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Short Communication

High-performance liquid chromatographic method for the enantioselective analysis of mefloquine in plasma and urine

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Abstract

An HPLC method for analysis of the enantiomers of the antimalarial drug mefloquine is presented. A complete resolution of (−)-(11*S*,2′*R*) and (+)-(11*R*,2′*S*) *erythro*-mefloquine from plasma and urine was obtained on a commercial AGP column. Mefloquine enantiomers were detected by UV at 222 nm. The separation factor (α) at +20°C was 1.50. The limit of determination (coefficient of variation 4.0%) for the enantiomeric ratio (11*S*,2′*R*)/(11*R*,2′*S*) is 15:1 at a total mefloquine concentration of 1.6 m*M*.

1. Introduction

Mefloquine (MQ), [(±)-(R^*, S^*)-2,8-bis(trifluoromethyl) - α - (2 - piperidinyl) - 4 - quinolinemethanol monohydrochloride], has been used for treatment of malaria caused by multiresistant *Plasmodium falciparum* since the mid 1980s. The drug is marketed as a racemic mixture of the erythro forms −(11*S*,2′*R*) and +(11*R*,2′*S*) here named *SR* and *RS*, respectively. Recently differences *in vitro* have been reported against *P. falciparum* [1].

Mild adverse reactions are common during mefloquine treatment and suggest a cholinergic mechanism. Ngiam and Go [2] found that the (−)*SR* enantiomer is a more potent inhibitor of acetylcholinesterase and butyrylcholinesterase than the (+)*RS* enantiomer. This shows that the potency for adverse reactions might be different for the two enantiomers.

There are several methods available for determination of MQ in body fluids [3]. The interaction between the MQ enantiomers and an AGP stationary phase has recently been investigated [4]. HPLC methods for determination of the two MQ enantiomers have been described [5,6]. One of these is designed for analysis of the drug in body fluids [6]. This HPLC method is, however, complicated and laborious and the chromatographic system does not accomplish full baseline resolution. These disadvantages and our previous experience in enantioselective analyses of the antimalarial drug chloroquine [7] moti-

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vated us to develop an improved method for the determination of the MQ enantiomers.

2. Experimental

2.1. Chemicals

The pure mefloquine enantiomers as well as the racemate were kindly supplied by Hoffman-La Roche (Basel, Switzerland and Stockholm, Sweden). Other drugs were obtained from tablets commercially available in Sweden. Methyl *tert.*-butyl ether (MTBE) and 1-propanol were of chromatographic grade (Merck, Darmstadt, Germany). All other chemicals and solvents were of analytical grade (Kebo, Stockholm, Sweden).

2.2. Chromatography

A Chiral-AGP 150 × 4.0 mm I.D. column (ChromTech AB, Norsborg, Sweden) was used with a guard column packed with the same material. The Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) was equipped with a 100-μl loop. The LC-9A pump from Shimadzu (Kyoto, Japan) was set at a flow-rate of 0.9 ml/min. The Shimadzu SPA-6A UV-detector was set at 222 nm. A water jacket (Alltech Associates, Deerfield, IL, USA) was used for thermostating the column. The chromatograms were recorded and the peak areas were calculated using the laboratory data system ELDS 900 (Chromatography Data Systems AB, Kungshög, Sweden). The eluent consisted of 10% (v/v) 1-propanol in a 0.05 *M* sodium phosphate buffer pH 4.85.

The influence of temperature on the chromatographic parameters was determined with a 0.5 μM MQ solution in mobile phase. The AGP column was mounted in a water jacket.

2.3. Extraction procedure

Plasma and urine samples were extracted in the same way and glass test tubes were used throughout the procedure. To a 1.0-ml sample we added 0.1 ml sodium hydroxide (1 *M*) and 2.0 ml of MTBE. After extraction for 15 min by slow rotation the phases were separated by centrifugation at 3000 *g* for 5 min.

The organic phase was extracted with 0.5 ml of 0.5 *M* phosphoric acid for 15 min. After centrifugation the organic phase was discarded and 1.0 ml of sodium hydroxide (1 *M*) was added to the remaining solution followed by 1.5 ml of MTBE. After extraction for 15 min and centrifugation the organic phase was transferred to a new tube and evaporated under nitrogen. The residue was dissolved in 0.3 ml of the eluent.

2.4. Extraction recovery

Racemic MQ was added to plasma to obtain a concentration of 0.5 μM. The samples were analysed after extraction. The resulting peak areas were compared with those of directly injected standards.

2.5. Precision and accuracy

The two enantiomers were added to plasma in three different proportions 1:1, 15:1 and 30:1 (*SR*:*RS*), the total MQ concentrations being 3.0, 1.6, and 1.5 μM, respectively. After extraction and chromatographic separation the measured ratios were compared with the expected values.

A similar test was done with urine using enantiomeric ratios of 1:1, 5:1 (*SR*:*RS*) at a total MQ concentration of 3.0 and 0.5 μM, respectively.

The method was also evaluated by spiking plasma and urine with a 0.5 μM MQ solution containing different ratios of the enantiomers 0:1, 1:9, 1:3, 2:3, 1:1 (*SR*:*RS*). The samples were analysed and the peak ratios were compared with the theoretical *SR*:*RS* ratios.

2.6. Interferences

Commercially available tablets were ground and suspended in water (sulphadoxine, cimetidine, ranitidin, verapamil, propranolol, atenolol, proguanil), water–ethanol (pyrimethamine, cycloguanil), or methanol (flunitrazepam, diazepam, hydroxychloroquine). An appropriate

volume was injected onto the chromatographic system.

Possible interference by chloroquine and its metabolite desethylchloroquine and by quinine and amitryptyline were tested by analysing samples from patients treated with these drugs.

2.7. Racemization

Plasma and urine samples containing single MQ enantiomers were stored at room temperature on the bench for one week and at −20°C during four months. Racemization was checked using the method described above.

3. Results and discussion

The mefloquine enantiomers were completely separated in our system (Fig. 1a,b). At +20°C a separation factor of 1.50 was obtained while it decreases to 1.40 at +25°C. Similar effects of temperature have been reported previously [8]. The temperature effect is probably due to either the weak protein–ligand interaction or to some conformational change of the protein which influences the binding.

Several organic modifiers including, 1-propanol, 2-propanol and acetonitrile were tested. The optimal peak shape was obtained with 1-propanol. This modifier afforded good separation at a reasonable retention time.

MQ shows two UV absorption bands, at 222 nm and at 285 nm. The absorption at 222 nm is *ca.* five times stronger as that at 285 nm. In order to determine low concentrations of MQ it is necessary to use the 222 nm wavelength despite the fact that at this wavelength problems arise with interference from endogenous compounds.

The over all extraction recovery was 50%, which may seem low. However, we accepted this low recovery since the extraction procedure efficiently reduced interferences from endogenous material (Fig. 1b).

We have evaluated the method by analysing plasma with different ratios of enantiomers at a total MQ concentration of 0.5 μM. The results are plotted *versus* the theoretical values. A

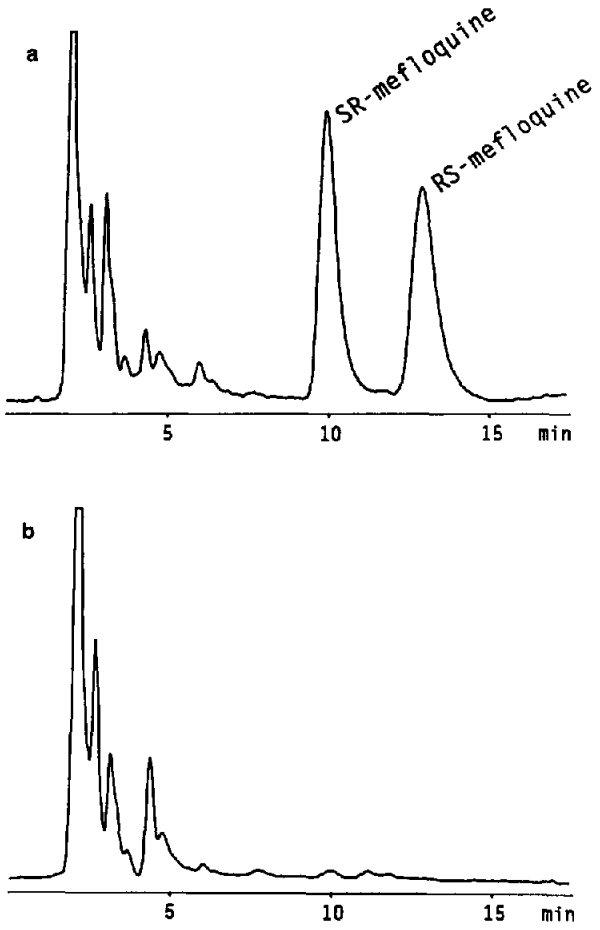

Fig. 1. (a) Chromatogram of extracted plasma containing racemic erythro mefloquine (0.5 μM). Column: Chiral-AGP (150 × 4.0 mm I.D). Eluent: 0.05 mM sodium phosphate buffer pH 4.85, 10% (v/v) 1-propanol. Flow-rate 0.9 ml/min. (b) Chromatogram of extracted blank plasma.

regression line is obtained with the equation $y = 0.9922x - 0.0069$ and a correlation coefficient of 0.993 (Fig 2a,b). The corresponding values for urine were $y = 1.0039x - 0.0034$ and 0.9996. The intercepts were negligible and the slopes were close to unity. This shows that the method gives an accurate estimate of the enantiomeric ratio (Table 1).

The concentration of a single enantiomer has to be at least 0.1 μM in plasma and urine to allow an accurate determination in a 1-ml sample.

No interferences were observed when spiked

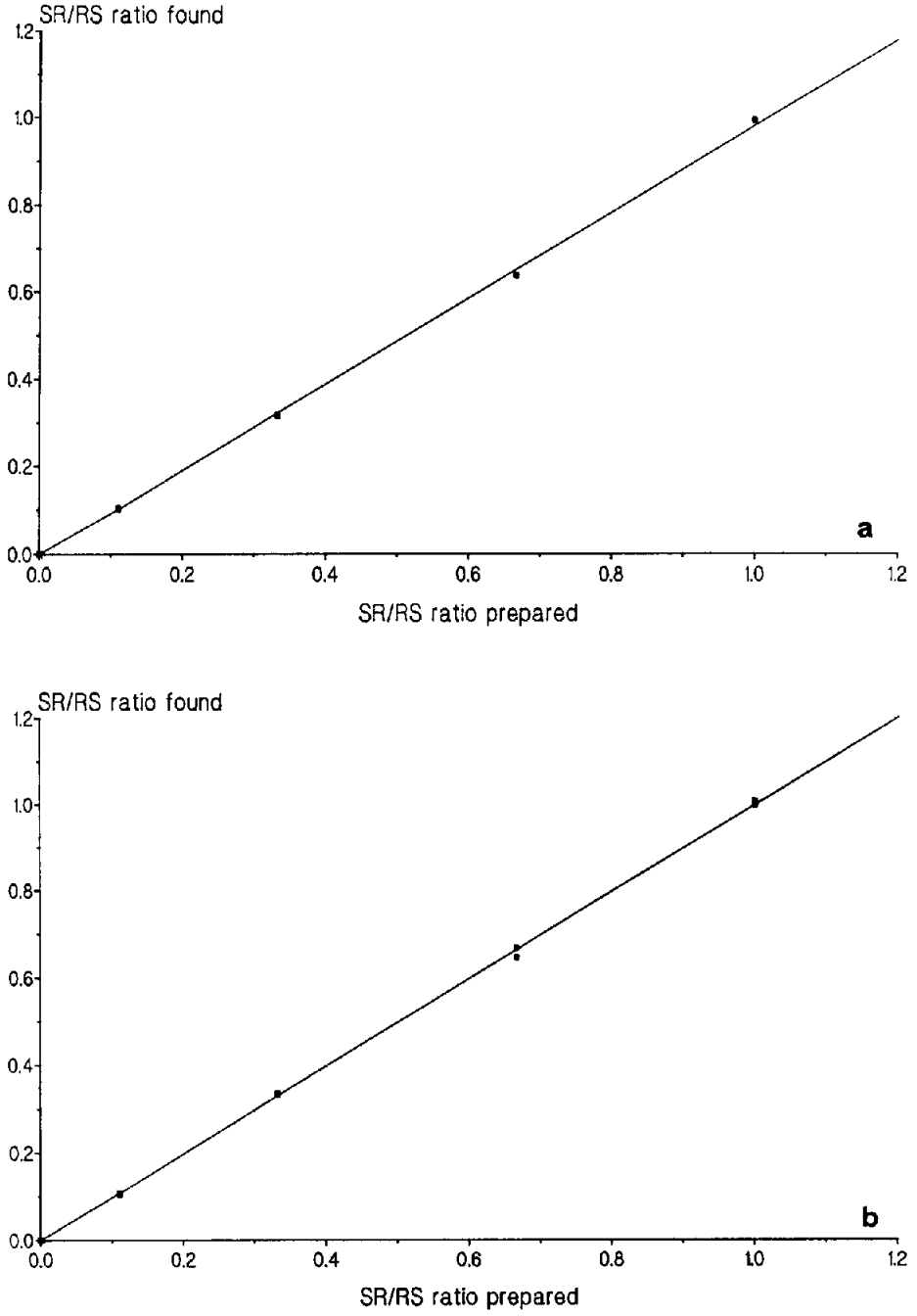

Fig. 2. Ratio of mefloquine enantiomers. (a) Plasma spiked with different ratios of the *SR*:*RS* enantiomers at a total mefloquine concentration of 0.5 μM. At a ratio of 0.1 the concentration of *SR* and *RS* was 0.05 and 0.45 μM, respectively. Duplicate samples were analysed. (b) Urine spiked with different ratios of the *SR* and *RS* enantiomers at a total mefloquine concentration of 0.5 μM. At a ratio of 0.1 the concentration of *SR* and *RS* was 0.05 and 0.45 μM, respectively. Duplicate samples were analysed.

Table 1
Precision and accuracy

SR-MQ (μM)	*RS*-MQ (μM)	Sample	*n*	Ratio *SR*/*RS*		C.V. (%)
				Prepared	Found	
1.50	1.50	Plasma	10	1:1	1.0:1	2.4
1.49	0.11	Plasma	9	15:1	17.0:1	4.0
1.45	0.05	Plasma	10	30:1	37.1:1	5.2
1.50	1.50	Urine	10	1:1	1.0:1	1.0
0.41	0.09	Urine	10	5:1	5.0:1	11.4

samples of commonly used antimalarials or other drugs were analysed.

Mefloquine is very stable towards decomposition and racemization. After one week at room temperature we could not detect any racemization in plasma or urine. We also kept urine and plasma samples frozen for 4 months without detecting any racemization. Sunlight had no influence on the stability of MQ which is in accordance with a previous report [9].

The present method determines the ratios between the erythro enantiomers at concentrations that are attained during treatment and prophylaxis. Several hundreds of samples from a group of travellers taking MQ as prophylaxis have been analyzed. No interfering peaks have been observed in the chromatograms despite the fact that many of the subjects were taking other drugs. The *SR* enantiomer concentration was 0.25–4.5 μM and the *RS* enantiomer 0.1–1.2 μM. In average the *SR*:*RS* ratio was 3.5 which illustrates the necessity to perform enantioselective pharmacokinetic studies. Similar results have been reported [6].

The present method is reliable, fairly simple and has sufficient sensitivity for clinical and pharmacokinetic studies.

4. Acknowledgement

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