3	7.6	5.8	5.6	5.0	3.1	2.5	1.6
Bias (%)	9.4	4.7	1.1	–1.0	–0.7	–1.8	–0.4	2.0
Extraction (%)	81.8	83.9	83.8	83.4	82.2	83.9	85.1	87.1

Regression equation $H/H_w = 0.023\ (\pm 0.0003)\ C + 0.017\ (\pm 0.004)$; $r = 0.9994$ where C = calculated concentration of ivermectin, H = peak-height of ivermectin, H_w = peak-height of imipramine hydrochloride (internal standard) and r = correlation coefficient.

calculated using the least square method, was $y = 0.023\ (\pm\ 0.0003)x + 0.017\ (\pm\ 0.004)$, $n = 6$. The correlation coefficient (r) of the standard curves (peak height–concentration) was 0.9994. Numerical data for precision and inaccuracy in within-day determinations are collected in Table 1.

To measure recovery, various concentrations of ivermectin were added to plasma and the samples were extracted as described above. The percentage recovery was determined by comparing the peak height of ivermectin extracted from samples with peak height obtained by direct injection of standard solutions.

Recovery was determined by comparing values of peaks height obtained with that achieved from standard solutions of equivalent concentrations of ivermectin and IS. Recovery studies were performed at eight different concentrations of ivermectin, ranging from 1 to 30 ng mL^{-1}. The values found lay between 81.8 and 87.1%, mean 83.9 ± 1.7%.

Concerning the selectivity of the method, the electrophoregrams corresponding to the samples of blank plasma reveal no peak interfering with ivermectin. Typical electrophoregrams of blank plasma sample (A) and plasma obtained from animal number one with a concentration of ivermectin of 10 ng mL^{-1} and imipramine hydrochloride IS 20 μg mL^{-1} (B) are shown in Fig. 1 for pigs. Figure 2 are those for horses. Ivermectin was detected with a migration time of 5.8 min and does not interfere with imipramine hydrochloride IS (time 5.1 min) and with peaks of endogenous constituents from plasma and reagents.

The precision of the assay, calculated as a relative standard deviation (RSD) for inter-assay variability, ranged from 1.6% for 30 ng mL^{-1} to 9.3% for 1 ng mL^{-1}. Inaccuracy of the method for within-day variability ranged from –0.1% for 12 ng mL^{-1} to 6.5% for 2 ng mL^{-1}. Numerical data are shown in Table 1. Table 2 lists precision and inaccuracy in between-day tests. As can be seen, precision ranged from 2.9% for 20 ng mL^{-1} to 9.7% for 2 ng mL^{-1}. Inaccuracy of the method for between-day variability ranged from –0.1% for 12 ng mL^{-1} to 6.5% for 2 ng mL^{-1}.

The limit of detection (LOD) was estimated as follows. All the reagents were added to 0.5 mL of the blank animal plasma, with the exception of ivermectin, and then analysed using the same procedures (described above). Then, from ivermectin-spiked plasma, several successive dilutions were carried out to obtain the lower concentration, giving an instrumental signal three times as large as background noise. In these procedural conditions, using 0.5 mL of pigs and horse plasma, the LOD for ivermectin was estimated at 0.3 ng mL^{-1} ($n = 6$).

The limit of quantification (LOQ), defined as the lowest concentration level at which the assay was validated (precisely and accurately with RSD% and Bias less than 10%), is 1 ng mL^{-1}. The numerical data are given in Table 1.

Stability studies

The stability of spiked plasma samples after 24 h storage at room temperature (20°C), after 2 months of storage at –20°C was evaluated. The effect of freezing and thawing cycles was studied using six parallel samples at two concentrations (12 and 20 ng mL^{-1}). The evaluation of the data was the same as for the long-term stability test. The results indicate that there was

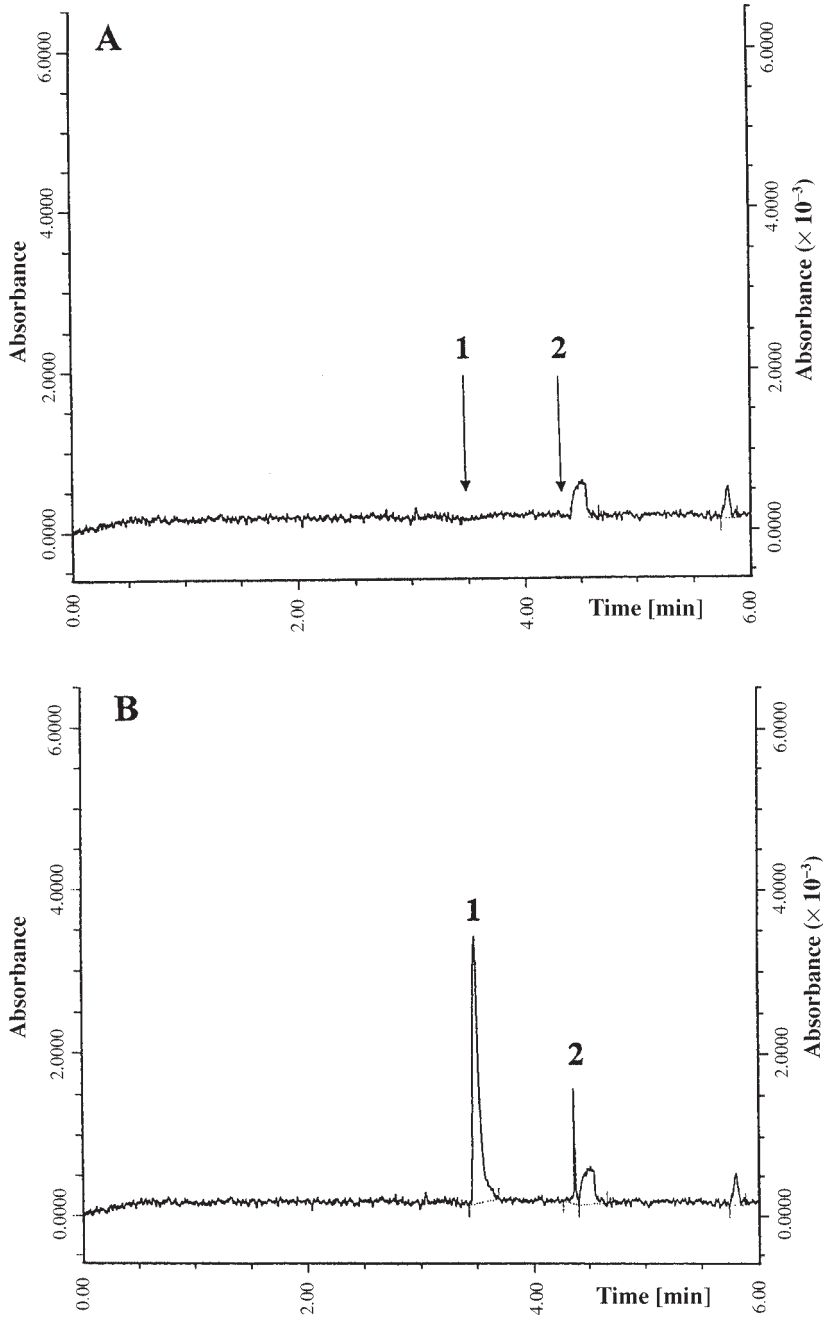

Figure 1. Electrophoregrams obtained from pigs plasma, respectively, of a blank plasma sample (A) and a plasma obtained from animal number one with concentration of imipramine hydrochloride (1) IS 20 μg mL^{-1} and ivermectin (2) 10 ng mL^{-1} (B).

no significant or relevant change (no more than 10%) of concentration of ivermectin during two cycles of freezing and thawing.

Pharmacokinetic studies

The pharmacokinetic parameters, such as peak concentration (C_{max}) and time to maximum concentration (T_{max}) were determined directly from the plasma concentration–time plots for each animal. The half-life time ($T_{0.5}$) was determined as 0.693/K. The area under the plasma concentration versus time curve up to the last time (t), in which concentration of ivermectin was measured (AUC_{0-t}), was determined using Purves' algorithm (Purves, 1992). The $AUC_{0-\infty}$ values were calculated by adding to AUC_{0-t}, the $C_{est} \times K_{el}^{-1}$ value. In order to avoid over- or under-estimation of the extrapolated area, the measured 96 h concentration was

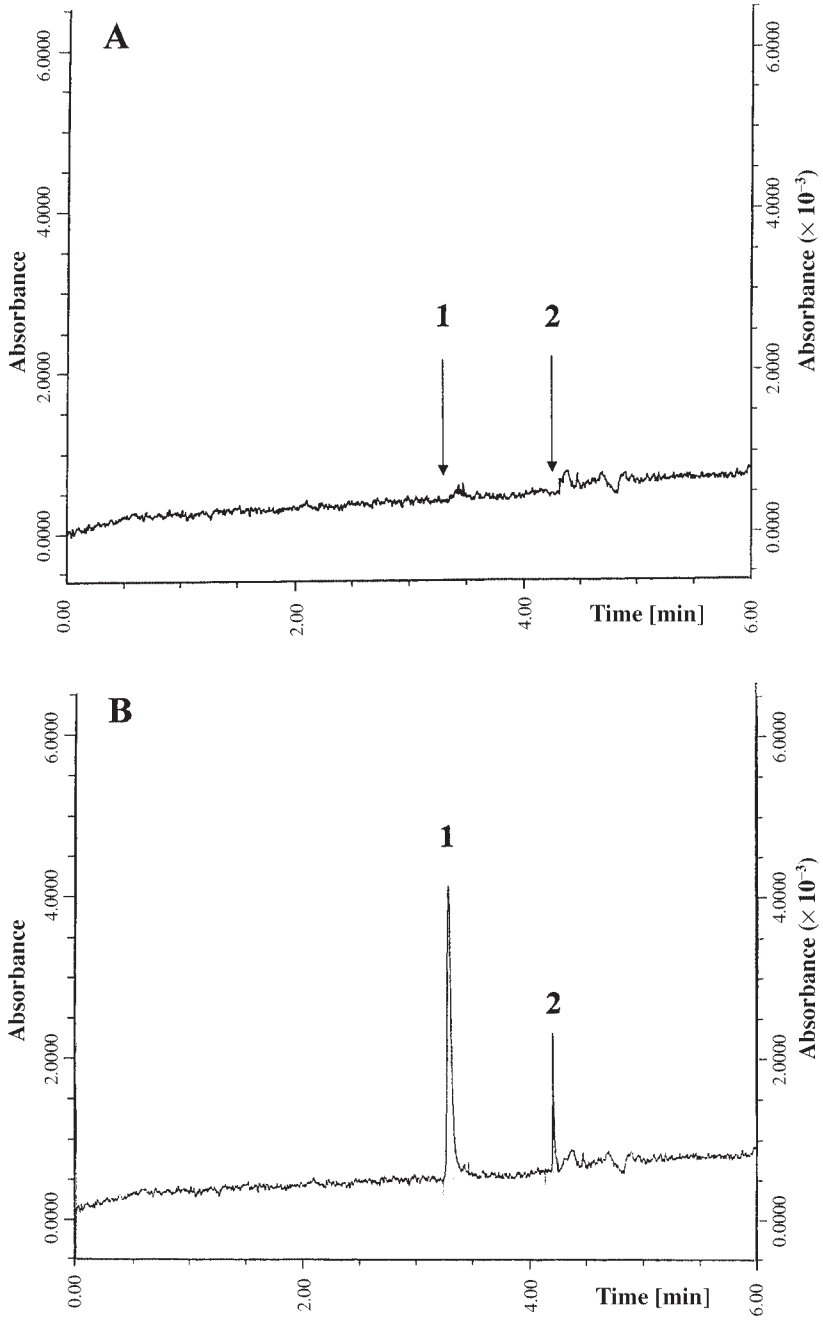

Figure 2. Electrophoregrams obtained from horses plasma, respectively, of a blank plasma sample (A) and a plasma obtained from animal number one with concentration of imipramine hydrochloride (1) IS 20 μg mL^{-1} and ivermectin (2) 10 ng mL^{-1} (B).

replaced by its estimate, which in turn was obtained from log–linear regression line.

The relative clearance (CL/F) was estimated as follows: CL/F = dose/$AUC_{0-\infty}$, in which F, the bioavailability, could not be determinated in the present study. The volume of distribution was obtained from the following expression: dose/($AUC_{0-\infty} \times K$).

Plasma concentration–time curve for each animal was analysed separately to estimate non-compartmental pharmacokinetic variables. Figure 3 shows mean plasma concentration–time curve after intragastric administration of ivermectin in the form Ivomec premix (for pigs) and Eqvalan paste (for horses).

The basic pharmacokinetic parameters are listed in Table 3. The results indicate that the pharmacokinetics of ivermectin did not differ significantly between pigs and horses. Nevertheless, pigs eliminate the drug rapidly, compared with horses. The area under the

Table 2. Precision and inaccuracy between days. Numbers in parentheses denote standard deviations at regression coefficients. *a* = slope, *b* = intercept

	Parameters of calibration curve				Nominal concentration (ng mL^{-1})		
	a	*b*	*r*	*s*	2	12	20
Days					Back calculated concentrations (ng mL^{-1})		
A	0.023 (0.001)	0.013 (0.02)	0.9988	0.01	1.61	12.39	19.65
B	0.024 (0.001)	0.010 (0.02)	0.9986	0.02	2.04	11.65	20.87
C	0.023 (0.0006)	0.018 (0.007)	0.9997	0.007	1.91	12.21	19.78
D	0.024 (0.001)	0.018 (0.02)	0.9987	0.02	1.65	11.17	20.30
E	0.022 (0.0003)	0.020 (0.004)	0.9998	0.004	2.00	11.78	19.22
F	0.023 (0.001)	0.018 (0.01)	0.9989	0.01	1.83	12.52	19.74
G	0.024 (0.001)	0.014 (0.01)	0.9990	0.01	2.17	11.91	21.04
H	0.023 (0.001)	0.016 (0.02)	0.9982	0.02	1.70	12.78	19.91
I	0.023 (0.001)	0.020 (0.02)	0.9985	0.02	1.87	12.52	19.52
J	0.023 (0.001)	0.009 (0.02)	0.9983	0.02	1.96	11.17	20.22
Average					1.87	12.01	20.03
SD					0.18	0.57	0.58
RSD (%)					9.69	4.72	2.91
Bias (%)					6.5	−0.08	−0.15

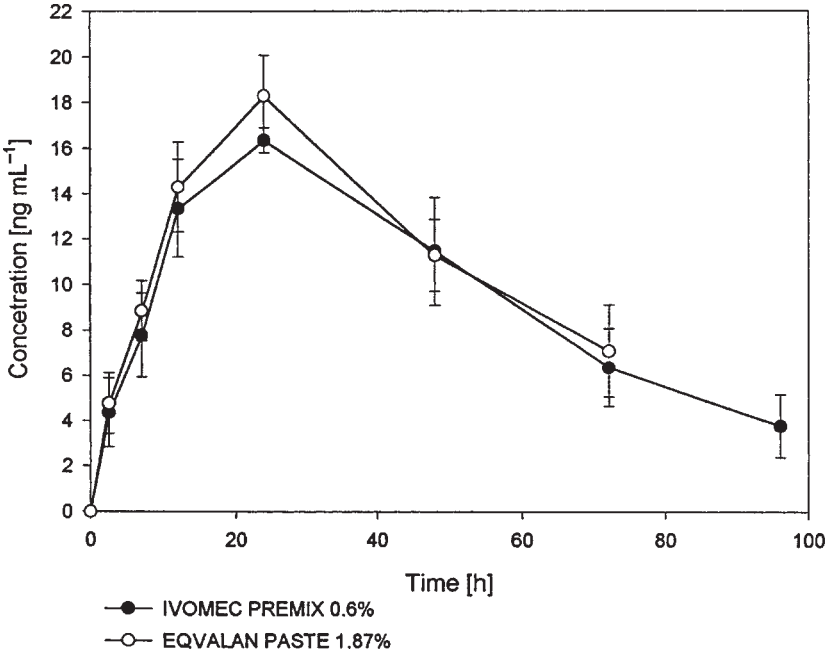

Figure 3. Mean plasma concentration-time curve after intragastric administration of ivermectin in the form Ivomec premix (for pigs) and Eqvalan paste (for horses).

curve up to the last time in which ivermectin was measured was higher in pigs than in horses, although the area under the curve extrapolated to infinity was higher in horses than in pigs. Mean plasma concentration of ivermectin increased to 16.4 ng mL^{-1} (for pigs) (18.3 ng mL^{-1} for horses), with the maximum concentration (C_{max}) appearing precisely 24 h after administration of both species. The volume of distribution at steady state V_{dss} was significantly greater in horses (5.7 L) than pigs (0.34 L). The mean residence time (MRT) and half-life ($T_{0.5}$) of ivermectin were insignificantly greater for horses than for pigs, thus could possibly explain a more prolonged anthelmintic effect. Moreover, this will be dependent upon the relative potency of the formulation of the drug and should be confirmed in efficacy studies. The plasma disposition kinetics of ivermectin in pigs and horses was similar following treatment with both formulations. None of the estimated kinetic

Table 3. Mean pharmacokinetic parameters of ivermectin after intragastric administration at the manufacturer's recommended therapeutic dose of Ivomec premix (0.1 mg kg^{-1} body weight) for pigs and Eqvalan paste (0.2 mg kg^{-1} body weight) for horses.

Parameter	Ivomec premix 0.6% (pigs)	Eqvalan paste 1.87% (horses)
$AUC_{0-\infty}$ (ng h mL^{-1})	1084.2 ± 149.0	1222.2 ± 245.5
AUC_{0-t} (ng h mL^{-1})	931.3 ± 100.0	866.7 ± 88.4
C_{max} (ng mL^{-1})	16.4 ± 0.5	18.3 ± 1.78
T_{max} (h)	24, 24–24	24, 24–24
K_{el} (h^{-1})	0.0283 ± 0.01	0.0225 ± 0.008
$T_{0.5}$ (h)	27.7 ± 9.8	33.5 ± 9.9
V_{dss} (L)	0.34 ± 0.03	5.7 ± 0.5
MRT (h)	54.1 ± 8.2	58.5 ± 12.3

$AUC_{0-\infty}$, area under the curve extrapolated to infinity; AUC_{0-t}, area under the curve up to the last time (t) in which ivermectin was measured; C_{max}, the maximum plasma concentration; T_{max}, the time to reach peak concentration; K_{el}, the apparent elimination rate constant; $T_{0.5}$, the apparent elimination half-life; V_{dss}, volume of distribution at the steady state; MRT, mean residence time.

parameters were statistically different between drug formulations.

CONCLUSIONS

The CE method eliminates many of the problems associated with classical separation techniques. The method has been validated by assessments of a range of factors such as linearity, selectivity, precision, inaccuracy and sensitive. The method uses small quantities of solvent and has a minimal number of steps. In contrast, classical methods for the separation of ivermectin from biological matrixes require large volumes of extracting solvents, multiple extractions and evaporation of large volumes of extracting solvents. The method is applicable for monitoring plasma levels during clinical and pharmacokinetic trials with ivermectin to evaluate its most efficacious dosage regimen. In conclusion, the method is particularly useful for processing large series of blood samples as obtained in extensive pharmacokinetic studies.

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