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Liquid-phase microextraction combined with high-performance liquid chromatography for the enantioselective analysis of mefloquine in plasma samples

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Abstract

A simple and rapid method, which involves liquid-phase microextraction (LPME) followed by HPLC analysis using Chiralpak AD column and UV detection, was developed for the enantioselective determination of mefloquine in plasma samples. Several factors that influence the efficiency of three-phase LPME were investigated and optimized. Under the optimal extraction conditions, the mean recoveries were 33.2 and 35.0% for (−)-(*SR*-)-mefloquine and (+)-(*RS*)-mefloquine, respectively. The method was linear over 50–1500 ng/ml range. Within-day and between-day assay precision and accuracy were below 15% for both enantiomers at concentrations of 150, 600 and 1200 ng/ml. Furthermore, no racemization or degradation were seen with the method described.
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Keywords: Mefloquine enantiomers; Liquid-phase microextraction; Liquid chromatography; Plasma samples

1. Introduction

Mefloquine (MQ) [*rac*-erythro-α-(2-Piperidyl)-2,8-bis(trifluoro-methyl)-4-quinolinemethanol] (Fig. 1) is a chiral drug administered orally as a racemic mixture for prophylaxis and treatment of malaria caused by multiple-drug-resistant strains of *Plasmodium falciparum* [1]. There are conflicting reports about the antimalarial activity of MQ enantiomers. In some reports, no significant difference was observed between antimalarial activities of enantiomers against *P. yoelli* [2] and *P. falciparum in vitro* [3]. In other report the (+)-(*RS*)-enantiomer was more active than the (-)-(*SR*)-enantiomer against different strains of *P. falciparum* [4]. MQ may cause neuropsychiatric adverse effects in some patients, but the toxicological relevance of enantioselectivity in this process has yet to be established [1]. After oral administration, MQ is mainly metabolized to the 4-carboxylic acid derivative, which has no *in vitro* antimalarial activity [5]. In man, higher concentrations of the (−)-(*SR*)-enantiomer have been observed in both plasma and brain, but the (−)/(+) ratio was higher in plasma [6,7]. Unlike humans, studies in rats have demonstrated that plasmatic concentrations of the (+)-(*RS*)-enantiomer are two to three times higher than those of (−)-(*SR*)-enantiomer [8].

Most of the available methods for stereospecific quantitation of MQ enantiomers in plasma utilize high-performance liquid chromatography (HPLC). Some of these methods require an achiral chromatographic separation of the antimalarial agent, followed by chromatographic resolution of the enantiomers in each fraction by use of chiral stationary phases [9,10]. There are some indirect methods reported for the enantioseparation of MQ using (−)-1-(9-fluorenyl)ethyl chloroformate and (+)-(*S*)-naphthylethylisocyanate as chiral derivatizing reagents [11,12]. In addition, some direct methods based on chiral stationary phases were also reported [13,14]. More recently, an electrophoretic method was developed to resolve MQ enantiomers [15].

The majority of the methods developed for the enantioselective determination of MQ in biological fluids uses liquid–liquid extraction (LLE) for sample preparation. However, LLE is considered to be a tedious, time-consuming procedure and needs

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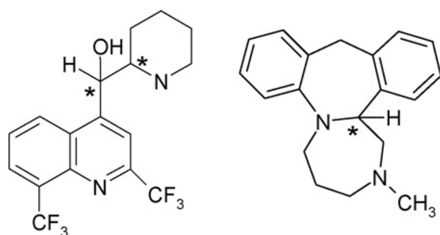

Fig. 1. Structures of mefloquine (*left*) and mirtazapine (*right*), used as internal standard ('*' denotes chiral center).

a large amount of organic solvents [16]. On another hand, new solvent-minimised techniques based upon liquid-phase microextraction (LPME), as an alternative to LLE, have been developed. Nowadays, the use of LPME with disposable hollow fibre membranes in drug analysis has been an increasing technique due to its simplicity and efficiency. At the same time, LPME combines extraction, concentration and sample clean-up in one step. It can be used in two modes: two or three phases, according to analyte or sample characteristics [17]. In the last mode, analytes of interest are extracted from aqueous samples, through a thin layer of organic solvent immobilized within the pores of a porous hollow fibre, and into an acceptor solution (acidic or alkaline) inside the lumen of the hollow fibre. In order to achieve greater recovery, this process can be optimized, varying some important factors, such as organic solvent, extraction time, agitation speed, pH of extraction and acceptor phase [18]. To our knowledge, LPME has not been used for the analysis of antimalarial drugs in biological samples.

The aim of this study was to develop a simple and efficient LPME-based extraction procedure followed by chiral HPLC analysis for the enantioselective determination of MQ in plasma samples. Analytical parameters investigated included: solvent type, extraction time, stirring rate, sample pH, acceptor phase type, salt and methanol addition. Finally, the optimized and validated method was applied to determine MQ enantiomers in rat plasma after administration of the racemic drug, in order to evaluate the application of this method to real samples.

2. Experimental

2.1. Chemicals

Rac-mefloquine was generously supplied by Hoffman-La Roche (Basel, Switzerland). *Rac*-mirtazapine (internal standard) was supplied by Analytical Control Labs., N.V. Organon (Oss, The Netherlands). Hexane, ethanol and methanol were all of chromatographic grade and purchased from Merck (Darmstadt, Germany). Analytical grade diethylamine was obtained from Fluka (Buchs, Switzerland). Di-*n*-butyl ether and *n*-octanol were purchased from Sigma (St. Louis, MO, USA), while di-*n*-hexyl ether (>96%) was obtained from Alfa Aesar, Johnson Matthey Company (Ward Hill, MA, USA). Sodium hydroxide was purchased from Nuclear (São Paulo, SP, Brazil). Purified water was obtained from a Milli-Q-system (Millipore, Mildford, MA, USA). All other chemicals and solvents were of either chromatographic or analytical grade.

2.2. Calibration and quality control solutions

MQ stock (1 mg/ml) and working solutions (4–120 μg/ml) were prepared in methanol, stored frozen at −20 °C and protected from light, remaining stable for at least 3 months. The internal standard solution (mirtazapine) was prepared in methanol at the concentration of 30 μg/ml.

Drug-free human plasma samples were obtained from healthy volunteers and stored frozen at −20 °C. Prior to use, the plasma samples were allowed to thaw at room temperature.

Plasma quality control samples (QC) spiked with 150, 600 and 1200 ng/ml of each enantiomers were prepared to measure the accuracy and precision of the method.

2.3. General liquid-phase microextraction procedure

Prior to extraction, the sample vial was filled with 1 ml plasma, the pH was adjusted to alkaline region with NaOH or phosphate buffer solutions and diluted with deionized water to a total volume of 4 ml. The LPME system (Fig. 2) consisted of a sample solution (donor) in a 4 ml vial (Supelco Inc., Bellefonte, PA, USA) containing a 6.5 cm Q3/2 Accurel KM porous polypropylene hollow fiber (Membrana, Wuppertal, Germany). The inner diameter of the hollow fiber was 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.2 μm. Two syringe needles 0.8 mm o.d. (Hamilton, Reno, NV, USA) were used to connect hollow fiber ends, one serving to introduce the acceptor solution, while the second was used for collection of final extract. The hollow fiber was soaked in an organic solvent (*n*-octanol, di-*n*-butyl ether or di-*n*-hexyl ether) for 10 s to immobilize the solvent in the pores, which the mefloquine was preferably soluble. Excess of solvent was removed by 15 s of ultrasonication in water. After impregnation, 20 μl of an acidic

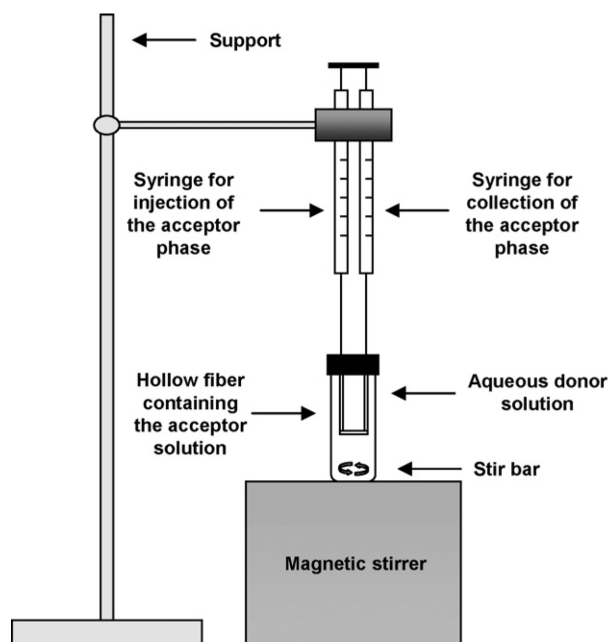

Fig. 2. Schematic representation of experimental set-up of LPME using hollow fiber membrane.

solution (perchloric, acetic or trifluoroacetic acid) were injected into the lumen of the hollow fiber with the inlet microlitre syringe and, subsequently, the fiber was placed in the sample solution. During the extraction, the samples were stirred using a PC-210 magnetic stirrer (Corning, New York, NY, USA) with cylindrical-shaped stirring bars (10 mm × 4 mm) at room temperature (23 ± °C). After extraction, the acceptor solution was withdrawn from the fiber by the outlet microlitre syringe and transferred into a conical tube. The addition of salt and methanol to the donor solution was also evaluated. For each extraction, a new hollow fiber was placed. The final fractions of LPME were evaporated to dryness under air flux. The dry residues were then dissolved in 80 μl of LC mobile phase prior to analysis. Hence, an aliquot of this solution (50 μl) was introduced into the LC system. Optimized conditions are described in Section 3.

2.4. High-performance liquid chromatography analysis

Analyses were conducted using a Shimadzu (Kyoto, Japan) liquid chromatograph, equipped with an LC-AT VP solvent pump unit and an SPD-10A UV–vis detector operating at 222 nm. Injections were performed manually through a 50 μl loop with a Rheodyne model 7125 injector (Rheodyne, Cotati, CA, USA). Data were collected using a Chromatopak CR6A integrator (Shimadzu, Kyoto, Japan).

The resolution of MQ enantiomers were carried out at 23 ± 2 °C on a Chiralpak AD column (250 mm × 4.6 mm i.d., 10 μm particle size, Chiral Technologies, Exton, PA, USA). A CN guard column (4 mm × 4 mm i.d., Merck, Darmstadt, Germany) was used to protect the analytical column. The mobile phase used for the analysis of MQ consisted of hexane-ethanol (97:3, v/v), plus diethylamine (0.05%), at a flow rate of 1 ml/min. Enantiomeric elution order was evaluated by analyzing pure enantiomers (obtained by semipreparative analysis under the conditions established) using a procedure described in Qiu et al. [13].

2.5. Method validation

A calibration graph using the optimized method was obtained analyzing spiked plasma samples ($n = 3$ for each concentration) over the concentration range of 50–1500 ng/ml (6 points) for each MQ enantiomer. *Rac*-mirtazapine was used as internal standard. The results were plotted on a graph of peak height ratio *versus* analyte concentrations and the best relationship was obtained by linear least-squares regression analysis. To verify method linearity, an ANOVA lack-of-fit test ($\alpha = 0.05$) was performed [19].

The absolute recovery of MQ enantiomers extracted from plasma samples ($n = 3$) spiked with 150, 600 and 1200 ng/ml of each enantiomer was determined using calibration curves obtained from the data of the analytes not submitted to extraction. Recovery was expressed as percentage of the amount extracted.

The sensitivity of the method was estimated by determining the quantification limit (LOQ). The LOQ was defined as the lowest concentration of MQ enantiomers that could be determined with accuracy and precision below 20% over five analytical runs, obtained from plasma samples spiked with 50 ng/ml of each enantiomer.

To assess within-day precision and accuracy, replicate analysis ($n = 5$) of plasma samples spiked at 150, 600 and 1200 ng/ml of each enantiomer were perfomed. For between-day assay, five determinations of each concentration level (as described for within-day assay) were analyzed over 3 days. Precision and accuracy were expressed as relative standard deviation (R.S.D.%) and deviation from the theoretical value, respectively.

Freeze–thaw cycles and short-term room temperature stability assays were also evaluated. To perform the freeze-thaw cycles stability assay, three aliquots at low (150 ng/ml) and high (1200 ng/ml) concentrations of the quality control samples were stored at −20 °C for 24 h and thawed at room temperature. When completely thawed, the samples were refrozen for 12 h under the same conditions. This cycle was repeated twice more. Finally, the samples were extracted and analyzed after the last cycle. For the determination of short-term room temperature stability assay, three aliquots of each quality control sample (at the same concentrations as described above) were prepared and kept at room temperature (23 ± 2 °C) for 12 h. After this period, the samples were analyzed. The peak height ratio obtained from both stability assays were compared to the peak height ratio obtained with freshly prepared samples. Student's *t*-test was applied, with the level of significance set at $p < 0.05$ [19].

Inversion of configuration of chiral drugs may occur during the extraction procedure [20]. In order to verify the occurrence of racemization, MQ enantiomers were separated and collected under chromatographic conditions established. After mobile phase evaporation, the residues were dissolved in methanol. Next, aliquots of 1.0 ml of plasma samples ($n = 3$) spiked with 25 μl of each enantiomer solution were submitted to the LPME procedure and subsequent chromatographic analysis.

2.6. Method application

To assess the applicability of the validated method, the plasma levels of MQ enantiomers were measured in rats after administration of the racemic mixture. Male Wistar rats weighing approximately 200 g were kept under standardized conditions with free access to food and water. Food was withheld overnight. The next day, rats received a single dose of 50 mg/kg (±)-MQ HCl by oral gavage ($n = 3$ at each time point). Food was not given to the animals until at least 1 h after dosing.

Following oral dosing, rats were anesthetized by ether inhalation and blood collected in heparinized tubes by open chest cardiac puncture at 2, 4, 8, 12, 16, 24, 48 and 56 h. Immediately following collection, each blood sample was centrifuged for 15 min at 2000 *g*. All samples were stored at −20 °C until analyzed for MQ enantiomers.

3. Results and discussion

3.1. Optimization of LPME procedure

In order to optimize the liquid-phase microextraction of MQ from plasma samples, analytical factors that potentially affect

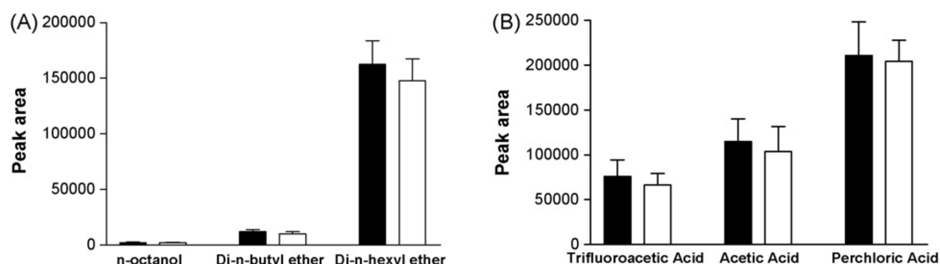

Fig. 3. Extraction efficiency (represented by peak areas) using different organic solvents (A) and acceptor phases (B) for (−)-(*SR*)-MQ (■) and (+)-(*RS*)-MQ (□). Extraction conditions: (A) extraction time: 30 min; stir speed: 870 rpm; 100 μl 4 M NaOH; acceptor phase: 10 mM HCl; (B) extraction time: 30 min; stir speed: 1100 rpm; 100 μl 10 M NaOH; organic solvent: di-*n*-hexyl ether. Chromatographic conditions as in Section 2.

sample extraction were studied. Such factors include solvent type, extraction time, stirring rate, sample pH, acceptor phase type, salt and methanol addition to the donor phase. In this stage, all experimental procedures were assayed in triplicate and no internal standard was used.

3.1.1. Organic solvent selection

Selection of a suitable solvent is a critical parameter in LPME. Ideally, the organic solvent should be compatible with the fiber, immiscible in water, stable enough over the extraction time and present high solubility for target analyte [21]. MQ has a log value of octanol–water distribution coefficient (log *D*) near 3.0. Three solvents were selected for study: *n*-octanol, di-*n*-butyl ether and di-*n*-hexyl ether. According to Fig. 3A, di-*n*-hexyl ether gave higher peak area response for both enantiomers and, besides, it fills all requirements listed above. On the basis of these considerations, di-*n*-hexyl ether was selected for subsequent experiments.

3.1.2. Extraction time

Since liquid-phase microextraction is a time-dependent process, the function of extraction time was examined over 10, 20, 30, 45 and 60 min. As observed in Fig. 4A, an increase in the extraction time resulted in higher enrichment of MQ enantiomers. The extraction kinetics are similar to those generally observed in SPME, which normally takes considerable time before reaching equilibrium. According to Halvorsen et al. [22], high sample viscosity is the main reason to the system requires a long period to reach equilibrium. It is therefore undesirable to use an extraction method based on equilibrium time [23]. Thus, from a practical point of view, 30 min was used for following experiments.

3.1.3. Effect of stirring rate

In LPME, the extraction can be accelerated by stirring the donor solution, thereby reducing the time required to attain thermodynamic equilibrium [24]. The effect of sample agitation was evaluated using a stirring speed between 250 and 1100 rpm. Sample agitation improved MQ extraction efficiency, where chromatographic peak areas increased with stirring rate (Fig. 4B). Therefore, 1100 rpm was selected on the basis of these observations.

3.1.4. Effect of sample pH

As in LLE, sample pH plays an important role in LPME [18]. MQ is a weak basic drug (pK_a 8.5) so it must be extracted in alkaline medium. Hence, the effect of pH in the range of 6.0–13.0 was evaluated (Fig. 4C). Peak areas for MQ enantiomers were lower when buffer solutions (0.05 M phosphate buffer – pH 6.0 and 12.0; 0.05 M borate buffer – pH 10.0) were used to control pH than when NaOH (100 μl 4 M—pH 13.0) was used. It may be due to high protein interaction presented with buffer solutions, which is broken when using NaOH. Another possible explanation is the fact that addition of NaOH could reduce the viscosity of the sample since it influences on the extraction speed [22]. Then, the next step was to select NaOH concentration (2, 4 and 10 M) keeping a constant volume (100 μL) and the final volume completed up to 4 mL with deionized water. It was noted that increasing NaOH concentration resulted in greater analyte recovery. Hence, 10 M NaOH was used for the next experiments.

3.1.5. Acceptor phase selection

Another crucial parameter is the pH value of the acceptor phase. For extraction of basic substances the maximal trapping of the extracted analyte in ionised form requires that the pH of the acceptor phase should be low [25]. Initially, 10 mM HCl was used as acceptor phase but, in order to prevent possible oxidation caused by this acid to the chromatographic system at regular use, it was decided to look for another acceptor solution. For the purpose to evaluate other acidic solutions, both mineral and organic acids were tested. Acceptor solutions of 10 mM $HClO_4$, 100 mM CH_3COOH and 100 mM trifluoroacetic acid were investigated. The acceptor phase made of 10 mM $HClO_4$ (a strong mineral acid) provided the highest analyte recovery (Fig. 3B). Further modification was carried out by adding ethanol to the acceptor phase, in order to improve the solubility of MQ, as observed for other analytes [26]. Experiments were performed with 10, 20 and 30% ethanol in 10 mM $HClO_4$. No improvement was noted (Fig. 4D); besides, proportions greater than 10% results in lack of acceptor phase during extraction. Thus, it was decided to keep acceptor phase only with 10 mM $HClO_4$.

3.1.6. Salt and methanol addition

The addition of salt or methanol to a bioanalytical sample can increase analyte recovery in microextraction procedures [27–29]. For that purpose, the addition of NaCl at concentrations

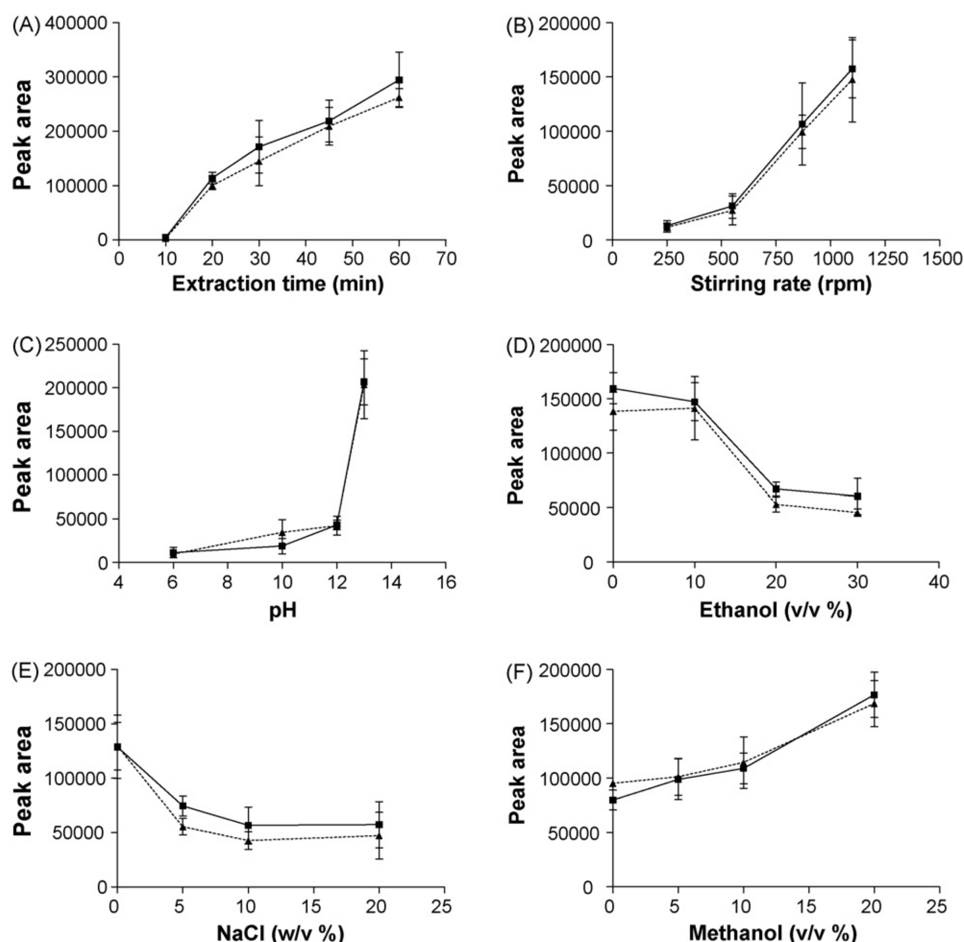

Fig. 4. Optimization parameters evaluated in LPME method development for (−)-(*SR*)-MQ (■ and (+)-(*RS*)-MQ (▲). Extraction efficiency represented by peak areas. (A) Extraction time (extraction conditions: stir speed at 870 rpm; 100 μl 4 M NaOH; organic solvent: di-*n*-hexyl ether; acceptor phase: 10 mM HCl), (B) Stirring rate (extraction conditions: 30 min; 100 μl 4 M NaOH; organic solvent: di-*n*-hexyl ether; acceptor phase: 10 mM HCl), (C) Sample pH (extraction conditions: 30 min; stir speed at 1100 rpm; organic solvent: di-*n*-hexyl ether; acceptor phase: 10 mM HCl), (D) Ethanol addition (extraction conditions: 30 min; stir speed at 1100 rpm; 100 μl 10 M NaOH; organic solvent: di-*n*-hexyl ether; acceptor phase: 10 mM $HClO_4$), (E) salt and (F) methanol addition (extraction conditions same as in ethanol addition experiment). Chromatographic conditions as in Section 2.

between 5 and 20% (w/v) was therefore evaluated. However, the extraction efficiency decreased significantly with the addition of salt (Fig. 4E). On the basis of this, no salt addition was done in the next experiments. MQ is highly (98%) protein bound [30]. Therefore, the addition of methanol at concentrations between 5 and 20% (v/v) was carried out in order to verify the inhibition of drug-protein binding. As illustrated in Fig. 4F, 20% methanol was required for an effective raise in analytes recoveries. However, as we are searching for solvent-minimising techniques (including LPME), 800 μl of methanol to each plasma sample is considered too high. Hence, it was decided to keep donor phase (plasmatic matrix) free of organic additives without any lack in method sensitivity.

3.1.7. Optimized extraction procedure

On the basis of the experiments discussed above, the optimal LPME conditions were di-*n*-hexyl ether as the extraction solvent, an extraction time of 30 min, a stirring speed of 1100 rpm, sample pH adjustment with 100 μl 10 M NaOH and 10 mM $HClO_4$ as the acceptor phase. Under these conditions, carboxymefloquine (main MQ metabolite) was not extracted due to its acidic characteristic.

3.2. Method validation

In order to evaluate the practical applicability of the LPME technique, optimal extraction conditions were validated prior to analysis of real samples. Some important parameters, such as linearity, recovery, precision, accuracy, limit of quantification, racemization and stability assays were studied. For this purpose, *rac*-mirtazapine (Fig. 1) was added as internal standard at 375 ng/ml each enantiomer (only peak 2 in Fig. 5 was considered for calculation purposes). This compound was appropriate because it was recovered from plasma approximately by the same factor and it was easily separated from MQ enantiomers by the chromatographic system (recovery values obtained for i.s. were *ca*. 40%).

Under the optimized conditions, LPME absolute recoveries were between 33 and 35%, with R.S.D. values lower than 10% for both enantiomers (Table 1). Although these recov-

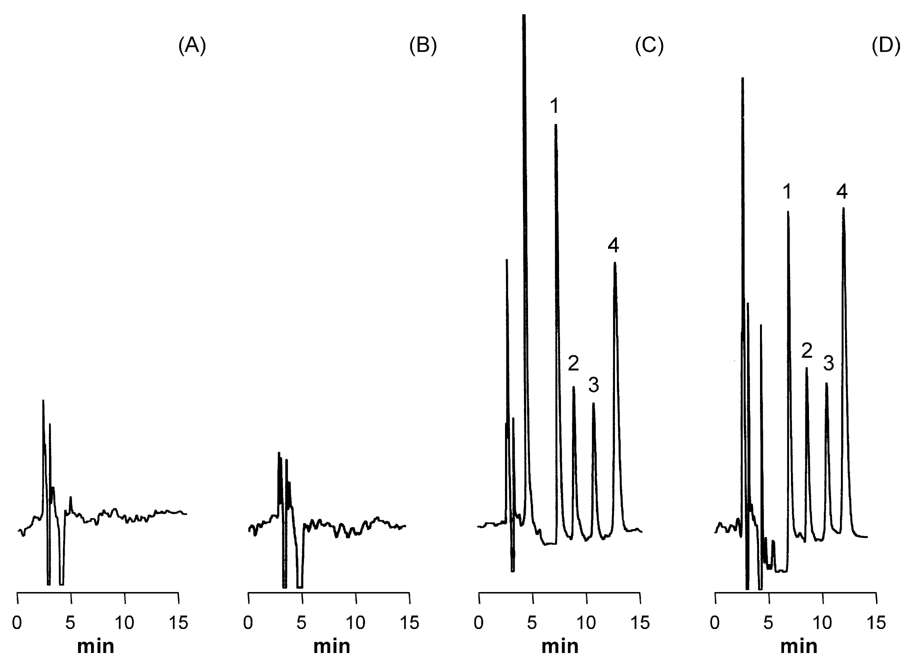

Fig. 5. Chromatograms referring to the analysis of MQ enantiomers after LPME procedure in: (A) human; (B) rat drug-free plasma; (C) plasma spiked with 600 ng/ml of (−)-(*SR*)-MQ (1) and (+)-(*RS*)-MQ (4) and 375 ng/ml of (±)-mirtazapine (2,3); (D) plasma sample from a rat collected 12 h after administration of 50 mg/kg oral (±)-MQ. Chromatographic conditions as in Section 2.

eries may seem low when compared to traditional extraction methods, they are considered suitable for LPME, due to the microscale characteristic of the technique [28]. Furthermore, it was accepted since extraction procedure efficiently reduced interference from endogenous materials, allowing low determinations of MQ enantiomers.

The method presented typical calibration curve equations determined as $y = 0.0029x + 0.0216$ and $y = 0.0018x + 0.0297$ for the (−)-(*SR*)- and (+)-(*RS*)- enantiomers of mefloquine, respectively, with correlation coefficients (r) >0.998 for both enantiomers. In order to check linearity of the calibration graph, an ANOVA lack-of-fit test was applied. Since the F values obtained (0.76 and 0.21 for *SR* and *RS*- enantiomers, respectively) were less than the critical value of F with a level of significance of 5% ($F = 3.63$), the linear model was adequate for the observed data.

The precision and accuracy validation data are summarized in Table 2. The within-day precision was <9.9% R.S.D. and the between-day precision was <14.4% R.S.D. for both enantiomers at the three concentrations evaluated. The within-day and between-day accuracies were found to be within −12.1 and 9.0% for both enantiomers over the concentration studied, indicating acceptable accuracy values [31]. The lowest concentration quantified by the method (LOQ) was 50 ng/ml (Table 2).

The stability study showed no statistically difference between freeze-thaw cycles and short-term room temperature stability assays with p-values >0.05 (Table 3).

Chromatograms of human and rat drug-free plasma samples and a plasma sample spiked with 600 ng/ml of MQ enantiomers and *rac*-mirtazapine (I.S.) are shown in Fig. 5. The extract is remarkably clean and no interfering peaks were detected in drug-free plasma samples, indicating that the method could be used for the enantioselective analysis of MQ in both plasma samples. The elution order established showed that the first peak corresponds to (−)-(*SR*)-MQ and the last one corresponds to (+)-(*RS*)-MQ. In addition, the analysis of plasma samples spiked with pure enantiomers did not demonstrate any racemization during the extraction procedure.

3.3. Method application

The concentrations of MQ enantiomers after oral administration of racemic mixture to rats are presented in Fig. 6. The plasma concentrations of (+)-(*RS*)-MQ were greater than

Table 1
Mean recoveries of MQ enantiomers in plasma samples

Plasma concentration (ng/ml, $n = 3$)	(-)-(*SR*)-MQ		(+)-(*RS*)-MQ	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
150	35.5	5.5	36.7	6.3
600	32.3	4.7	33.1	5.9
1200	31.9	8.3	35.3	9.5
Range (150–1200)	33.2	5.9	35.0	5.1

n, number of samples; R.S.D., relative standard deviation.

Table 2
Precision, accuracy and quantification limit for the analysis of MQ enantiomers in plasma samples

Nominal standard concentration (ng/ml)	Analyzed concentration (ng/ml)		Precision[a]		Accuracy[b]	
	(−)-(*SR*)-	(+)-(*RS*)-	(−)-(*SR*)-	(+)-(*RS*)-	(−)-(*SR*)-	(+)-(*RS*)-
Within-day[c]						
50[d]	55.4	57.3	9.9	12.5	10.8	14.6
150	154.5	150.1	9.6	9.8	3.0	0.1
600	564.8	544.9	4.5	8.5	−5.8	−9.1
1200	1301.3	1308.8	7.1	9.4	8.4	9.0
Between-day[e]						
150	150.8	144.3	12.7	12.4	0.5	−3.7
600	541.1	527.0	6.6	7.9	−9.8	−12.1
1200	1166.2	1179.6	12.7	14.3	−2.8	−1.6

[a] Expressed as relative standard deviation.
[b] Expressed as deviation from theoretical values.
[c] Number of samples.
[d] Quantification limit, $n = 5$.
[e] Number of days.

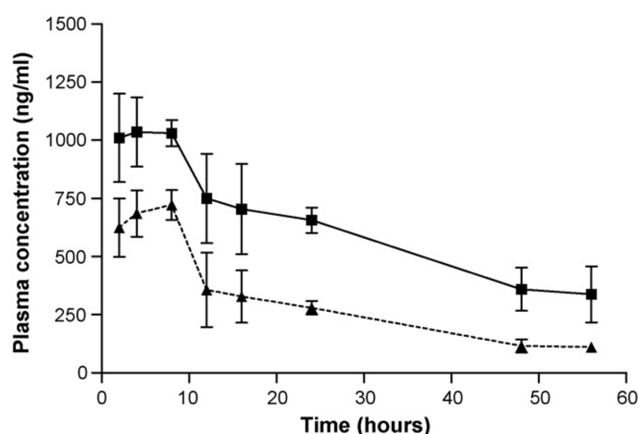

Fig. 6. Mean (±S.D.) plasma concentrations *vs.* time curves of MQ enantiomers in rats ($n = 3$ each time point) following a single oral dose of 50 mg/kg (±)-MQ HCl. (▲) (−)-(*SR*)-MQ; (■)-(+)-(*RS*)-MQ.

its antipode at all measured times. This stereoselectivity might be explained by one or more stereoselective pharmacokinetic processes, for example absorption, excretion, metabolism and distribution. It is interesting to note that plasma stereoselectivity in man is opposite of that in the rat [8]. After administration of the racemic mixture, higher concentrations of the (−)-enantiomer are observed in humans [6]. This discrepancy can be explained on the basis of differences in protein binding and the metabolism of the two enantiomers observed in rats [32].

Table 3
Stability test for the analysis of MQ enantiomers in plasma samples

Nominal concentration (ng/ml)	(-)-(*SR*)-MQ	(+)-(*RS*)-MQ
	p-Values	*p*-Values
Freeze-thaw cycles		
150	0.18	0.21
1200	0.06	0.08
Short-term room temperature		
150	0.32	0.23
1200	0.07	0.18

4. Conclusions

In the present study, a three-phase LPME method for the enantioselective analysis of MQ in plasma samples was developed and validated. This method is simple, cost-effective and solvent minimising procedure. The optimized LPME technique using hollow fiber membrane in conjuction with chiral HPLC is reliable, precise and linear over 50–1500 ng/ml range. Sample clean-up was highly effective, with no interfering peaks from matrix compounds. Finally, the method has a proven viability for quantitative analysis of MQ enantiomers in plasma samples.

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