Disposition of mefloquine and enpiroline is highly influenced by a chronic Schistosoma mansoni infection

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Running title: Disposition of arylmethanols in S. mansoni infection

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Abstract

Chronic *Schistosoma mansoni* infections lead to severe tissue destructions of the gut wall and liver and can influence drug disposition. This study aimed to investigate the impact of a chronic *S. mansoni* infection on pharmacokinetic (PK) parameters of two promising antischistosomal lead candidates (mefloquine and enpiroline) in mice. Studies were conducted in two different mouse cohorts (*S. mansoni*-infected and non-infected mice) for both drugs. Plasma samples were collected after oral treatment (200 mg/kg) with study drugs at various time points. An HPLC method was validated to analyze enpiroline and mefloquine in plasma. Livers and intestines were collected from infected animals to determine the onset of action, hepatic shift and worm burden reductions. Following mefloquine administration hepatic shifting and significant worm burden reductions (79.2%) were observed after 72 h. The majority of worms had migrated one week post treatment with enpiroline to the liver and significant worm burden reductions were observed (93.1%). The HPLC-method was selective, accurate (87.8 - 111.4%) and precise (<10%) for the analysis of both drugs in plasma samples. PK profiles revealed increased half-lives and AUCs for both drugs in infected animals when compared to uninfected animals. Considerable changes were observed for mefloquine with a 5-fold increase of t\(_{1/2}\) (182.7 h versus 33.6 h) and 2-fold increase of AUC (1116517.8 ng*h/mL versus 522409.1 ng*h/mL).

*S. mansoni* infections in mice influence the PK profiles of enpiroline and mefloquine leading to delayed clearance. Our data confirm that drug disposition should be carefully studied in schistosomiasis patients.
Introduction

Schistosomiasis is one of the most important neglected tropical diseases. Based on latest estimates, 780 million people are at risk of an infection with schistosomes, with the majority of cases in sub-Saharan Africa. Schistosomiasis is a chronic disease caused by the immunological response to eggs trapped in tissue and organs. For example, in the case of *Schistosoma mansoni*, eggs get mainly trapped in the gut wall so as liver leading to severe tissue destructions, hepatosplenomegaly, periportal liver fibrosis, and portal hypertension.

Treatment with praziquantel is the mainstay of control and widely used in preventive chemotherapy programs. The liver is one of the main organs in charge of drug metabolism and the gut wall is significantly involved in the absorption process of drugs. It is therefore not surprising that pharmacokinetic (PK) changes occur in patients with schistosomiasis, depending on the extent of disease. For example, a study in Egyptian schistosomiasis patients treated with praziquantel demonstrated that PK parameters increased proportional to the degree of hepatic insufficiency. In addition, PK studies undertaken with praziquantel in *S. mansoni*-infected mice documented elevated plasma levels in infected animals compared to non-infected mice. Furthermore, the infection resulted in significant inhibition of microsomal cytochrome P450 activities compared with activities in non-infected mice.

The endemic areas of schistosomiasis and other infectious diseases such as malaria and soil-transmitted helminths overlap geographically. Co-endemicity as well as known similarities of both parasites such as hemoglobin digestion were the key rationales for in-depth studies on the potential antischistosomal effects of antimalarials. In the past years, the antimalarial mefloquine emerged as promising lead structure. The drug presented remarkable *in vitro* as well as *in vivo* activities against major schistosome species and high egg reduction rates in combination with artesunate in an exploratory clinical trial in *S. haematobium*-infected school-aged children. Recently, a clinical trial in pregnant malaria-infected women revealed a positive effect of mefloquine on *S. haematobium* co-
infections.(14) A population PK study of mefloquine observed altered PK profiles in malaria-
infected persons compared to uninfected people (personal communication Piero Olliaro).
Nevertheless, the impact of a schistosome infection on the drug disposition of mefloquine
has not been studied to date.

The aim of the present study was to investigate the PK parameters of mefloquine and
enpiroline in the chronic S. mansoni mouse model. Enpiroline was included in this study
since recent in vitro and in vivo studies on mefloquine related arylmethanols identified
enpiroline as promising lead candidate with excellent activity on S. mansoni so as S. haematobium.(12) Drug plasma levels were analyzed between 1 and 168 h after oral
treatment with mefloquine and enpiroline. For that purpose we adapted a previously
established HPLC- UV method (15), re-validated the method for mouse plasma samples and
expanded it for the quantification of enpiroline. Finally we determined the onset of action of
both drugs following administration to infected animals.

Material and Methods

Drugs and Chemicals:

Mefloquine ((2,8-Bis(trifluoromethyl)quinolin-4-yl)(-piperidin-2-yl)methanol) was kindly
provided by Cilag AG (Switzerland). Two mefloquine metabolites, one human metabolite (Ro
21-5104) and one animal metabolite (Ro 14-0518) were gifts of Roche (Switzerland). WRAIR
(Washington, USA) kindly provided us with enpiroline (threo-α-(2-piperidyl)-2-trifluoromethyl-
6-(4-trifluoromethylphenyl)-4-pyridinemethanol). Chlorpromazine (2-Chlor-10-(3-
dimethylaminopropyl) phenothiazin) was purchased from Sigma Aldrich (Switzerland).
Mefloquine and enpiroline were dissolved in DMSO and chlorpromazine in ethanol (10
mg/mL stock solutions). All analytes are depicted in Figure 1. Methanol (Sigma Aldrich) and
acetonitrile (Biosolve BV, Netherlands) were purchased in HPLC-grade. Monobasic
potassium phosphate and phosphoric acid (85%) were acquired from Sigma Aldrich.

Animals and Parasites:
Animal studies were carried out at the Swiss Tropical and Public Health Institute (Basel, Switzerland) under protocols approved by Swiss national and cantonal animal welfare regulations. Three-week old (weight 14 g), female NMRI mice (n=119) (Charles River, Sulzfeld, Germany) were allowed to adapt in the animal facilities for one week under controlled conditions (temperature ca. 22 °C; humidity ca. 50 %; 12-hour light and 12-hour dark cycle; free access to rodent diet and water) before infection. Half of the mice (infected cohorts) were subcutaneously infected with 100 S. mansoni (Liberian strain) cercariae following standard procedures of our laboratory. Animals were then left under controlled conditions for 7 weeks to establish an early stage chronic schistosome infection with visible impairment of liver (granulomatous tissue) and gut (swelling, inflammation). The remainder of the mice (uninfected cohorts) was likewise kept under controlled conditions for 7 weeks.

Pharmacokinetic studies:
Pharmacokinetic studies were conducted in two different mouse cohorts for both drugs, one in S. mansoni-infected and a second in non-infected NMRI mice. Oral formulations of enpiroline and mefloquine were prepared 3 h before treatment in 10% vol/vol Tween/Ethanol and 90% vol/vol water. Groups of mice (n=3 per group) were treated orally with 200 mg per kg bodyweight of enpiroline and mefloquine by gavage and sacrificed by the CO2 method at selected time points post-treatment (1, 2, 4, 8, 12, 24, 48, 72 or 168 h). Whole blood samples of 0.5-1 mL were collected by cardiac puncture of each mouse. Each cohort had untreated control mice (n= 8 infected, n= 3 non-infected) and each of the four treatment arms (MFQ: infected/non-infected; EP: infected/non-infected) consisted of 27 mice, 3 mice per sampling.
timepoint. Blood samples were collected into lithium-heparin coated microtainers (Sarstedt) and centrifuged to obtain plasma samples which were stored at -80 °C until analysis.

Studies on the onset of antischistosomal action in mice:
The onset of action was determined at different time points following treatment with single oral doses of enpiroline and mefloquine administered to mice (n = 3 per group). After each PK sampling point (1, 2, 4, 8, 12, 24, 48, 72 or 168 h post treatment) liver and gut including the portal vein and mesenterial veins were dissected from infected mice. Livers were pressed and examined under the microscope, all worms were sexed and counted.(16) In addition, worms within the portal vein and mesenterial vein system were picked and counted separately as described before.(16) Worm burdens (average number of alive worms) of treated mice were compared to control (non-treated) animals (average number of alive worms) and reductions of worm burden (reduction of alive worms) calculated for each time point. Furthermore the onset of action, visible as migration of worms from the mesenteric veins to the liver was determined based on the distribution of worms (alive and dead) within portal and mesenterial veins and pressed livers (hepatic shift).

HPLC-UV analysis and sample extraction:
A validated high performance liquid chromatographical analytical method introduced by Lai and colleagues (15) for mefloquine in human plasma was used. The method was adapted in order to detect mefloquine and enpiroline simultaneously under similar conditions. Important validation parameters such as selectivity, intra and inter-day-accuracy and precision, bench-top stability and extraction recovery were determined according to the bioanalytical method validation guidance for industry by the Food and Drug Administration (FDA).(17) Plasma samples (100 μL) were processed using protein precipitation with methanol containing internal standard (Chlorpromazine, conc: 5 μg/mL). Each mouse sample was extracted three times on three different days. Samples were vortex mixed and centrifuged
(Eppendorf Centrifuge 5415C) at 10000 g for 10 min. The supernatant was transferred to a microtube and evaporated to dryness in a Speedvac (Labcanco) at 38 °C for 2 h. The residue was reconstituted in 100 µL acetonitrile: 0.05 M phosphate buffer (1:1) solution and transferred to an autosampler vial.

Analyte working solutions were prepared by serial dilution in acetonitrile: KH₂PO₄ buffer (0.05M) (1:1) to final concentrations of 4, 8, 20, 40, 80, 200, 400 and 800 µg/mL. Calibration curves were established by diluting analyte working solutions with blank mouse plasma (purchased from DUNN Technik, Germany) (1:20, V_total = 100 µL). Each calibration set consisted of one blank plasma sample (plasma sample processed without internal standard (IS)), one zero sample (plasma sample spiked with IS) and 8 calibration samples (0.2, 0.4, 1, 2, 4, 10, 20 and 40 µg/mL). The lowest and highest calibrator corresponded to the lower (LLOQ) and upper limit (ULOQ) of quantification, respectively. The LLOQ was selected as the minimal concentration in plasma samples, which could be analyzed with a precision of ≤20% (CV%) and accuracy between 80% and 120% (signal: noise ratio > 5:1).

In addition, quality control (QC) samples (n=6) were prepared at low (0.75 µg/mL), medium (7.5 µg/mL), and high (30 µg/mL) concentrations covering the entire calibration range. Selectivity was determined by examination of blank mouse plasma obtained from different origins (non-infected NMRI mice and infected mice without treatment (n=6 each) and commercially acquired mouse plasma) for interferences with endogenous substances using the above-described extraction procedure but without adding the IS working solution.

Accuracy and precision of the method was evaluated by analyzing QC samples (n = 6 per concentration). The intra- and inter-day accuracy/precision was determined within a single run and between different assays (n = 3), respectively. Freshly prepared calibration standards were used for analyses. The precision was calculated using the coefficient of variation (CV [%]). The accuracy represented the measured concentration as fraction of the nominal concentration in percentage. A precision of ±15% (LLOQ: ±20%) and accuracy between 85% and 115% (LLOQ: 80–120%) was accepted in our study.
Relative recoveries (RRE) of mefloquine and enpiroline were determined by comparing the absolute peak areas of blank plasma samples spiked before and after the extraction at 0.75, 7.5, and 30 µg/mL (n = 5 per concentration). Matrix effects of mefloquine and enpiroline were assessed as the ratio of the absolute peak areas of blank plasma samples spiked after the extraction to the absolute peak areas of the analytes solved in a mixture of acetonitrile-

KH$_2$PO$_4$ Buffer (1: 1, v/v).

Autosampler stability and extended benchtop stability studies were included in our method validation. QC samples were used to test stabilities under different conditions. Autosampler stability was evaluated by the analysis of QC samples (n = 6 per concentration) over a period of 72 h. Extended benchtop stability was evaluated after samples were kept at benchtop conditions for 8 h. The concentrations of these samples were compared with concentrations of freshly prepared QC samples. The drug solutions were considered as stable with a deviation of not more than ±15% and ±20% at the LLOQ.

For the HPLC-UV detection an aliquot (50 µL) of each sample was injected onto an Inertsil 8-3 analytical column (2.1 mm × 30 mm, 3.5 µm, GL Sciences, Japan). Separation was carried out using an isocratic elution method with a mobile phase consisting of 35% methanol, 25% acetonitrile and 40 % KH$_2$PO$_4$ buffer (0.05M) adjusted to pH 3.9 with 0.05% phosphoric acid. Flow rate was set at 1 mL/min and increased to 1.5 mL/min from 11 min onwards. UV signals were detected at 284 nm. The HPLC system Agilent 1100 series (Agilent Technologies Inc), consisted of binary pumps, micro vacuum degasser, analytics autosampler, column heater (T=25°C) and UV-Vis detector.

Pharmacokinetic Analysis and Statistics:

Analytical raw data (AUC) of plasma samples were processed with help of the ChemStation Software (Agilent). Average values as well as calibration lines and animal data were calculated using Microsoft Excel 2007. Statsdirect (Statsdirect Ltd., Cheshire, UK) was used to determine whether worm burden reductions were significant (Kruskal-Wallis-Test). The mean value from the three animals at each time point was plotted against time to give
plasma concentration time profiles. PK parameters of mefloquine and enpiroline were determined by non-compartmental analysis using WinNonLin (Version 5.2, Pharsight Corporation, USA). The following parameters are presented here: maximal plasma concentration (c_{\text{Max}}; \text{ng/mL}); time to achieve maximal plasma concentration (t_{\text{max}}; \text{h}); area under the plasma concentration-time profile (AUC_{0-168h}; \text{ng}*\text{mL/min}); area under the plasma concentration-time profile extrapolated to infinity (AUC_{0-\infty}; \text{ng}*\text{mL/min}); and terminal elimination half-life (t_{\text{1/2}}; \text{h}). T_{\text{max}} and c_{\text{max}} are the observed data from the mean concentration time curve. The terminal elimination rate constant is the first order rate constant associated with the terminal elimination phase. It is estimated via linear regression of time vs. log concentration. The extrapolated times and areas after the last data point are extrapolated to infinity. For any of the AUC calculation methods, the AUC rule after t_{\text{last}} is the log rule.

Results

Studies on the onset of antischistosomal action of enpiroline and mefloquine in mice:

We studied the worm distribution 1 h to 168 h after treatment. Following mefloquine administration schistosomes started to migrate into the liver (“hepatic shift”) 8 - 12 h post treatment. Approximately half of the worms (live and dead) were found within the liver 1-2 days post-treatment until nearly all worms (86.5%) were observed in the liver from day 3 onwards (Figure 2). A worm burden reduction of 79.2% was calculated 72 h post-treatment, while a worm burden reduction 46.9% was determined for the group of mice (n=3) 1 week post-treatment with mefloquine.

Worms shifted slowly into the liver following enpiroline treatment and an equal distribution between liver and mesenterial veins was reached 72 h post-treatment. The majority of worms had migrated to the liver 1 week post-treatment. Enpiroline evolved its full antischistosomal activity 1 week post treatment revealing a significant worm burden reduction of 93.1%.
Validation of the HPLC-UV method:

The calibration curves of mefloquine and enpiroline were linear from 200 - 40000 ng/mL mouse plasma ($R^2 > 0.999$). The applied method was found selective, since retention times of analytes did not interfere with any endogenous substances (signal: noise ratio > 5:1) so as any tested mefloquine metabolites (human, animal metabolite). The mean extraction recovery estimated for mefloquine and enpiroline was 76.6% and 71.3%, respectively and was consistent over the whole calibration line with a CV of less than 12%. Only little matrix effects (<20%) were present (Table 1).

The intra-day accuracy estimated for both analytes ranged from 87.8% to 111.4% with a precision $\leq 10$. Inter-day accuracy was always within 90-110% margin with a maximal imprecision of 10.5% (Table 2). An LLOQ value of 200 ng/mL plasma was determined for mefloquine with an inter-day-accuracy of 97.4% ± 3.8% and for enpiroline 113.5% ± 4.4%, respectively.

Results obtained from stability experiments demonstrated that the samples were stable under increased benchtop conditions ($t= 8$ h) and autosampler conditions (10 °C, $t= 72$ h). Variations of plasma samples following 72 h at autosampler conditions were below 2.9% and accuracies range from 90.2-103.5%. Stability was likewise observed for our samples when left at room temperature (23-25 °C) for 8 h.

Disposition of enpiroline and mefloquine in S. mansoni-infected and uninfected mice

The established HPLC-UV method was used to study the pharmacokinetics of mefloquine and enpiroline in S. mansoni-infected and non-infected NMRI mice following administration of a single oral dose of 200 mg/kg mefloquine or enpiroline. The pharmacokinetic parameters are summarized in Table 3.
A twofold increase of the AUC_{inf} for mefloquine and enpiroline was observed in S. mansoni-infected mice. C_{max} levels were increased 30-40% within the non-infected cohorts (10223 ng/mL versus 7238 ng/mL for mefloquine ($R^2=0.94-0.98$) and 7641 ng/mL versus 5746 ng/mL for enpiroline ($R^2=0.99$), respectively). The time to achieve maximal concentrations ($T_{max}$) altered only for enpiroline, with $T_{max}$ being reached 24 h later in the non-infected animals compared to infected mice (24 versus 48 h). The half-life ($t_{1/2}$) of mefloquine and enpiroline were 5 and 2.5 fold, respectively elevated (182.7 h versus 33.6 h for mefloquine and 182.1 h versus 72 h for enpiroline) in the infected population. The plasma concentration time profiles of both drugs in infected and uninfected mice are presented in Figure 3.

Discussion

Chronic S. mansoni infections cause substantial pathological and physiological changes in infected patients leading to clinical symptoms such as abdominal pain, diarrhea, blood in the stool and finally to liver cirrhosis and portal hypertension.(18)

The liver and gut, which are mostly affected by a S. mansoni infection play an important role in drug metabolism and disposition.(3) (19) Changes in absorption, distribution, metabolism, and elimination of orally applied drugs are therefore very likely in S. mansoni-infected individuals. An altered drug disposition has been shown in several clinical trials in patients with schistosomiasis.(6) (7)

In the past few years drug metabolism and PK studies became increasingly integrated into early stages of the drug discovery process. However in the field of schistosomiasis few studies have been carried out to date elucidating pharmacokinetic/ pharmacodynamic (PK/PD) relationships and studying the influence of the parasitic infection on drug disposition.

It was therefore our aim to investigate the PK properties of the two antimalarials mefloquine and enpiroline in S. mansoni-infected and uninfected animals.

We successfully adapted an analytical HPLC-UV method, which was originally used to determine artesunate, its active metabolite dihydroartemisinin and mefloquine in human...
plasma (15) to analyze enpiroline and mefloquine in mouse plasma. Similar accuracy, precision and sensitivity values were documented for our analytical method compared to the original work.

The speed of onset of action represents a key pharmacodynamic parameter. We determined the hepatic shift (a well described parameter in antischistosomal drug discovery) which monitors the forced migration of schistosomes to the liver after treatment with active drugs. Interestingly, the hepatic shift of all worms started rather late, only 72 h post-treatment for mefloquine and 168 h following drug administration for enpiroline. The hepatic shift of mefloquine observed in our study was comparable to two previous studies in S. mansoni-infected mice using a single oral dose of 400 mg/kg. (21, 22) Mefloquine acts slightly faster in S. japonicum infected mice. (22, 23) The slow onset of action of mefloquine on schistosomes in vivo has been reported within other studies. (23) In contrast, praziquantel acts much faster, the hepatic shift was completed already 30 min post oral drug administration (unpublished observations), which might be explained by the drugs action on the muscles of schistosomes. In addition, in vivo studies showed that extensive structural changes to worms occurred within 15 min after treatment, and adult worms died within 24 h following treatment with praziquantel. (21, 24)

Interestingly, the majority of worms exposed to mefloquine was still not affected after 7 days (WBR 46.9 %) post treatment in our study. On the other hand, previous studies presented worm burden reductions of 74.1% already one week post treatment with mefloquine (400 mg/kg) and a worm burden reduction of 72.3 % was observed for the dosage of 200 mg/kg, 2-weeks post treatment. (22) These differences in worm burden reductions might be explained by the rather small group of three mice per time point in our study setup.

Additionally the speed of action of the 200 mg/kg dosage might be slower.

The rather late onsets of actions might correlate with the determined long half-lifes for mefloquine (T_{1/2}: 182.7 h) and enpiroline (T_{1/2}: 182.1 h) in infected animals, which are even 2.5- to 5-fold increased compared to non-infected animals. A previous PK study with mefloquine in uninfected mice revealed a half-life of 17 h after oral treatment with 8-10
mg/kg, which is in a similar range as described from our estimation of 33.6 h. (25) However, it is important to emphasize that a rather small sample size was used in the present work and inter-individual variations between the animals are high. Furthermore, the estimated AUC is 2-fold higher in infected animals than in non-infected mice for both drugs. The slow clearance of both drugs might be attributed to a decreased activity of the cytochrome P450 enzyme system caused by the *S. mansoni* infection. (8) It is known that the AUC and the elimination half-life of mefloquine are significantly increased in humans by inhibition of CYP3A4 with inhibitors (e.g. ketoconazole) (Information Leaflet Lariam®). Surprisingly, both drugs presented rather decreased maximal plasma concentrations ($c_{\text{max}}$) in infected mice. The heavy inflammation of the gut wall, which was observed microscopically, might result in decreased drug absorption, which could be an explanation for this finding. It is interesting to note that in contrary to our findings increased $c_{\text{max}}$ values were determined in infected rodents for praziquantel. (8)

Mefloquine presented plasma concentrations above the determined *in vitro* IC$_{50}$ (half maximal inhibitory concentration) value (24 h post exposure. MFQ: 4.7 ± 2.8 µg/mL) for approximately 32 h. (12) Interestingly, the plasma level of enpiroline did not reach the determined 24 h-*in vitro* IC$_{50}$ value (IC$_{50}$: 7.4 ± 2.7 µg/mL). However enpiroline is a slow acting drug *in vitro* presenting an IC$_{50}$ value of 3.1 µg/mL after 72 h drug exposure. (12) The plasma level remained above this concentration for up to 150 h. Since $c_{\text{max}}$ levels are reached much earlier (12 h for mefloquine and 24 h for enpiroline) than the onset of action (24-72 h for mefloquine and 168 h for enpiroline) one can speculate that the antischistosomal activity of both drugs is rather AUC than $c_{\text{max}}$ driven. However, further rigorous PK/PD studies are required to strengthen our hypothesis.

In conclusion, we showed that a *S. mansoni* infection triggers considerable changes in the drug disposition of mefloquine and enpiroline. Pharmacokinetic studies with antischistosomal lead candidates in infected animals should be integrated early in the drug discovery process. In addition, PK studies should be conducted in *S. mansoni* and *S. haematobium* infected
children, the key target group of preventive chemotherapy, since children highly depend on
safe efficacious and controlled dosage regimens. Our findings are of great public health
relevance, since mefloquine is widely used in areas where schistosome infections are
common. Over-dosing might occur and lead to toxic adverse events, which might be of
relevance in light of reported neurotoxicity of mefloquine.(26) Furthermore, influences on the
pharmacokinetic profiles of antimalarials could lead to prolonged plasma-levels beneath the
minimal inhibition concentration and hence result in sub-optimal dosing selecting for drug-
resistant parasites. Additional, in depth studies should therefore be conducted with
antimalarials in patients co-infected with S. mansoni and P. falciparum.

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References


water resources development: systematic review, meta-analysis, and estimates of

368:1106-1118.

Stothard JR, Gabrielli AF, Clements AC, Kabatereine NB, Toure S, Dembele R,
Nyandindi U, Mwansa J, Koukounari A. 2009. The Schistosomiasis Control

Parasitology 136:1719-1730.


Table 1: Relative Recovery (RRE) and matrix effect of mefloquine and enpiroline extracted from mouse plasma samples (n=5) by protein precipitation with methanol.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Nominal concentration (ng/mL)</th>
<th>RRE (%)</th>
<th>Mean±CV (%)</th>
<th>Matrix effect (%)</th>
<th>Mean±CV (%)</th>
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</thead>
<tbody>
<tr>
<td>Mefloquine</td>
<td>750</td>
<td>78.4</td>
<td>76.6±7.9</td>
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<td>71.3±12</td>
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<td>84.3±11.1</td>
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Table 2: Intra-day and inter-day accuracy and precision. Intra-day values are mean values of 6 samples. One representative experiment is shown. Inter-day values are mean values of 3 independent sets of experiments. RSD: Relative Standard Deviation

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<tr>
<th>Analytes (regression model)</th>
<th>Nominal concentration (ng/mL)</th>
<th>Intra-day Mean concentration found (ng/mL)</th>
<th>RSD (%)</th>
<th>Accuracy (%)</th>
<th>Inter-day Mean concentration found (ng/mL)</th>
<th>RSD (%)</th>
<th>Accuracy (%)</th>
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<td>(linear, R²=0.9995)</td>
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<td>835.3</td>
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<tr>
<td>(linear, R²=0.9996)</td>
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Table 3: Pharmacokinetic parameters of mefloquine and enpirole following oral administration of mefloquine or enpirole at 200 mg/kg. Data present mean values obtained by 3 mice per time point.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mefloquine</th>
<th>Enpirole</th>
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<td>Dosage</td>
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<td>200 mg/kg p.o.</td>
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<tr>
<td>S. mansoni infection</td>
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<tr>
<td>T_{1/2} (h)</td>
<td>182.7</td>
<td>33.6</td>
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<tr>
<td>AUC_{last 0-168h} (ng*h/mL)</td>
<td>567845.5</td>
<td>503623.5</td>
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<td>AUC_{inf 0-∞} (ng*h/mL)</td>
<td>1116517.8</td>
<td>522409.1</td>
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<tr>
<td>C_{max} (ng/mL)</td>
<td>7238</td>
<td>10223</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>12</td>
<td>12</td>
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Figure 1. Chemical structures of mefloquine, enpiroline and chlorpromazine (internal standard).
Figure 2. Worm distribution (alive and dead) between liver and mesenterial vein system presented over time post treatment with 200 mg/kg mefloquine (A) or enpiroline (B).
Figure 3. Mean plasma concentration-time profile of mefloquine (A) and enpiroline (B) after oral administration of 200 mg/kg to 3 mice per time point to either infected (dashed line) or non-infected animals (black line).