ABSTRACT

Mefloquine, as a racemic mixture, is used for the treatment and prophylaxis of malaria. Stereoselective pharmacodynamic and pharmacokinetic differences have been observed for mefloquine. In this study a modified stereoselective HPLC method is presented for determination of mefloquine (MFQ) enantiomers in whole blood. The assay involved liquid-liquid extraction of MFQ from biological fluids with methyl tert-butyl ether in the presence of sodium hydroxide and derivatization of the residue by (+)-1-(9-fluorenyl) ethyl chloroformate (FLEC) as chiral derivatizing reagent. Separation of the resulting diastereomers was performed on a Nova pack C18 reversed-phase cartridge column using acetonitrile, water, glacial acetic acid (730:270:0.7, v/v/v) as the mobile phase with a flow-rate of 1 mL/min. Using 500 µL of whole blood, the limit of determination was 50 ng/mL with fluorescence detection with excitation at 263 nm and emission at 475 nm for both enantiomers. This method is comparatively simple and practical for the determination of small amounts of mefloquine enantiomers.

Keywords: Mefloquine, Enantiomer, Derivatization, Whole blood, RP-HPLC

MATERIALS AND METHODS

Chemicals and reagents. Racemic MFQ-HCl was purchased from Roche (Basel, Switzerland). The (-)-(SR)- and (+)-(RS)-MFQ enantiomers were resolved with (+)-3-bromo-8-camphorsulphonic acid ammonium salt (Aldrich, Milwaukee, WI, USA) according to Carroll and Blackwell [1]. The derivatizing reagent, (+)-1-(9-fluorenyl) ethyl chloroformate (FLEC) (18 mM in acetone) was purchased from Fluka (Buchs, Switzerland). The optical purity of the reagent was higher than 99.5%. All other chemicals and solvents were of either chromatographic or analytical reagent grade from Merck (Darmstadt, Germany).

Standard solutions. A stock solution of rac-MFQ was prepared by dissolving 43.85 mg MFQ-HCl in 10 mL methanol to a final concentration of 4 mg/mL. Standard solutions were prepared by subsequent dilution of the stock solution with methanol. All solutions were stored at +4°C.

A solution of 36 µM (+)-FLEC was prepared by diluting 200 µL 18 mM (+)-FLEC in acetonitrile. The reagent solution was stored at -20°C until used.
**Chemical structure of mefloquine (MFQ) and derivatizing reagent.**

Borate buffer (43 mM) was prepared by dissolving 0.26 g boric acid and 0.32 g potassium chloride in approximately 80 mL distilled water, adjusted to pH 8.5 with 1M sodium hydroxide solution and made to 100 mL volume with water.

**Table 1. Accuracy and precision in spiked blood (n= 9; three sets for three days)**

<table>
<thead>
<tr>
<th>Concentration added (ng/mL)</th>
<th>(+)-(SR)-Mefloquine</th>
<th>(+)-(RS)-Mefloquine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calculated (mean±S.D.)</td>
<td>%C.V.</td>
</tr>
<tr>
<td><strong>Intra-day (n=3)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>49.6 ± 5.8</td>
<td>11.6</td>
</tr>
<tr>
<td>100</td>
<td>100.2 ± 5.4</td>
<td>5.4</td>
</tr>
<tr>
<td>500</td>
<td>508.4 ± 22.9</td>
<td>4.5</td>
</tr>
<tr>
<td>2000</td>
<td>1976.6 ± 58.4</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>Inter-day (n=9)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>51.6 ± 7.0</td>
<td>13.5</td>
</tr>
<tr>
<td>100</td>
<td>100.5 ± 4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>500</td>
<td>505.1 ± 31.9</td>
<td>6.3</td>
</tr>
<tr>
<td>2000</td>
<td>1999.6 ± 47.1</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

The proposed HPLC technique is based on the method reported by Bergqvist et al [11] with some modifications. Derivatization of MFQ enantiomers was performed using (+)-FLEC instead of (-)-FLEC. Various columns and different combinations of mobile
phases were tested to find the optimum condition of chromatography. Best result was obtained by using Novapack C18 cartridge column and a mixture of acetonitrile, water and acetic acid (730:270:0.7, v/v/v) as mobile phase. Good baseline resolution of both enantiomers was achieved ($\alpha > 1.3$) without any interfering peak. In this system two unequal peaks of (-)-(SR)- and (+)-(RS)-MFQ was observed with retention times of about 10 and 12.7 min (Fig 2). The elution order of the diastereomers was identified using pure enantiomers. The fluorescence response of the (-)-(SR)-MFQ diastereomer was about 4.5 times higher than the (+)-(RS)-MFQ diastereomer.

![HPLC chromatograms of MFQ diastereomers](image)

**Fig 2.** HPLC chromatograms of MFQ diastereomers. (A) Drug free blood; (B) Blood sample spiked with MFQ enantiomers (1 $\mu$g/mL of each enantiomer). Peaks: 1, (+)-(SR)-MFQ diastereomer; 2, (+)-(RS)-MFQ diastereomer.

Optimal conditions of derivatization reaction were selected by studying the influence of (+)-FLEC concentration, borate buffer concentration, the reaction time and the temperature. The best result was achieved with 43 mM borate buffer and 36 $\mu$M (+)-FLEC as derivatizing reagent. The reagent concentration was about 1/10 of the reported concentration for (+)-FLEC. The reaction was allowed to proceed at different time intervals at room temperature. The maximum peak intensity of the diastereomers was observed after 40 min which is consistent with that reported by Bergqvist et al [11]. The standard calibration curves constructed by plotting peak areas of MFQ diastereomers against the concentrations of each enantiomer exhibited good linearity ($r^2 > 0.98$). Typical equations describing the linearity were $Y=278.7 \times X-3158.6$ for (-)-(SR)-MFQ and $Y=74.3 \times X+734.1$ for (+)-(RS)-MFQ over the range of 50-2000 ng/mL.

The accuracy and precision data for the determination of MFQ enantiomers in whole blood are presented in Table 1. The data shown in this table is in support of validation of the proposed method. The limit of determination of each enantiomer was 50 ng/mL with a C.V. better than 13.5% using 500 $\mu$L of whole blood.

### Conclusion

A modified indirect method for the determination of MFQ enantiomers in blood is reported using (+)-FLEC as derivatizing reagent. Based on a commercially available reagent and fluorescence detection, this method is practical and suitable for determination of small amounts of MFQ enantiomers for pharmacokinetic studies.

### References


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