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Application of negative-ion chemical ionization isotope dilution gas chromatography–mass spectrometry to single-dose bioavailability studies of mefloquine

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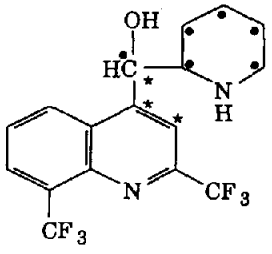
Abstract

An electron-capture negative-ion chemical ionization gas chromatographic–mass spectrometric assay for mefloquine, an antimalarial drug used in the treatment of drug-resistant *Plasmodium falciparum* malaria, is described. The method, developed in support of bioavailability studies involving the co-administration of different tableted formulations of the drug and an aqueous solution of its $^{13}C_3$-labeled analog, enables quantification of both dosage forms. Quantitative analysis of extracted plasma samples was performed on the O-*tert.*-butyldimethylsilyl (*t*-BDMS) derivative of the drug by selected-ion monitoring, using a VG Trio 2000 quadrupole mass spectrometer and monitoring the $[M - t\text{-BDMSOH}]^-$ ions of the analytes. The method, incorporating $[^2H_6]$mefloquine as an internal standard, demonstrated good accuracy and precision over the 1–200 ng ml^{-1} range, with correlation coefficients greater than 0.990 for all standard curves and a detection level of 50 fg on-column. Replicate analysis of plasma samples over a 90-day period exhibited a mean intra-day and inter-day variation of less than 4.5% and 5.5%, respectively. The high stability and sensitivity of the assay, combined with the inherent selectivity of mass spectrometric detection, make the method well-suited for such studies.

1. Introduction

The antimalarial drug mefloquine (I, Fig. 1), racemic *erythro*-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinoline-methanol, is formulated in Europe and the United States and has been proven effective against chloroquine- and quinine-resistant strains of *Plasmodium falciparum*. To evaluate the possible differences in the bioavailability of these two tableted dosage forms, each of the unlabeled formulations was orally co-administered with an aqueous solution



I

(*) [$^{13}C_3$]mefloquine
(•) [$^2H_6$]mefloquine

Fig. 1. Structure of mefloquine (I).

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of isotopically-labelled [$^{13}C_3$]mefloquine to adult subjects and blood samples drawn over a 84-day period. Such an evaluation requires an analytical method capable of distinguishing between the administered labeled and unlabeled forms of mefloquine at drug concentrations as low as 1 ng ml^{-1}.

Of the several gas chromatographic (GC) methods that have been developed for the analysis of mefloquine [1–5], only that of Schwartz and Ranalder has incorporated mass spectrometric (MS) detection techniques [6]. However, the loss of specificity and sensitivity due to the low abundance of high-mass ions generated by this electron ionization (EI) GC–MS method has precluded EI from use with regard to the positions in the molecule readily accessible for ^{13}C isotopic labeling and the sensitivity requirements of the assay. This paper describes an electron-capture negative-ion chemical ionization (NCI) automated GC–MS assay for the quantitation of mefloquine and [$^{13}C_3$]mefloquine in human plasma, utilizing a stable isotope-labeled internal standard ([$^2H_6$]mefloquine) and offers an assessment of the sensitivity, precision and accuracy of the technique in the quantification of these compounds.

2. Experimental

2.1. Chemicals and materials

[$^{13}C_3$]Mefloquine (>95 atom% $^{13}C_3$) from the same lot as that administered to subjects and [$^2H_6$]mefloquine, employed as internal standard, were used (weight corrected to free base) as supplied by Hoffman-La Roche (Nutley, NJ, USA) as their hydrochloride salts. The $^2H_6$ compound was 30.9% of the $^2H_6$ isotopomer and 0.3% $^2H_2$, 3.5% $^2H_3$, 8.7% $^2H_4$, 25.9% $^2H_5$, 19.7% $^2H_7$, 8.4% $^2H_8$, 2.4% $^2H_9$ and 0.3% $^2H_{10}$. HPLC-grade acetonitrile and reagent-grade methanol, ethyl acetate and anhydrous diethyl ether were obtained from J.T. Baker (Phillipsburg, NJ, USA). N-Methyl-N-(*tert.*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) containing 1% *tert.*-butyldimethylchlorosilane (*t*-BDMCS) was purchased from Regis (Morton Grove, IL, USA). Methane (99.99% pure), used as the moderating gas for NCI, was procured from Airco Industrial Gases (Murry Hill, NJ, USA). Extraction was performed using glass screw-cap culture tubes (16 × 125 mm) with Teflon-lined caps and disposable culture tubes (16 × 125 mm) without special cleaning considerations. Final evaporation and derivatization was carried out using fine-tipped glass disposable concentration tubes purchased from Lab Research (Lomita, CA, USA).

2.2. Instrumentation

GC–MS analysis of the derivatized extracts was performed on a VG Trio 2000 quadrupole mass spectrometer (VG BioTech, Cheshire, UK) fitted with an Hewlett-Packard 5890 Series II gas chromatograph equipped with a capillary splitless injector and an Hewlett-Packard 7673A autosampler (Hewlett-Packard, Palo Alto, CA, USA). A DB-5 fused-silica capillary gas chromatograph column (30 m × 0.32 mm I.D., 0.25 μm film thickness) was used (J and W Scientific, Rancho Cordova, CA, USA), and operated with helium (head pressure, 10 psi) as carrier gas with a 3 ml min^{-1} septum purge through the injector. Samples were injected in the splitless mode (injector temperature, 250°C) and the solute cold-trapped on the column at 140°C. After one minute, injector purge was initiated and the column oven temperature programmed linearly to 290°C at a rate of 30°C/min and held for 1 min.

The mass spectrometer was operated in the electron-capture NCI mode, using methane as a moderating gas (~1 Torr), with a filament emission current and electron energy of 200 μA and 70 eV, respectively. The ion source temperature was 200°C and the GC–MS interface was held at 300°C. NCI response was optimized as a function of source temperature and pressure using the O-*t*-BDMS derivative of mefloquine. The selection of the ion windows (+/−0.25 Da) for selected-ion monitoring (SIM) data acquisition and daily tuning of the quadrupole was accomplished using a 486DX50 PC and VG Lab-Base software. Ions monitored were *m/z*

360.1, 363.1 and 366.1, corresponding to the $[M - t\text{-BDMSOH}]^-$ of mefloquine, $[^{13}C_3]$mefloquine and the $[^2H_6]$mefloquine internal standard, respectively. The dwell-times were set at 300 ms per ion.

2.3. Sample preparation

After thawing and mixing by inversion, 1-ml aliquots of plasma samples were transferred to screw-capped tubes to which 50 μl (50 ng) of $[^2H_6]$mefloquine internal standard had been previously added. After addition of 2 ml of methanol–acetonitrile (1:1, v/v), the tubes were capped and horizontally shaken for 10 min, (reciprocation rate ~270 cycles/min), to effect protein denaturation. After centrifugation for 10 min at 560 *g*, the supernatant was decanted into a clean screw-capped tube. Extraction was accomplished by the addition of 2 ml of 0.2 *M* potassium phosphate buffer (pH 8.3) followed by 7.5 ml of ethyl acetate–diethyl ether (1:1, v/v) and shaking and centrifuging as described in the previous steps. The upper organic layer was transferred to a disposable culture tube, dried by vortex-mixing with ~0.5 g of magnesium sulfate and decanted into a concentration tube after centrifugation for 10 min at 560 *g*. Following evaporation to dryness under dry nitrogen, the samples were exposed to vacuum (~0.2 Torr) for 0.5 h, capped and stored at room temperature. For the generation of standard curves, 50-μl aliquots each of seven methanolic stock solutions containing equal amounts of mefloquine and $[^{13}C_3]$mefloquine at concentrations of 0.00, 0.02, 0.10, 0.20, 1.00, 2.00 and 4.00 μg ml^{-1}, respectively, were added to 1-ml aliquots of blank plasma and processed as above. In addition to the seven plasma standards, three quality assurance (QA) plasma samples were assayed to monitor the precision of the method for each tray of samples. Stocks of these samples, containing 5, 90 and 150 ng ml^{-1} of the mefloquine analytes, were stored at −20°C and processed throughout the study. Prior to GC–MS analysis, a solution of 15% MTBSTFA in acetonitrile (45 μl) was added to each sample. After vortex-mixing, the derivatized extracts were transferred to 100-μl glass liners, which were sealed in autosampler vials, for subsequent GC–MS analysis using 1-μl injections. Typically, each sample tray would contain 93 vials: seven standards in duplicate (for calibration purposes), two sets of the three QA samples and 71 unknowns. The total cycle time between injections was 10.3 min. Individual ion intensities were integrated using standard VG Lab-Base software. Response ratios, based on the observed peak area for each analyte relative to the internal standard, were calculated and used for the generation of standard curves and the determination of the analyte concentration in patient and control samples.

3. Results and discussion

Formation of the O-*tert.*-butyldimethylsilyl derivative using a 15% solution of the MTBSTFA in acetonitrile was found to be facile, proceeding to completion within 15 min at room tempera-

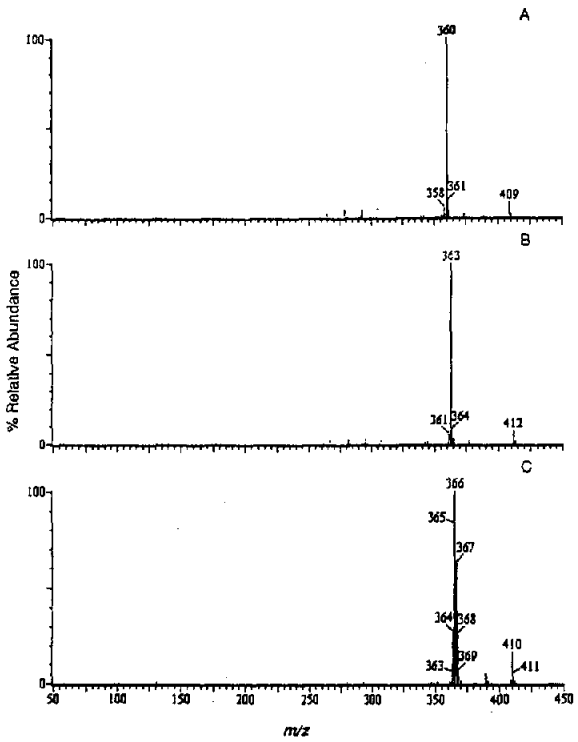

Fig. 2. Mass spectra of the *tert.*-butyldimethylsilyl derivatives of (A) mefloquine, (B) $[^{13}C_3]$mefloquine and (C) $[^2H_6]$mefloquine (internal standard).

ture with no evidence of derivatization of the piperidyl amine. In addition, the *t*-BDMS ether was found to be stable in the final solution for at least 7 days at room temperature without measurable degradation, unlike the TMS derivative reported by Heizmann and Geschke [2].

The methane NCI mass spectra of the derivatized mefloquines showed no molecular ions (Fig. 2). Intense fragment ions corresponding to the neutral loss of the *tert.*-butyldimethylsilyl alcohol ($[M - t\text{-BDMSOH}]^{-\cdot}$) were found at m/z 360, 363 and 366 in the mass spectra of mefloquine ($M_r = 492$), ($[^{13}C_3]$) ($M_r = 495$) and ($[^2H_6]$) ($M_r = 498$), respectively. Exhibiting no loss of label, these ions were chosen for quantitation by the SIM method. Less intense fragment ions corresponding to the loss of 1,2-dehydropiperidine ($[M - C_5H_9N]^{-\cdot}$) from the molecular ions of mefloquine and respective labeled analogs, were found at m/z 409, 412 and 410. The mass shifts of 3 and 1 Da associated with the appearance of the $[M - C_5H_9N]^{-\cdot}$ fragment ion in the spectra

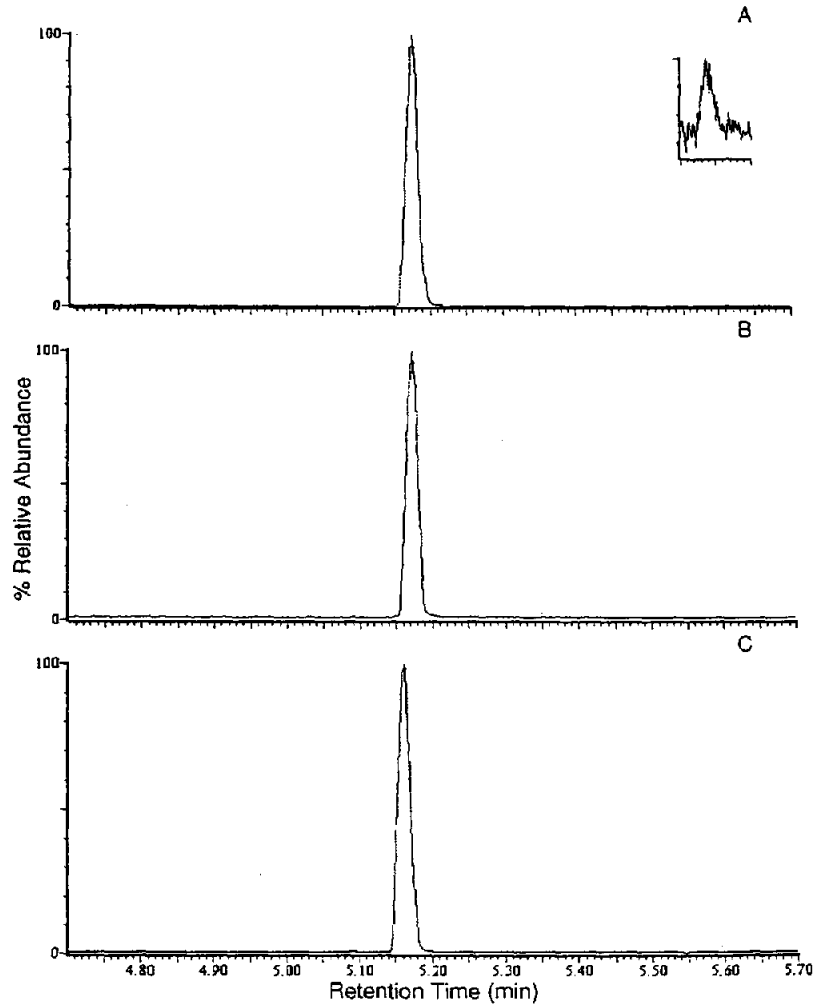

Fig. 3. Selected-ion current chromatograms of a derivatized plasma extract. The ions monitored and compounds being monitored in each trace are as follows: (A) m/z 360, mefloquine at a concentration of ~2 ng ml^{-1}, inset: signal for 50 fg of mefloquine (S/N ~3:1); (B) m/z 363, $[^{13}C_3]$mefloquine; and (C) m/z 366, $[^2H_6]$mefloquine (internal standard).

for [$^{13}C_3$] and [$^{2}H_6$]mefloquine, respectively, were consistent with the known sites of isotope incorporation (see Fig. 1) for these synthesized analogs.

Representative selected-ion current chromatograms obtained from the analysis of the plasma taken from an adult subject during the bioavailability study are depicted in Fig. 3. The *t*-BDMS derivative has good chromatographic properties, giving a sharp peak with no observed interferences in a reasonable gas chromatographic run time, allowing the analysis of a complete sample tray (93 + samples) within a 16-h period. The inset in Fig. 3A exemplifies the high detection sensitivity in NCI for this compound with a detection limit of ~50 fg injected.

For the generation of standard curves, peak-area ratios of the analytes to the internal standard were plotted against the known mefloquine concentrations. Over the concentration range 1–200 ng ml^{-1} for both analytes the standard curves were found to be linear with correlation coefficients greater than 0.990 in all cases. Typical standard curves for mefloquine and [$^{13}C_3$]mefloquine in plasma are shown in Fig. 4. As expected, the observed isotopic contribution of $^{2}H_3$ in the internal standard (Fig. 2C) to the measured abundance at *m/z* 363 resulted in a significant positive *y*-intercept for the standard curves for [$^{13}C_3$]mefloquine, corresponding to the ratio of *m/z* 363 to *m/z* 366 in the [$^{2}H_6$]mefloquine used in this assay.[7]

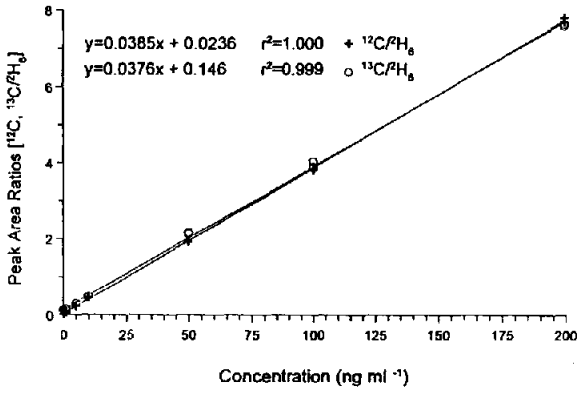

Fig. 4. Representative calibration curves for mefloquine and [$^{13}C_3$]mefloquine in plasma. Peak-area ratios were obtained by monitoring the $[M-132]^{-}$ ions of the *t*-BDMS derivatives of the analytes (*m/z* 360 and 363) and [$^{2}H_6$]mefloquine (*m/z* 366).

Assay precision and the recovery of the analytes were determined by the use of quality assurance (QA) plasma samples prepared and stored as described earlier. The intra-day precision was calculated from five sets of QA samples analysed over a 24-h period, whereas the inter-day precision was obtained by the analysis of QA samples as unknowns on different days, using each day's calibration over a three-month period. The intra- and inter-day mean coefficients of variation for the assay were found to be 4.2% and 4.9% for mefloquine; and 4.5% and 5.5% for [$^{13}C_3$]mefloquine, respectively, in the range 5–150 ng ml^{-1}. Precision data and accuracy data are summarized in Table 1. Recovery was assessed by comparing the response ratios of mefloquine and its $^{13}C_3$-analog to internal standard for two sets of six 90 ng ml^{-1} QA samples differing in work-up by the pre- and post-extraction addition of [$^{2}H_6$]mefloquine. The respective mean recoveries of these compounds using this method were found to be 81.0 ± 1.7% and 82.0 ± 3.9%.

The plasma concentration vs. time curves resulting from the oral co-administration of mefloquine and [$^{13}C_3$]mefloquine to a subject participating in this bioavailability study are shown in Fig. 5. Data for concentrations exceeding 200 ng ml^{-1} were obtained by reducing the volumes of plasma used in the analysis to ensure that the response fell within the linear range of the calibration curve. As may be seen, the absorption, distribution (Fig. 5, inset) and elimination phases of the drug are clearly discernable.

4. Conclusions

An NCI GC–MS method has been developed and validated, which allows the quantification of mefloquine and its $^{13}C_3$ analog as their *t*-BDMS ethers from plasma. The increased stability of this derivative over its TMS counterpart makes it well-suited for other GC detection methods. The high sensitivity and specificity of the NCI GC–

Table 1
Intra- and inter-day coefficients of variation for mefloquine and [$^{13}C_3$]mefloquine in plasma control samples

Nominal concentration (ng/ml)	Intra-day variation ($n = 5$)		Inter-day variations ($n = 20$)	
	Mean (ng/ml)	C.V. (%)	Mean (ng/ml)	C.V. (%)
150.00	140.7 ± 3.95 (141.5 ± 2.91)	2.81 (2.05)	148.6 ± 6.1 (153.2 ± 7.2)	4.1 (4.7)
90.00	94.5 ± 6.99 (95.2 ± 7.31)	7.39 (7.69)	96.6 ± 4.2 (94.5 ± 2.8)	4.3 (3.0)
5.00	5.81 ± 0.13 (5.68 ± 0.21)	2.25 (3.66)	5.7 ± 0.4 (5.8 ± .05)	6.2 (8.8)

MS detection method in combination with the exact control of sample recovery, using [$^2H_6$]mefloquine as an internal standard, provides precise and accurate determination of the analytes, with a detection limit of 50 fg on-column. The described assay has proven to be rapid and reliable, and has been utilized to measure mefloquine concentrations ranging from 1 to 300 ng ml^{-1} in over 1000 plasma samples during a three-month period.

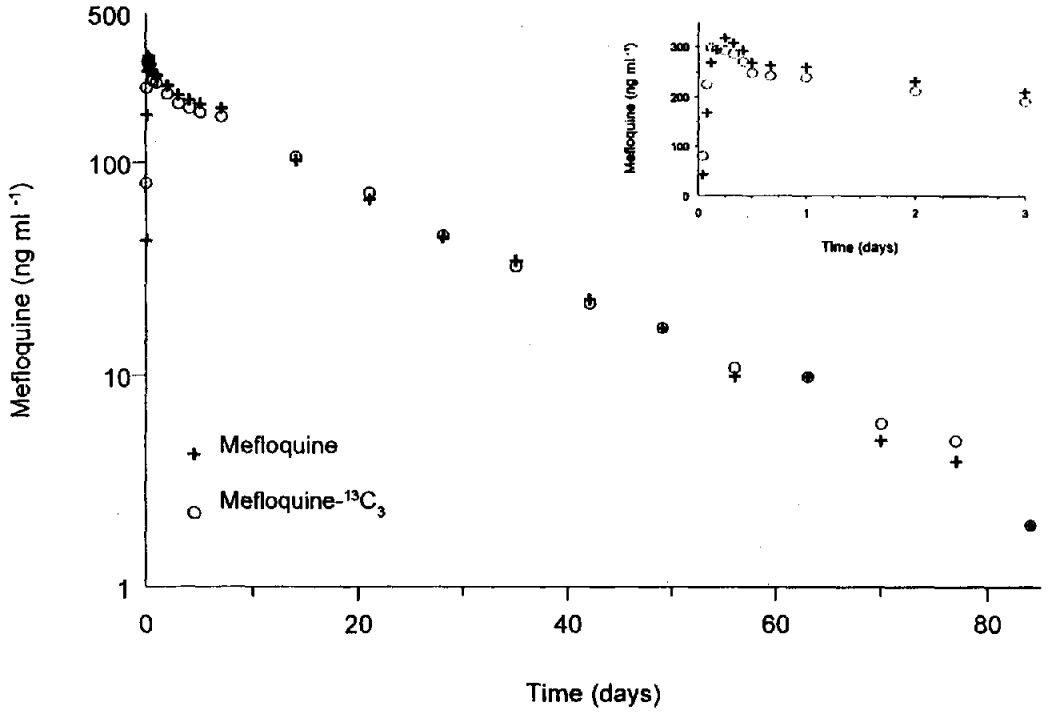

Fig. 5. Semi-log plot of mefloquine plasma concentration (ng ml^{-1}) vs. time (days) after oral co-administration of a tableted mefloquine formulation and its $^{13}C_3$ analog in water. Inset: magnification showing early time course.

Acknowledgement

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