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## Parvaquone and buparvaquone: HPLC analysis and comparative pharmacokinetics in cattle

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### Summary

A high performance liquid chromatographic (HPLC) method for the determination of the antitheilerial drugs parvaquone and buparvaquone in plasma was developed. Both compounds were extracted from plasma with ether. After evaporating the extracts to dryness the residue was dissolved in methanol and an aliquot was injected onto a column (10 cm × 5 mm, i.d.) of ODS-Hypersil (5 µ) with a mobile phase of 0.05 M – Na acetate buffer (pH 3.6) – methanol (15:85, v/v). Detection was at 252 nm. The mean recovery for both compounds was about 92%. This method was used to elucidate their pharmacokinetics in 6 calves after intramuscular administration. The maximum plasma concentration for parvaquone was  $6.36 \pm 0.58$  µg/ml after  $0.84 \pm 0.08$  h. The corresponding values for buparvaquone were  $0.102 \pm 0.030$  µg/ml and  $3.17 \pm 0.39$  h, respectively. The decay in plasma concentrations for the two drugs was biexponential and the terminal elimination half lives were  $11.12 \pm 1.63$  h and  $26.44 \pm 2.81$  h for parvaquone and buparvaquone, respectively.

**Key words:** parvaquone; buparvaquone; HPLC; pharmacokinetics; cattle.

### Introduction

*Theileria parva* infection in cattle (East Coast fever, ECF) causes high morbidity and mortality each year in many parts of Africa due to lack of effective drugs. Some novel hydroxynaphthoquinone compounds have been reported to exhibit remarkable efficacy against *Theileria* and other protozoon parasites including *Plasmodia* and *Eimeria* species (McHardy et al., 1976; Boehm et al., 1981; McHardy et al., 1983; Dolan et al., 1984; Hudson et al.,

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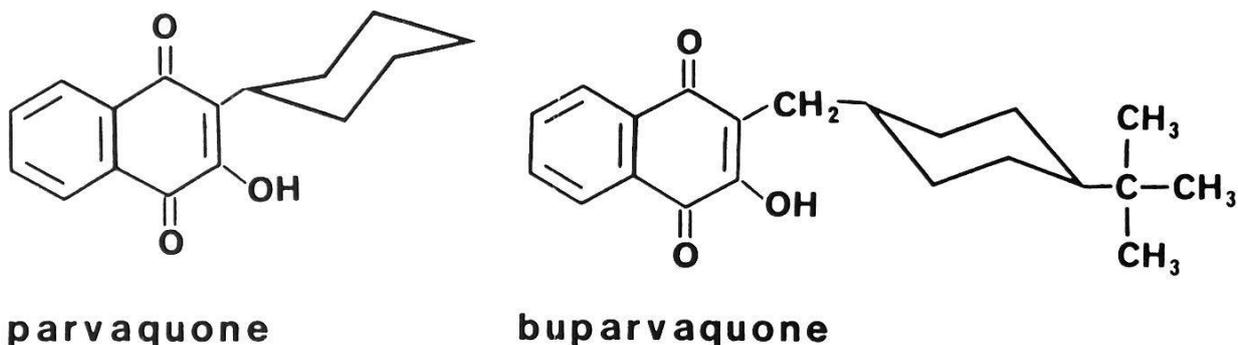


Fig. 1. Chemical structures of parvaquone and buparvaquone.

1985). One of these compounds, parvaquone (PVQ) (Clexon, Coopers Animal Health Ltd., England), has already been introduced in the field for treatment of ECF. In naturally occurring ECF, PVQ has been shown to provide a recovery rate of about 80% (Mbwambo et al., 1987) to 90% (Chema et al., 1986). Another hydroxynaphthoquinone derivative which has been found recently to be even more potent than PVQ against *Theileria* is buparvaquone (BPQ) (BW720C., McHardy et al., 1985; Minami et al., 1985). BPQ was synthesised as part of a programme to find a compound which is metabolised more slowly than PVQ and it is probable that it may also be marketed soon. Fig. 1 shows the chemical structure of PVQ and BPQ. Information on the pharmacokinetics of these compounds is limited (McHardy and Mercer, 1984) and such studies would be valuable in selecting suitable dosage regimens.

In this study we developed an analytical method suitable for the determination of PVQ and BPQ in plasma and evaluated their pharmacokinetics in cattle using this method.

### Materials and Methods

*Cattle.* Twelve experiments were carried out in six clinically healthy *Bos taurus*, mixed breed calves weighing 120 to 240 kg. They were fed hay and concentrates and had free access to water.

*Drugs.* PVQ and BPQ as pure compounds for standard solutions and as injectable preparations for the animal experiments were kindly gifted by Coopers Animal Health Ltd., England. PVQ (Clexon) and BPQ (Buparvaquone) were formulated as solutions for injection containing, respectively, 15% and 5% of the active ingredient in a mixture of organic solvents and emulsifiers.

*Administration and sampling.* The drug treatments were performed in a crossover design, allowing a minimum period of 16–30 days between treatments. All drugs were administered into the neck muscles. PVQ was administered at a dose of 20 mg/kg and since all the injection volumes were more than 20 ml half was injected into each side of the neck. BPQ was given at a dose of 2.5 mg/kg. Blood samples were drawn from the jugular vein over a period of 0–54 h. Plasma was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  till analysed.

*Analytical method.* The HPLC system consisted of a solvent delivery pump (Gilson Model 802, Scotlab Instrument Sales Ltd., Strathclyde) connected to an ODS-Hypersil ( $5\ \mu$ ) column (10 cm  $\times$  5 mm, i.d.) and a UV-detector (Model CE 2012, Cecil Instruments, England) operated at 0.05 absorbance units full scale. The effluent was monitored at 252 nm. The mobile phase was 0.05 M – Na acetate buffer (pH 3.6) – methanol (15:85; v/v) pumped at a flow rate of 0.8 ml/min for PVQ and 1.2 ml/min for BPQ.

Sample preparation was done by ether extraction. To 4 ml of plasma, 2 ml of water was added followed by 25 ml of ether. The mixture was shaken on a rotary mixer for 15 min and then on a vortex mixer for 30 sec. Twenty millilitres of ether were transferred to a 50 ml glass tube, a further 25 ml of ether added to the sample and shaken for 15 min. Twenty five ml were removed and pooled with the first extract and evaporated at 50° C under nitrogen to a volume of 5 ml which was then transferred to a 10 ml conical tube and evaporated to dryness. The walls were rinsed with ether and the washings evaporated again to dryness. A minimum of 200 µl of methanol were added to the residue and sonicated for 30 sec and an aliquot (20 µl) was injected on to the column. Samples with high drug concentrations were diluted with methanol.

*Quantitative evaluation.* Recoveries of analytes from spiked plasma were determined by reference to peak heights resulting from direct injection of methanolic standards.

The concentration of both drugs in actual plasma samples were calculated by reference to spiked plasma samples taken through the analytical procedure.

*Pharmacokinetic analysis.* The plasma drug concentration versus time data obtained for each calf were individually analysed by a curve stripping computer programme CSTRIP (Sedman and Wagner, 1976). The experimental constants derived from this were then used to calculate the pharmacokinetic parameters (Baggot, 1977).

*Statistical analysis.* The significance of the difference between mean values was assessed by the Student's t-test for paired data and a  $P < 0.05$  was considered significant. The results are expressed as mean  $\pm$  SEM.

## Results

*Chromatograms.* PVQ and BPQ were eluted with symmetrical peaks and without interference from endogenous compounds. PVQ had a retention time of 3.2 min with a capacity factor of 1.5. The corresponding values for BPQ were 5.8 min and 5.4. Typical chromatograms are shown in Fig. 2.

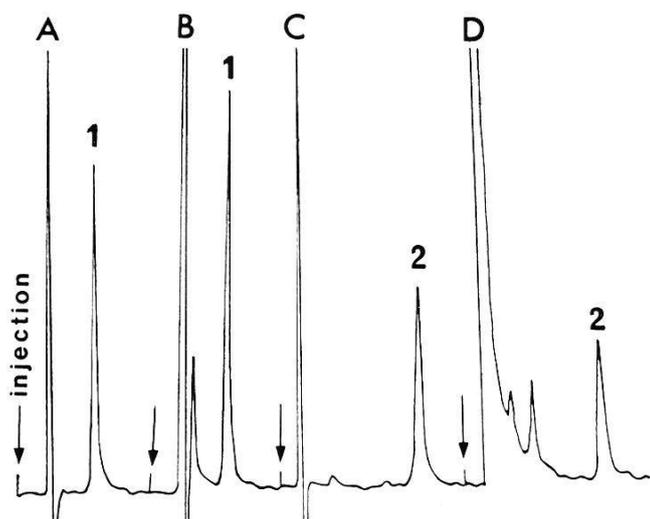


Fig. 2. Typical chromatograms obtained using the method on samples from calves dosed with clexon and buparvaquone injection. A = parvaquone standard, 2 µg/ml, B = plasma at 6 h after administration of clexon, C = buparvaquone standard 5 µg/ml, D = plasma at 4 h after administration of buparvaquone injection. Peaks: 1 = parvaquone, 2 = buparvaquone.

Table 1. Recovery of parvaquone from spiked bovine plasma

Spiked concentration µg/ml	Number of assays	Measured concentration µg/ml	Recovery %
0.10 .....	4	0.094±0.001	94.25
0.20 .....	4	0.195±0.002	97.64
0.25 .....	3	0.238±0.006	95.40
0.50 .....	4	0.43 ±0.01	86.13
1.0 .....	3	0.96 ±0.05	95.96
2.0 .....	4	1.72 ±0.01	86.16
4.0 .....	4	3.57 ±0.08	89.24
Mean±SEM .....			91.56±0.99

Table 2. Recovery of buparvaquone from spiked bovine plasma

Spiked concentration µg/ml	Number of assays	Measured concentration µg/ml	Recovery %
0.05 .....	3	0.048±0.002	96.01
0.20 .....	7	0.171±0.006	90.22
2.0 .....	4	1.80 ±0.09	90.16
4.0 .....	5	3.59 ±0.16	91.12
Mean±SEM .....			91.88±0.64

*Statistical validation and sensitivity.* Tables 1 and 2 show the recoveries of PVQ and BPQ, respectively, from the replicate samples. The calibration curves were rectilinear ( $r = 0.99$ ) over the concentration ranges studied (0.05–4.0 µg/ml). Recovery for PVQ from plasma was  $91.56 \pm 0.99\%$  ( $n = 26$ ) and within and between day coefficients of variation were 5.52 and 4.31%, respectively. BPQ had a similar recovery of  $91.88 \pm 0.69\%$  ( $n = 19$ ) and within and between day coefficients of variation were 3.04 and 8.81%, respectively. The limit of detection for both compounds was 0.015 µg/ml.

*Plasma concentration time profiles.* The curves of PVQ and BPQ concentrations in plasma as a function of time, plotted on arithmetic coordinates are shown in Fig. 2. The maximum plasma concentration ( $C_{\max}$ ) for PVQ was  $6.36 \pm 0.58$  µg/ml and this was rapidly attained after  $0.84 \pm 0.08$  h ( $t_{\max}$ ) whereas  $C_{\max}$  for BPQ was only  $0.102 \pm 0.030$  µg/ml at  $t_{\max} = 3.27 \pm 0.39$  h. The  $C_{\max}$  values for BPQ showed a wider intersubject variation (0.050–0.208 µg/ml) and appeared to be scattered ranging from  $t_{\max}$  of 2–4 h. At 54 h after drug administration, concentrations of PVQ in plasma were still much higher ( $0.04 \pm 0.006$  µg/ml,  $n = 3$ ) than those of BPQ ( $< 0.015$  µg/ml,  $n = 3$ ).

Table 3. Disposition kinetics of parvaquone in calves (n = 6) after intramuscular administration of clexon (20 mg/kg)

Parameter units	Calf No.						Mean±SEM
	A	B	C	D	E	F	
C <sub>max</sub> ..... µg/ml	4.89	6.16	7.56	8.32	4.99	7.41	6.36±0.58
t <sub>max</sub> ..... h	1.02	1.0	0.97	0.52	0.80	0.77	0.84±0.08
K <sub>ab</sub> ..... h <sup>-1</sup>	2.66	2.01	2.89	2.86	2.30	2.95	2.61±0.15
t <sub>1/2</sub> K <sub>ab</sub> ..... h	0.27	0.35	0.23	0.25	0.30	0.23	0.27±0.05
α ..... h <sup>-1</sup>	0.269	0.714	0.401	0.692	0.595	0.578	0.54±0.07
t <sub>1/2</sub> α ..... h	2.58	0.97	1.73	1.0	1.17	1.20	1.44±0.25
β ..... h <sup>-1</sup>	0.083	0.037	0.079	0.077	0.063	0.064	0.067±0.007
t <sub>1/2</sub> β ..... h	8.30	18.93	8.77	8.93	10.98	10.82	11.12±1.63
Vd area/F ..... l/kg	6.22	19.25	6.54	7.92	13.83	11.86	10.92±2.07
AUC ..... µg · h/ml	38.73	28.08	38.72	32.80	22.95	26.35	31.27±2.69
Clb/F ..... ml/kg/h	516	712	517	610	872	759	664±58

Table 4. Disposition kinetics of buparvaquone in calves (n = 6) after intramuscular administration of buparvaquone injection (2.5 mg/kg)

Parameter units	Calf No.						Mean±SEM
	A	B	C	D	E	F	
C <sub>max</sub> ..... µg/ml	0.083	0.050	0.064	0.077	0.208	0.188	0.102±0.030
t <sub>max</sub> ..... h	4.12	2.02	2.08	3.93	2.93	3.93	3.17±0.39
K <sub>ab</sub> ..... h <sup>-1</sup>	1.86	4.24	2.29	0.92	1.0	0.67	1.83±0.54
t <sub>1/2</sub> K <sub>ab</sub> ..... h	0.37	0.16	0.30	0.75	0.70	1.03	0.55±0.13
α ..... h <sup>-1</sup>	0.113	1.73	0.095	0.202	0.733	0.227	0.52±0.26
t <sub>1/2</sub> α ..... h	6.15	0.40	7.30	3.43	0.95	3.05	3.55±1.12
β ..... h <sup>-1</sup>	0.026	0.034	0.031	0.019	0.022	0.035	0.028±0.003
t <sub>1/2</sub> β ..... h	26.92	20.72	22.58	37.35	31.42	19.67	26.44±2.81
Vd area/F ..... l/kg	27.24	51.42	55.62	37.81	18.33	15.87	35.38±6.84
AUC ..... µg · h/ml	3.53	1.43	1.45	3.48	6.20	4.50	3.43±0.74
Clb/F ..... ml/kg/h	708	1748	1724	718	403	556	976±245

*Disposition kinetics.* The decay in plasma concentration for the two drugs in all the calves was biphasic. The disposition kinetics are presented in Tables 3 (PVQ) and 4 (BPQ). There were no significant differences in the absorption rates (t<sub>1/2</sub> K<sub>ab</sub>) and the distribution rates (t<sub>1/2</sub> α) between the two drugs. The elimination half life (t<sub>1/2</sub> β) for PVQ ranged from 8.30–18.93 h with an average of 11.12 h (Table 3). This was significantly lower than that for BPQ which ranged from 19.67–37.35 h and averaged 26.44 h (Table 4).

In Tables 3 and 4 the apparent volume of distribution (Vd area) and total body clearance (Clb) are given with the fraction absorbed (F) as denominator since the intravenous route was not used.

## Discussion

The method described in the present study is considered simple and sufficiently sensitive for the determination of PVQ and BPQ in bovine plasma. From Fig. 2 it is evident that the compounds were chromatographed with symmetrical peaks and without interference. The eluent composition and the different flow rates used were designed as a compromise for optimal retention times, sensitivity and resolution from endogenous plasma compounds. The recoveries of both drugs from spiked plasma were similar and high (92%) and reproducible as indicated by the low coefficients of variation. The suitability of the method was demonstrated by the plasma concentration versus time profiles obtained after administration of the drugs to calves. Plasma concentrations for PVQ and BPQ could be measured and compared for up to 54 h after drug administration.

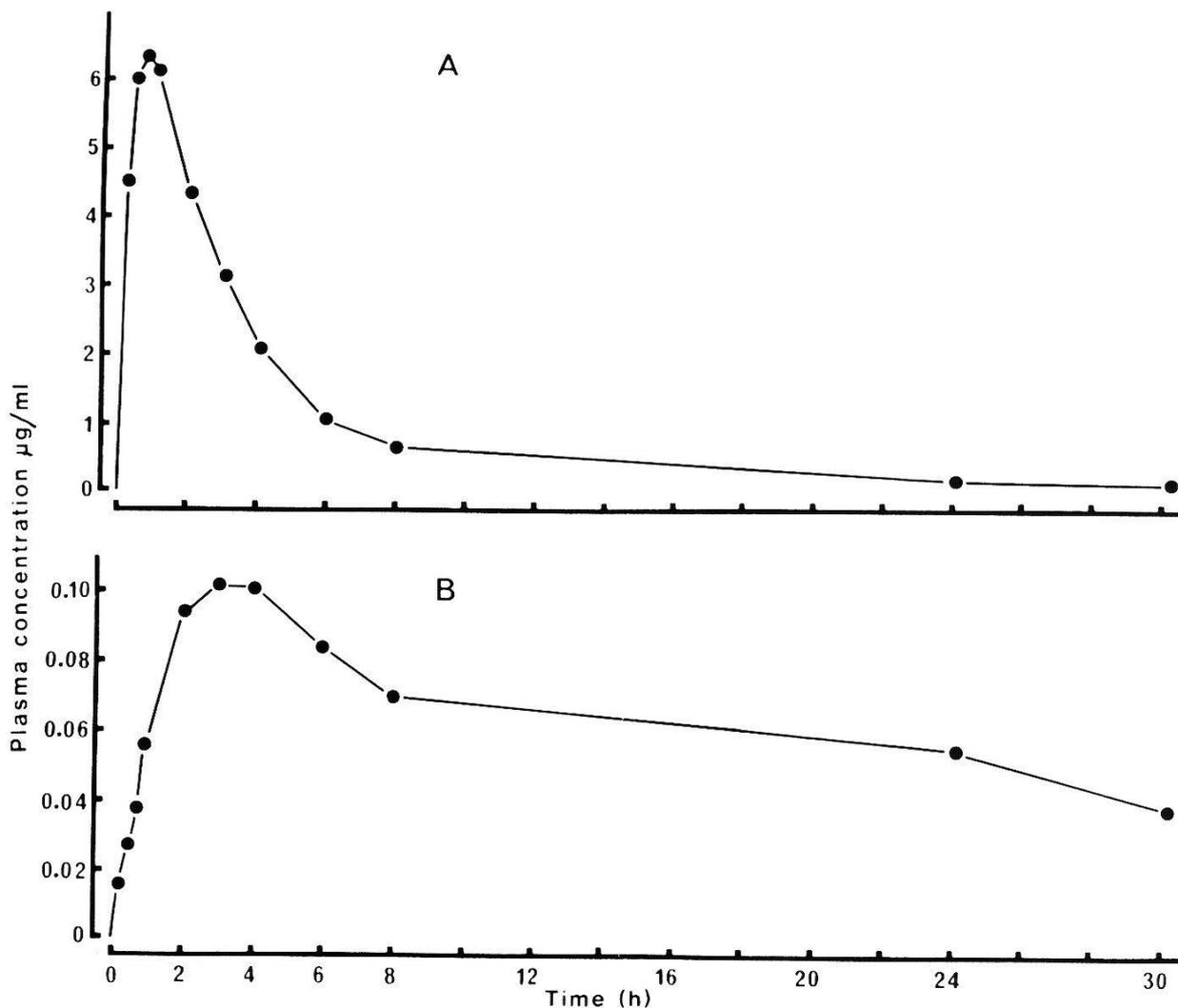


Fig. 3. Plasma concentration-time profiles of parvaquone (A) and buparvaquone (B) after intramuscular administration to calves. Each point represents the mean of 6 animals.

Fig. 3 shows that  $C_{\max}$  for PVQ is about 60 times higher than the  $C_{\max}$  for BPQ and it is attained 3 times faster. While the difference in  $C_{\max}$  between the 2 drugs may be attributed to the different doses, the difference in  $t_{\max}$  may be explained by their structural differences (Fig. 1). The butyl group at the 4-position on the cyclohexyl ring in BPQ was designed primarily to prevent metabolic deactivation (Hudson et al., 1986; McHardy et al., 1985). Because there were no significant differences in the absorption and distribution rates between the 2 drugs (Tables 3 and 4) it seems that the longer  $t_{\max}$  for BPQ is a reflection of a slower metabolic rate. This is in good agreement with the longer elimination half life for BPQ compared to that for PVQ (Tables 3 and 4). The shorter elimination half life for PVQ may be due to rapid excretion and metabolism which has been described (Fieser et al., 1948) to involve hydroxylation of the cyclohexyl ring at the 4-position (Fig. 1).

From Tables 3 and 4 it will be seen that values for some of the pharmacokinetic parameters for the drugs showed wide variations between subjects. The intersubject variation of AUC was small whereas variability in the elimination half life, apparent volume of distribution/F and total body clearance/F were of a wider range. Also  $C_{\max}$  values for BPQ showed a wide variation between individuals. Factors which may account for this variability may be related to the area of absorptive surface of the injected volume, the drug formulation and individual variations in eliminating the drugs (MacDiarmid, 1983).

The concentration-activity relationships for PVQ and BPQ in vivo have not been defined. Using a bioassay method, PVQ in cattle after intramuscular injection of 20 mg/kg has been found to attain maximum serum concentrations of about 1  $\mu\text{g/ml}$  3 h after administration and remained above the  $\text{EC}_{50}$  against *T. parva* (0.006  $\mu\text{g/ml}$ ; the concentration of drug required to reduce 50% the proportion of cells containing schizonts) for about 48 h (McHardy and Mercer, 1984). The activity of BPQ against *T. parva* ( $\text{EC}_{50} = 0.0003 \mu\text{g/ml}$ ) in cattle at a dose rate of 2.5 mg/kg, has been demonstrated in plasma by bioassay for a longer period of up to 10 days (McHardy and Wekesa, 1984). According to McHardy et al. (1985) BPQ has been found to be about 20 times more active than PVQ in vitro and to provide a higher recovery rate (100%) than PVQ (90%) in cattle experimentally infected with *T. parva*. In their clinical trial they used the same doses employed in the present work. The recovery rates of about 80% (Mbwambo et al., 1987) to 90% (Chema et al., 1986) reported for PVQ in naturally occurring ECF were achieved with 2 doses of 10 mg/kg given 48 h apart.

These findings, taken together with the data obtained in this study which demonstrate the pharmacokinetic basis of the superiority of BPQ (McHardy et al., 1985), show that BPQ should be a better choice of drug in the treatment of ECF. Also the finding that BPQ offers some prophylactic activity against both *T. parva* and *T. annulata* infections (McHardy and Wekesa, 1984; Dhar et al., 1987) makes it more promising than PVQ.

## Acknowledgments

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