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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF MEFLOQUINE AND ITS MAIN METABOLITE BY DIRECT PLASMA INJECTION WITH PRE-COLUMN ENRICHMENT AND COLUMN SWITCHING TECHNIQUES

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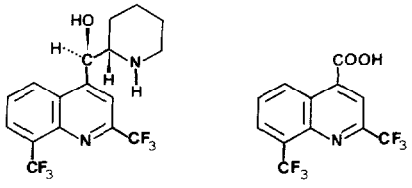
SUMMARY

For the simultaneous determination of mefloquine and its main metabolite from plasma using high-performance liquid chromatography with pre-column enrichment and column switching techniques, two parameters proved crucial: use of phosphoric acid for acidification of plasma samples to ensure pre-column extraction of mefloquine and its main metabolite; careful observation of the pH of the eluent to ensure baseline separation of mefloquine and metabolite and separation from interfering plasma peaks. The described method employs direct plasma injection, is rapid, specific and sensitive, with an analysis time of 15 min and column lifetimes of *ca.* 200 injections.

INTRODUCTION

Mefloquine, D,L-erythro-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol I, (Fig. 1) was synthesized for the treatment and prophylaxis of chloroquine-resistant falciparum and vivax malarias[1]. Giving a therapeutic dose of *ca.* 1 g of mefloquine, peak levels of *ca.* 1 mg/l unchanged drug and *ca.* 1 mg/l main metabolite 2,8-bis(trifluoromethyl)-4-quinolinecarboxylic acid (Fig. 1) are found in plasma.



Mefloquine Mefloquine metabolite

Fig. 1. Structure of mefloquine and its main metabolite.

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All published assays for the determination of mefloquine in plasma (thin-layer chromatography, high-performance liquid chromatography (HPLC)[1,9] gas chromatography, gas chromatography–mass spectrometry[2–7]) call for a time-consuming extraction step during sample preparation and prescribe the use of an internal standard. Only one describes the simultaneous determination of mefloquine and its main metabolite[3].

The aim of the present study was therefore to develop a rapid and precise method for the simultaneous determination of mefloquine and its major metabolite that is capable of handling large sample numbers in a suitable time for pharmacokinetic studies.

MATERIALS AND METHODS

Reagents

All reagents were of at least analytical grade. Acetonitrile (Lichrosolv), sodium sulphate (reagent grade) and phosphoric acid were obtained from Merck (Darmstadt, F.R.G.).

Apparatus

The chromatographic equipment consisted of a high-performance liquid chromatograph HP 1090 A with a UV diode array detector 1040 A (Hewlett-Packard, Böblingen, F.R.G.), a Latek column-switching valve HMV-P DUO and a Milton Roy minipump supplied by Latek (Heidelberg, F.R.G.). The chromatograms were evaluated using a Labor Automation System 3357 from Hewlett-Packard.

We used the column switching technique described by Roth *et al.*[8]. Following acidification of the samples (substances in water or plasma), 250 μl of sample were injected on a pre-column and purged with water adjusted to pH 3 with concentrated phosphoric acid. Backflushing with the mobile phase onto the analytical column yielded the respective chromatograms.

Sample preparation

Since mefloquine and its major metabolite differ vastly in their acidity, the impact of amount and nature of acid added to the samples on extraction efficiency was examined. We used perchloric, sulphuric or phosphoric acids in amounts between 0.12 and 1.2 mmol H^+ per 500 μl plasma sample.

Pre-column enrichment

For pre-column enrichment, C_{18}, phenethyl and diphenyl reversed-phase materials were used and column lengths ranging from 5 to 40 mm were tested. Purging was performed using water adjusted to pH 3 with phosphoric acid (86%, w/v) at a flow-rate of 0.8 ml/min for 3.5 min.

Analytical separation

In order to optimize the separation of mefloquine from its main metabolite and interfering substances, various acetonitrile–water mixtures were used as eluents, trying phosphate and acetate buffers as well as sodium sulphate as tailing reducers. The pH was adjusted between 2 and 6 using different amounts of phosphoric acid

(86%, w/v). Reversed-phase materials from different manufacturers were tested for suitability: C_{18}, C_8, C_2, phenyl and CN. The particle sizes were 3 and 5 μm, respectively. The column sizes were 60 mm × 4.6 mm I.D. in all cases.

Optimized method

Optimal results were achieved as follows.

Sample preparation. Mix 25 μl of phosphoric acid (40%, w/v) with 500 μl of plasma.

Pre-column enrichment. Pre-column: 10 mm × 4.6 mm I.D., Bonded-Phase-Phenethyl, 40 μm (J. T. Baker, Phillipsburg NJ, U.S.A.); mobile phase: water, adjusted to pH 3 with phosphoric acid (86%, w/v); flow-rate: 0.8 ml/min; purging time: 3.5 min.

Analytical separation. Column: 60 mm × 4.6 mm I.D., Nucleosil C_{18}, 3 μm (Macherey & Nagel, Düren, F.R.G.); mobile phase: water containing 50 mmol/l sodium sulphate adjusted to pH 2.84 with phosphoric acid–acetonitrile (67:33, v/v); flow-rate: 0.5 ml/min; detection: UV absorption at 220 nm; injection volume: 250 μl; external standardization.

RESULTS AND DISCUSSION

Since under physiological pH the metabolite of mefloquine is present mostly as carboxylate, only mefloquine is extracted from plasma by the pre-column (Fig. 2). The vast differences in polarity between the analytes were overcome by lowering the pH of the plasma samples. The addition of perchloric or sulphuric acid resulted in precipitation of plasma proteins together with mefloquine. Only by using phosphoric acid did we obtain clear plasma samples from which mefloquine and the metabolite could be extracted by the pre-column (Fig. 2).

The extraction yield for mefloquine and metabolite from plasma was measured against water samples. Table I gives the results for mefloquine and the metabolite. The extraction is practically quantitative over the whole concentration range of mefloquine; the metabolite is extracted with *ca.* 80% efficiency in the higher concentration range and quantitatively in the lower concentration range. Since calibration curves and recoveries were done with plasma samples, no correction was made for extraction yield.

The within-assay precision of the method is given in Table II. The coefficient of variation (C.V.) varies between 1.6 and 17%.

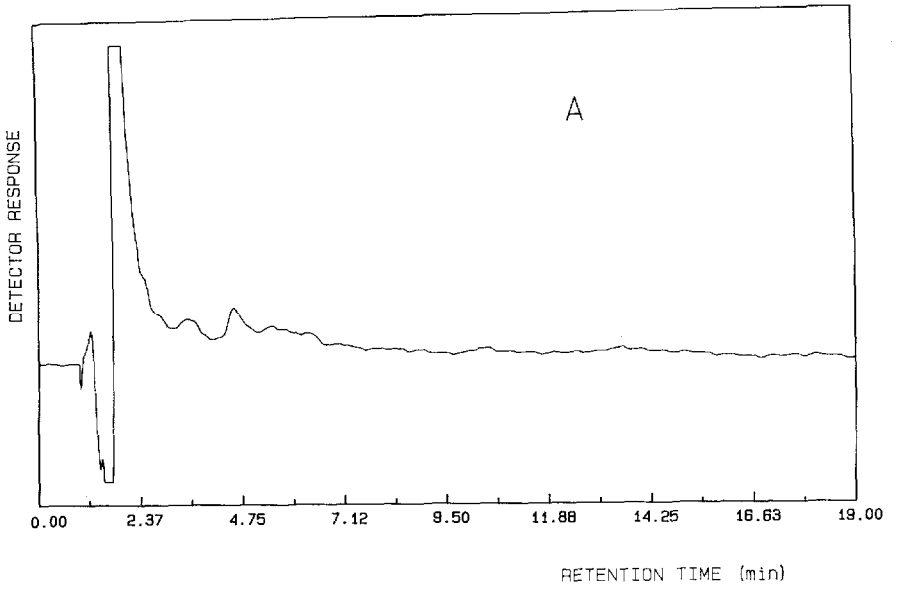
The method was calibrated in the ranges 0–1402 ng/ml (mefloquine) and 0–1281 ng/ml (metabolite), respectively, using eight points with six replicates each. Second-order polynomials were fitted to the calibration data according to the least-squares method. The equations of the best fits were:

mefloquine:

$$y = 5.8 + 1.509 \cdot 10^{-3}x - 0.540 \cdot 10^{-10}x^2$$

metabolite:

$$y = 9.2 + 1.447 \cdot 10^{-3}x - 1.26 \cdot 10^{-10}x^2$$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DETECTOR RESPONSE
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9.50
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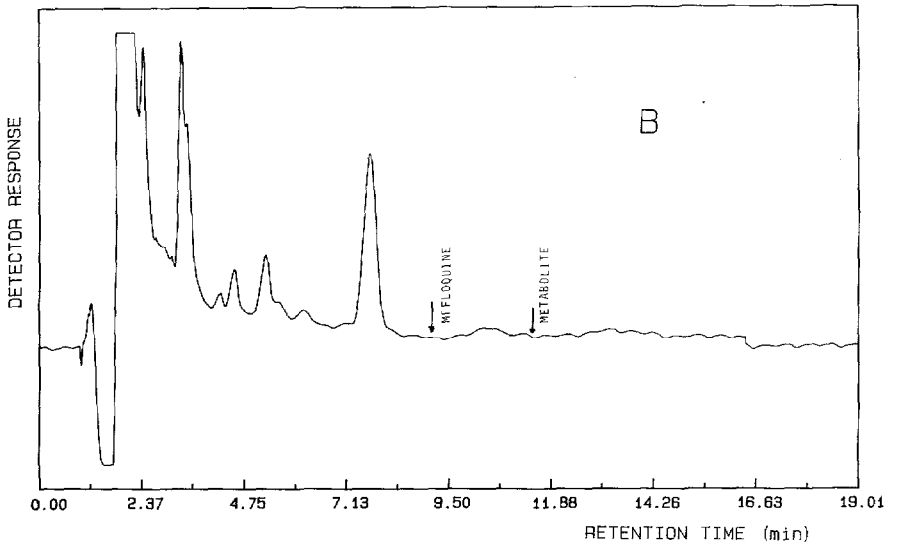
14.25
16.63
19.00
RETENTION TIME (min)


B
DETECTOR RESPONSE
MEFLOQUINE
METABOLITE
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RETENTION TIME (min)

Fig. 2.

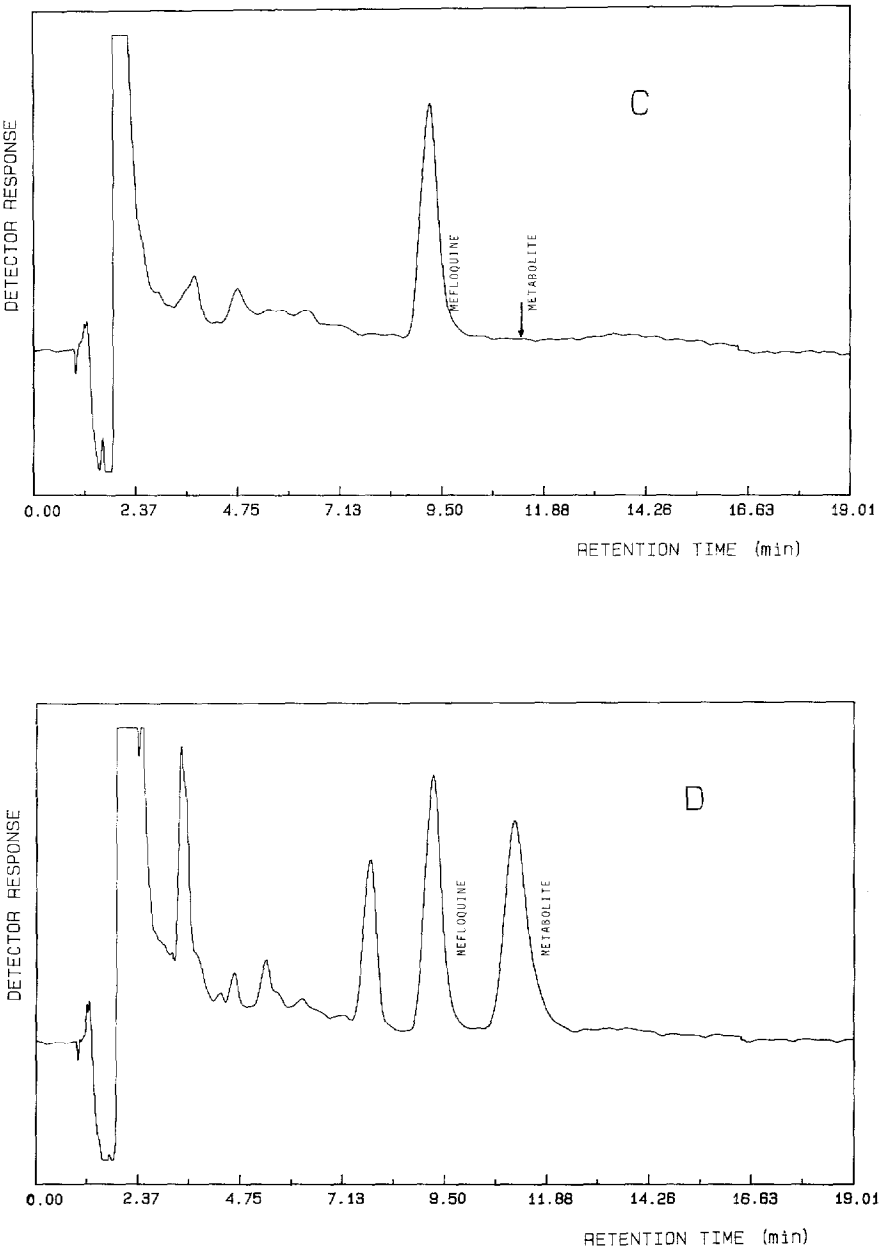
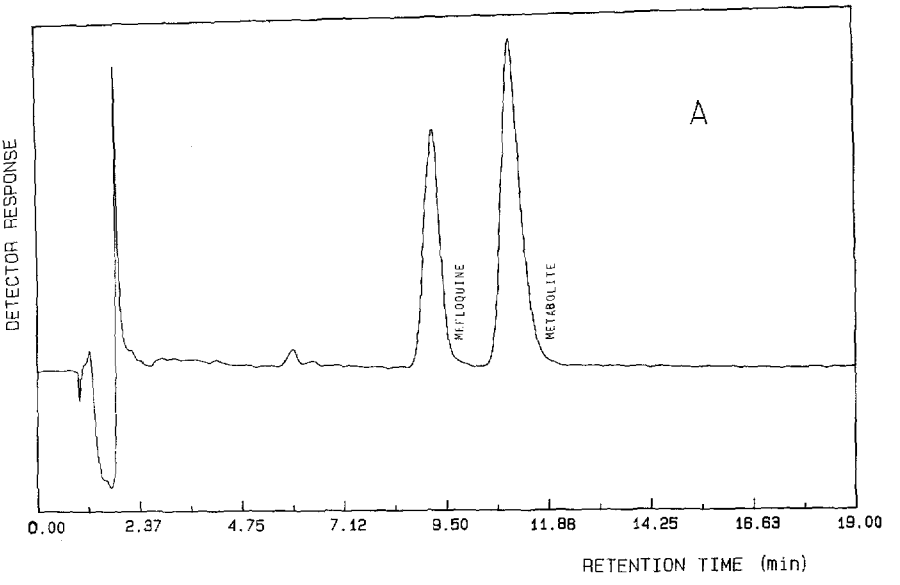


Fig. 2. Influence of addition of phosphoric acid to plasma on extraction yield. (A) Blank plasma without addition of phosphoric acid; (B) blank plasma with addition of 25 μl of phosphoric acid (40%, w/v) per 500 μl of plasma; (C) plasma sample containing 200 ng/ml mefloquine and 200 ng/ml metabolite without addition of phosphoric acid; (D) same plasma sample with addition of 25 μl of phosphoric acid (40%, w/v) per 500 μl of plasma. Conditions: pre-column: 10 × 4.6 I.D., mm; bonded-phase phenethyl, 40 μm; mobile phase: diluted phosphoric acid (pH 3.0) at 0.8 ml/min for 3.5 min; injection: 250 μl; analytical column: 60 × 4.6 mm I.D., Nucleosil C_{18}, 3 μm; mobile phase: 50 mmol sodium sulphate (pH 2.84)–acetonitrile (66:33) at 0.5 ml/min, oven temperature: 45°C; detection: UV 220 nm.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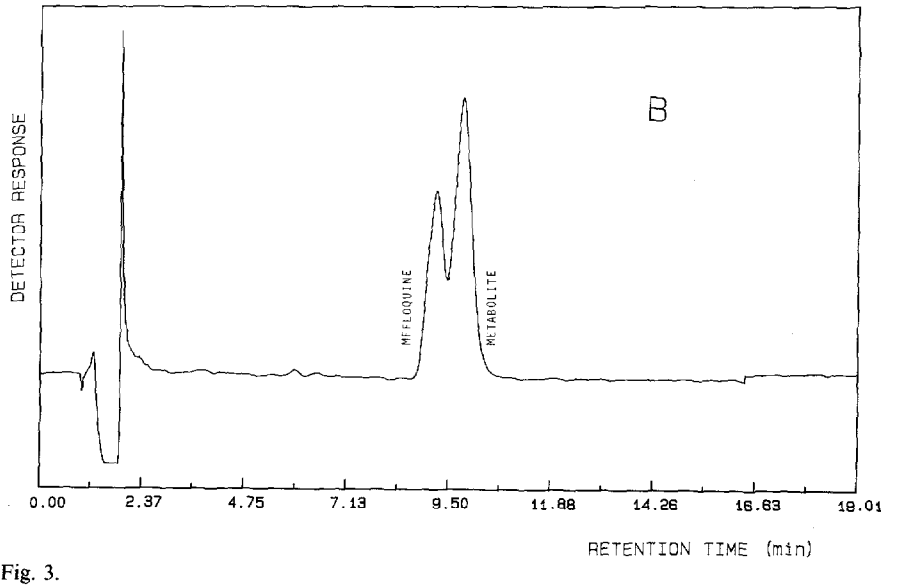
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Fig. 3.

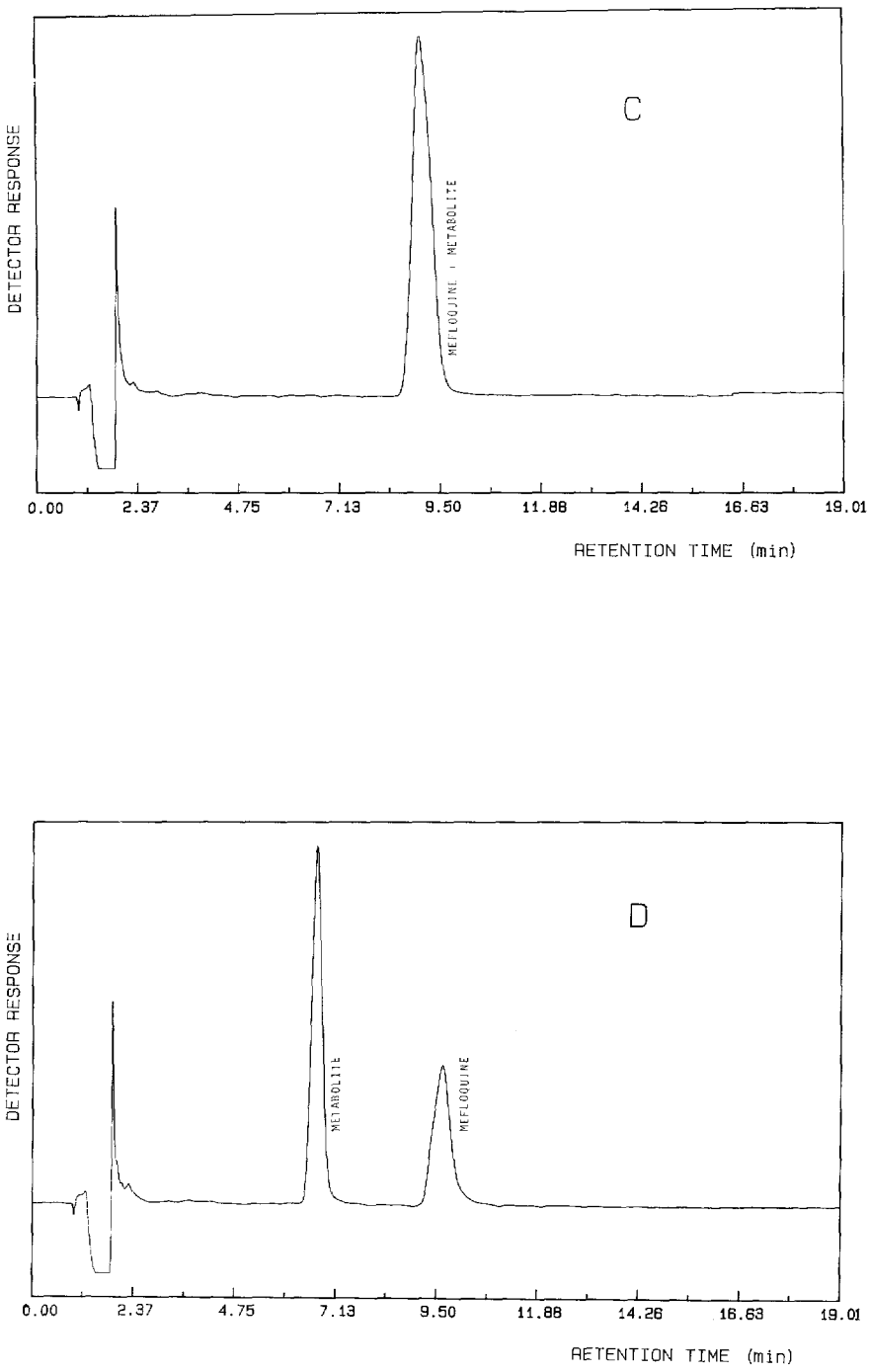

Fig. 3. Influence of pH of the eluent on the separation of mefloquine from its main metabolite. Sample containing 200 ng/ml mefloquine and 200 ng/ml metabolite in water. Eluent pH 2.84 (A), 2.9 (B), 3.1 (C), 3.5 (D). For conditions, see Fig. 2.

TABLE I

EXTRACTION YIELD

	Concentration (μg/l)	*Extraction yield (%)*	*C.V. (%)*	*n*
Mefloquine	14.0	102.9	4.9	5
	1402	95.3	2.7	6
Metabolite	12.8	102.1	5.1	5
	1281	79.0	0.5	6

TABLE II

WITHIN-ASSAY PRECISION FOR MEFLOQUINE AND METABOLITE

Mefloquine			*Metabolite*		
Concentration (μg/l)	*C.V. (%)*	*n*	*Concentration (μg/l)*	*C.V. (%)*	*n*
14	7.5	6	12.8	17.0	6
35.1	10.1	6	32	13.0	5
70.1	9.1	6	64.1	8.5	6
140.2	3.1	6	128.1	4.0	6
350.5	4.6	5	320.3	6.1	4
701	1.6	6	640.5	3.9	6
1402	1.6	6	1281	2.2	5

where y = plasma concentration (ng/ml) and x = peak area (arbitrary units). The 3-s limit of detection was calculated as 10 ng/ml for either substance.

Day-to-day accuracy was tested in a six-week period using 48 spiked plasma samples. The regression lines of concentration found *vs.* concentrations given were: mefloquine found = $(-4.39 \pm 4.9) + (1.040 \pm 0.008) \times$ given (ng/ml), $r = 0.9988$; metabolite found = $(-3.3 \pm 7.0) + (1.003 \pm 0.012) \times$ given (ng/ml), $r = 0.9967$.

CONCLUSION

For direct measurement of mefloquine and its major metabolite from plasma, using column switching techniques, two main points are essential: (i) addition of phosphoric acid to the plasma sample and (ii) pH adjustment of the eluent for the separation of mefloquine from its main metabolite (Fig. 3).

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