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Short Communication

Improved column-switching liquid chromatographic method for the determination of the enantiomers of mefloquine

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ABSTRACT

A liquid chromatographic method for the determination of the enantiomers of mefloquine has been improved. The chromatography involved two columns: an achiral cyanopropyl stationary phase for the quantification of (+/−)-mefloquine and a chiral naphthylurea stationary phase for the determination of the enantiomeric ratio. Compared with the previous method, which needed two detectors, this one used one detector–integrator to which the two columns are connected alternately by an automated column-switching system. The method is suitable for the quantification (0.05 μg/ml) of mefloquine and the determination of enantiomeric ratios from 500-μl plasma samples with ultraviolet detection.

INTRODUCTION

Mefloquine (MQ) is an antimalarial agent commercialized as a racemic form of *erythro*-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol. MQ plasma concentra-

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tions have been determined using gas chromatographic (GC) [1–4], liquid chromatographic (LC) [5–9] and supercritical [10] methods. However, none of these methods was able to separate the enantiomers. Two chiral methods have been described. One assay [11] allowed the separation of the four diastereoisomers (*threo* and *erythro*) on an AS cellulose column, but it was not applied to biological samples. The other [12] described the separation of MQ enantiomers in plasma and whole blood on a (*S*)-naphthylurea column. It was based on a coupled achiral–chiral chromatographic method and used two complete chromatographic systems (pump, detector and integrator). We have developed a similar achiral–chiral method using a double switching system. Compared with the previous method, this one has the advantage of using only one detector–integrator system.

EXPERIMENTAL

Chemicals

Racemic MQ was a gift from Roche (Basel, Switzerland). MQ enantiomers were obtained using the Carroll and Blackwell method [13]. The internal standard quinidine (QN), *tert.*-butyl methyl ether and triethylamine were purchased from Aldrich-Chimie (Saint Quentin, France). *n*-Hexane was purchased from OSI (Paris, France). 2-Propanol and methanol were purchased from Carlo Erba (Paris la Défense, France).

Extraction procedure

MQ was extracted from plasma according to a modified version of the method of Mount *et al.* [9]:to 500 μl of plasma were added 50 μl of a methanolic solution of the internal standard QN (10 mg/l), 500 μl of a 20% aqueous solution of trisodic phosphate and 2 ml of *tert.*-butyl methyl ether. The mixture was vortex-mixed for 30 s and centrifuged at 2000 *g* for 10 min. The aqueous phase was frozen using dry ice in acetone, and the organic phase was decanted. A second extraction was carried out using an additional 2 ml of *tert.*-butyl methyl ether, and the organic phases were pooled and evaporated under gentle stream of nitrogen. The residue was reconstituted in 70 μl of mobile phase, and 50 μl were injected.

Because of the adsorption of MQ on glass, all extraction procedures were carried out using siliconed glass tubes (Terumo, Saint Quentin, France).

Apparatus

Achiral and chiral chromatography was performed with two Beckman Chromatem A110 pumps (Beckman, Gagny, France) connected to a Wisp 720 Waters autosampler (Millipore, Montigny-le-Bretonneux, France). The detection was performed with a Shimadzu SPD 6A detector, set at 285 nm, and a Shimadzu C-R6A integrator (Shimadzu, Touzart & Matignon, Vitry, France).

Analytical chromatography. The column used for the separation of MQ and the internal standard from biological matrix was a 5-μm Nucleosil cyanopropyl (250 mm × 4.6 mm I.D.) (S.F.C.C./Shandon, Eragny, France) with a mobile phase of hexane–2-propanol–methanol (82:4:14, v/v/v), modified with 125 μl of a solution of triethylamine diluted in methanol (1:40, v/v). Analyses were carried out at ambient temperature at a flow-rate of 2.0 ml/min.

The column used for the separation of MQ enantiomers was a chiral 5-μm (*S*)-naphthylurea column (250 mm × 4.6 mm I.D.) (S.F.C.C./Shandon) with a mobile phase of hexane–2-propanol–methanol (50:5:45, v/v/v) modified with 125 μl of a solution of triethylamine diluted in methanol (1:40, v/v). Analyses were performed at room temperature at a flow-rate of 1.5 ml/min.

Double switching achiral–chiral coupled system (Fig. 1). The two chromatographic systems were connected through an EPS 130 HP2P automatic switching system equipped with two Rheodyne 7000 switching valves and a 5-μm Kromasil silica guard column (60 mm × 4 mm I.D.) (Informatiques & Technologies, Le Blanc Mesnil, France).

The switching system was programmed by the Shimadzu C-R6A integrator, which was itself programmed by the autosampler. The integrator was also connected to the detector in order to programme an autozero.

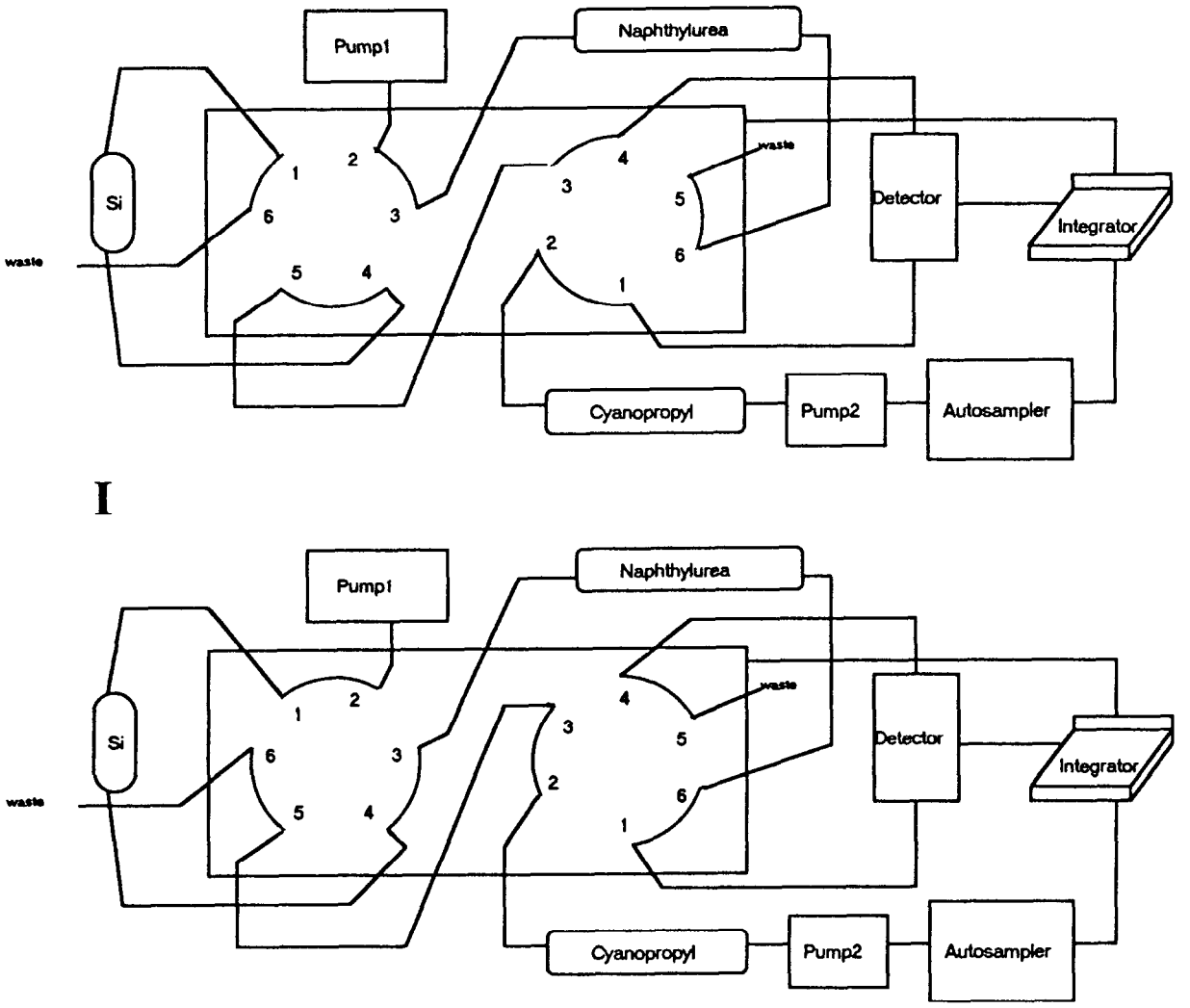

Fig. 1. Column-switching chromatographic system. (I) Configuration used to quantify (±)-MQ in the achiral cyanopropyl column and to load (±)-MQ into the silica guard column. (II) Configuration used to inject (±)-MQ from the guard column into the chiral (*S*)-naphthylurea column and to determine enantiomeric ratios.

After liquid extraction, the sample was injected into the achiral column with the system in position I (Fig. 1). MQ and the internal standard were detected and pumped to the guard column, where only MQ enantiomers were trapped. The double valve system was then switched in position II in order to backflush (±)-MQ into the chiral column and the detector. By switching the system in position II, the mobile phase of the achiral system was replaced in the detector by the mobile phase of the chiral system. The difference of absorptivity between the two phases was compensated by an autozero of the detector programmed by the integrator.

Both MQ enantiomers were then separated in the chiral column and the enantiomeric ratio was determined.

Method validation: achiral chromatography

Linearity. Standard curves were determined by spiking drug-free plasma with racemic MQ at the following concentrations: 0.2, 0.5, 0.75, 1.0, 1.25 and 1.5 μg/ml. This assay was performed in triplicate. The samples were analysed and evaluated by linear least-squares regression.

Precision and reproducibility. Five samples of the low (0.5 μg/ml) and the high (1.5 μg/ml) concentrations were analysed for the calculation of the coefficient of variation (C.V.). Analyses were carried out for intra-day and inter-day validation.

Accuracy. The accuracy of the method was investigated by analysing a control sample (1.0 μg/ml) prepared by another analyst during the study. Concentrations found were compared with theoretical values, and the percentage error was determined.

Recovery. The recovery of the method was calculated by comparing the peak areas of the samples of low (0.5 μg/ml) and high (1.5 μg/ml) concentrations after extraction with the peak areas of standard solutions at the same concentrations. These analyses were performed on three days.

Limit of quantification. Plasma samples were spiked with decreasing concentrations of MQ and analysed. This limit was considered to be the lowest concentration of MQ that could be measured with a stated level of confidence.

Method validation: chiral chromatography

During this same validation, enantiomeric ratios (+)-MQ / (−)-MQ for the racemic form were determined and compared with unity.

RESULTS AND DISCUSSION

Column switching system

When a column-switching system is used, different factors must be optimized: the nature and length of the guard column, the selectivity between the internal standard and the compound of interest, and the time of trapping into the guard column.

In this case, the nature and the length of the guard column had to be such as to trap and retain MQ, but to elute the internal standard QN as quickly as possible in the waste. We tested a cyanopropyl and a silica guard column. With the cyanopropyl guard column, QN was quickly eliminated into the waste; however MQ was only weakly retained and so could have been eluted before the switching. The selectivity between these two compounds had to be sufficient in order to prevent contamination of MQ with the internal standard. With a high selectivity factor between the two compounds ($\alpha = 2.5$), the internal standard QN was already eliminated into the waste at the time of MQ detection and switching.

The trapping time also had to be optimized, and was a compromise between increasing it in order to trap the maximum amount of MQ and decreasing it in order to prevent broadening of the enantiomer peaks after their elution in the chiral column. The system was switched from position I to position II 3 min after detection of the MQ peak (Figs. 1 and 2) and was switched back to position I just at the end of each analysis. The switching times were controlled by a time programme on the integrator.

Achiral chromatography

Under the chromatographic conditions described for this study, the internal standard QN and MQ were separated from the plasma matrix with capacity factors of 5.35 and 13.65, respectively.

Standard curves were linear over the range investigated. The equation describing the curve was $y = 0.621x + 0.015$, with a coefficient of correlation of 0.9974. The limit of quantification was 0.05 μg/ml (C.V. = 12.7%), and the percentage recoveries were 84% for low concentrations (0.5 μg/ml) and 77% for high concentrations (1.5 μg/ml).

The intra-day ($n = 5$) reproducibility had a C.V. of 6.1% for high concentrations (1.5 μg/ml) and 5.6% for low concentrations (0.5 μg/ml). The inter-day ($n = 5$) reproducibility had a C. V. of 8.9% for high concentrations (1.5 μg/ml) and 10.5% for low concentrations (0.5 μg/ml). The values obtained for the control sample (1.0 μg/ml) were 0.94–1.03 μg/ml, with a mean value of 0.97 μg/ml ($n = 8$).

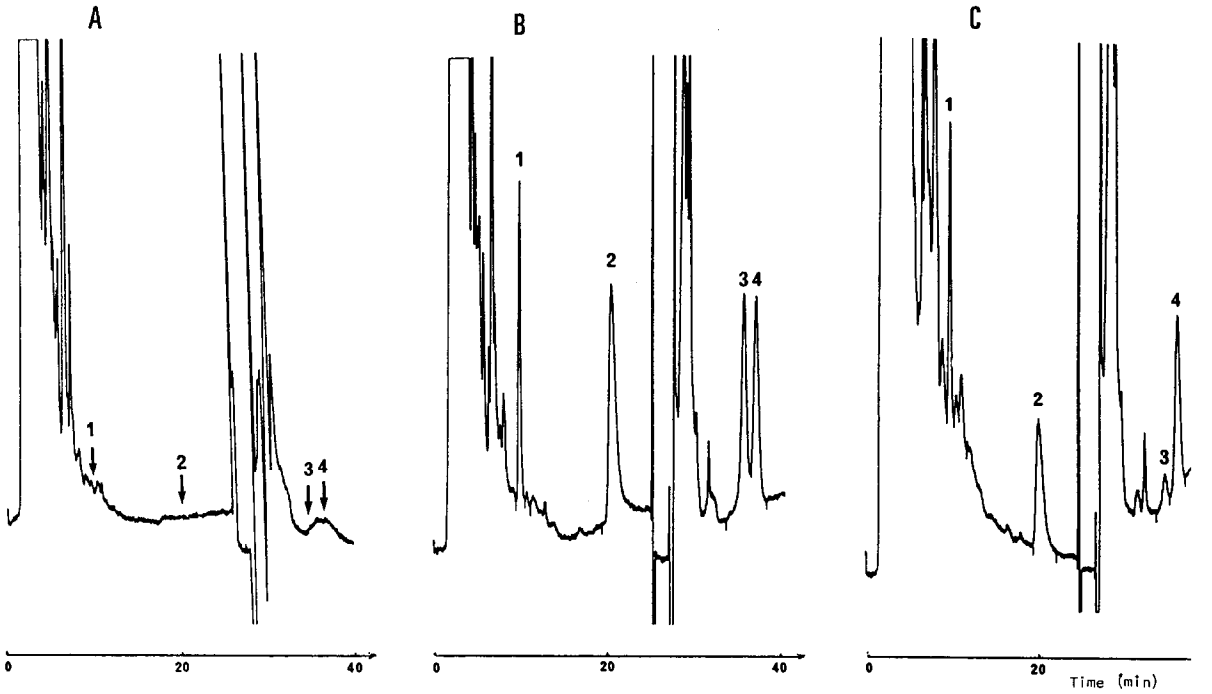

Fig. 2. Chromatograms of plasma samples obtained on the achiral–chiral column-switching system. (A) Blank plasma, (B) plasma spiked with 1.0 μg/ml of (±)-MQ; (C) plasma from a healthy volunteer after oral administration of repeated doses of 250 mg of racemic MQ. Peaks: 1 = internal standard (quinidine); 2 = MQ; 3 = (+)-MQ; 4 = (−)-MQ.

Chiral chromatography

The mobile phase used on the chiral column was more strongly eluting than that used on the achiral system (with a higher percentage of methanol). This compressed and sharpened both enantiomer peaks after backflushing. The elution order was determined by injecting the separated enantiomers into the chiral system. Under the chromatographic conditions used in this study, the capacity factors of (+)-MQ and (−)-MQ were 3.66 and 4.33, respectively. The selectivity factor was 1.18, and the resolution factor 2.7.

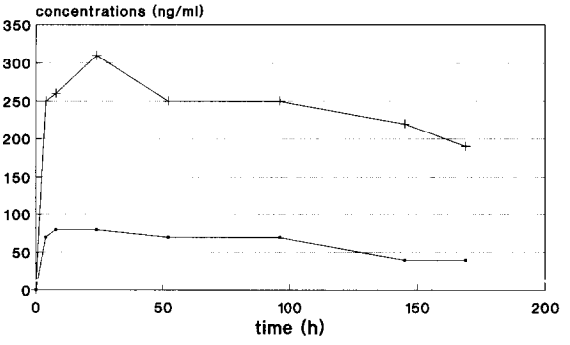

Fig. 3. Plasma concentration–time curves of the enantiomers after oral administration of 250 mg of racemic MQ: (■) (+)-MQ; (+) (−)-MQ.

During the validation, the enantiomeric ratio (+)-MQ / (−)-MQ varied from 0.97 to 1.02. Fig. 2 shows chromatograms obtained for biological samples (blank plasma, spiked plasma, and plasma after the administration of repeated 250-mg doses of racemic MQ) on the achiral–chiral system.

A plasma concentration–time profile of the enantiomers after oral administration of 250 mg of racemic MQ is shown in Fig. 3.

In conclusion, the described HPLC method is sensitive enough for the determination of the enantiomers of MQ in plasma at the usual dosage for preventive and curative treatments. Although

the system is complicated and analysis times are long (40 min for one analysis), it can be automated and used for pharmacokinetic studies.

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