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Note

Determination of primaquine in biological fluids by reversed-phase high-performance liquid chromatography

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Since its introduction in the 1950s primaquine, (8-(4-amino-1-methylbutylamino)-6-methoxyquinoline), has been the drug of choice to treat the tissue schizont stage of *P. vivax* and *P. ovale* malarias. Primaquine levels have been measured in plasma and urine by gas chromatography (GC) [1] and gas chromatography—mass spectrometry (GC—MS) [2]. These methods are complex and require lengthy sample treatment. MS is, in addition, inherently expensive. A high-performance liquid chromatographic (HPLC) method for the determination of primaquine in biological fluids has been developed which is suitable for use in clinical pharmacokinetic studies.

MATERIALS AND METHODS

Chemicals

Primaquine diphosphate was supplied by Aldrich (Gillingham, U.K.) and the internal standard 8-(3-amino-1-methylpropylamino)-6-methoxyquinoline was a gift from the Walter Reed Army Medical Research Centre (Washington, DC, U.S.A.), [^{14}C]primaquine (Specific activity 1.55 mCi/mmol) was synthesised by New England Nuclear (Boston, MA, U.S.A.). Dimethyldichlorosilane, toluene, 880 ammonia and orthophosphoric acid of analytical grade were obtained from B.D.H. (Poole, U.K.). Octanesulphonic acid was supplied by Aldrich. All other reagents were of HPLC grade (Rathburn Chemicals, Walkerburn, U.K.). Glass Culture (Sovirel) tubes and polytetrafluoroethylene (PTFE) lined screw caps were supplied by V.A. Howe (London, U.K.).

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Chromatography

The method was developed on a Spectra Physics liquid chromatograph. The system consisted of an SP 8700 solvent delivery system with an SP 8750 organiser module equipped with a Rheodyne valve injection system and coupled to an SP 8300 fixed-wavelength UV absorbance detector fitted with a 254-nm filter. The separation was carried out on a Partisil ODS III (10 μm particle size) reversed-phase column (20 cm × 0.6 cm O.D., HPLC Technology, Wilmslow, U.K.). The mobile phase consisted of water—acetonitrile—methanol (60:30:10) containing octanesulphonic acid (5×10^{-4} *M*) as an ion-pair reagent, buffered to pH 3.5 with orthophosphoric acid and flowing at 1.5 ml/min.

Extraction procedure

The extraction was carried out in 10-ml capacity glass culture tubes pretreated with dichlorodimethylsilane in toluene (5%, v/v) in order to minimise adsorption. To samples of plasma or urine (0.5—1.0 ml) containing internal standard (0—200 ng) was added ammonia solution (0.88 specific gravity, 2 ml), followed by vortex mixing for 30 sec. This mixture was extracted twice by mechanical tumbling for 10 min with a combination of hexane and ethyl acetate (9:1, total volume 5 ml). After centrifugation (1000 *g* for 10 min) and separation, the organic phases were combined, evaporated to dryness under a steady stream of nitrogen and reconstituted in methanol (50 μl). An aliquot of 5—25 μl was injected onto the column.

Standard curves

Standard curves were prepared by adding known quantities of primaquine (5—200 ng) to a fixed concentration of internal standard (100 ng) in drug-free plasma or urine. Samples were analysed as described above and the peak height ratio of drug to internal standard was plotted against the corresponding weight ratio. Peak height ratios of unknown samples were similarly determined and concentrations calculated from the standard curve. The extraction efficiency of primaquine was calculated from the recovery of ^{14}C-radioactivity following extraction of plasma or urine spiked with [^{14}C]primaquine (10,000 dpm/ml).

Volunteer study

Four healthy male volunteers aged 24—45 years, who were taking no other drugs, each received 45 mg primaquine orally after an overnight fast. Venous blood samples were taken pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 11 and 24 h. Blood was centrifuged (1000 *g* for 15 min) and the plasma removed and stored at −20°C until the time of analysis. Urine was collected for 1 h pre-dose and from 0—24 h. Urine volume and pH were measured and a sample was frozen and stored (−20°C). All samples were covered with aluminium foil to protect them from the light.

Calculations

The peak plasma concentration of primaquine and the time to attain peak plasma concentrations was measured by inspection and the plasma elimination

half-life was calculated by regression analysis of the post-distributive log linear portion of the plasma concentration versus time curve.

RESULTS AND DISCUSSION

The extraction procedure resulted in simple sample preparation. Chromatograms of an extract of pre-dose plasma sample and a plasma extract obtained after a single 45-mg oral dose of primaquine are shown in Fig. 1 (A and B). The plasma extract from the volunteer receiving primaquine showed a distinct peak with a retention time of 7 min 36 sec corresponding to primaquine (120 ng/ml). This peak was completely resolved from that of the internal standard, retention time 5 min 12 sec. The peak eluting prior to the internal standard was an impurity in the internal standard stock and was not observed in extracts of blank plasma. Chromatograms of blank urine and a urine specimen collected after a single oral dose of primaquine are shown in Fig. 2 (A and B).

The acetylated and carboxylated metabolites of primaquine described by Baker et al. [3] did not interfere with the assay. Although these metabolites appeared in the chromatograms of aqueous stock solutions at retention times of 14 min and 17 min, respectively, they did not appear in any plasma or urine extracts possibly due to poor analytical recoveries under the conditions of the extraction.

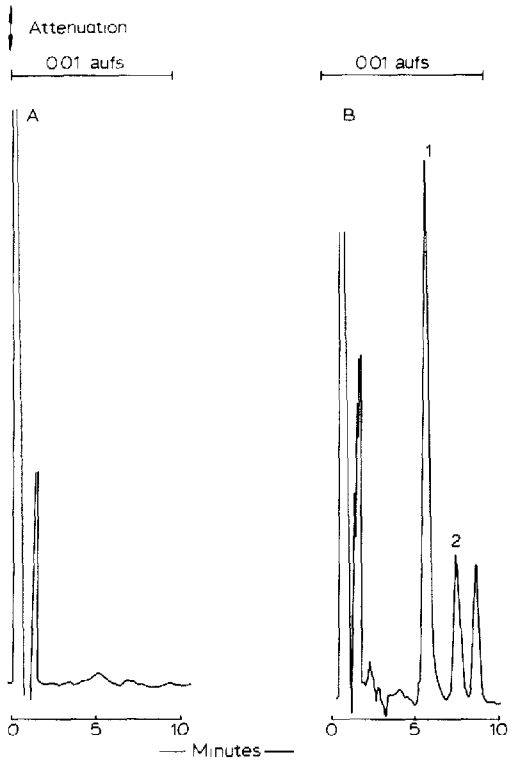

Fig. 1. Chromatograms of a blank plasma extract (A) and an extract of plasma obtained after a single 45-mg oral dose of primaquine (B) (primaquine, 120 ng/ml). Peaks: 1 = internal standard, 2 = primaquine.

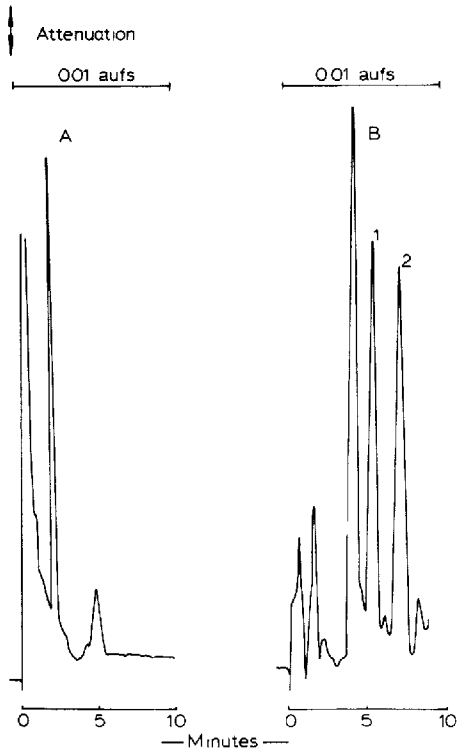

Fig. 2. Chromatograms of a blank urine extract (A) and an extract of urine obtained after a single oral dose of primaquine (B) (primaquine, 154 ng/ml). Peaks: 1 = internal standard, 2 = primaquine.

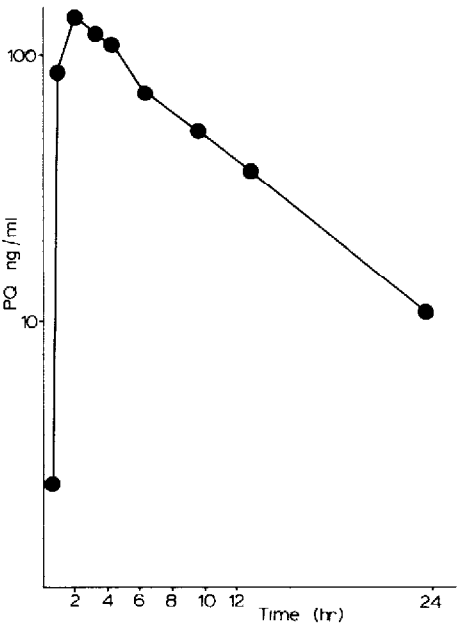

Fig. 3. Plasma levels of primaquine after a 45-mg dose (free base) orally. Volunteer 1.

We found no interference with chromatographic separation from the commonly used antimalarial drugs chloroquine and pyrimethamine or from endogenous compounds in plasma. A clearly resolved component of the extract from plasma obtained from a normal subject receiving primaquine (Fig. 1B) did not appear in blank plasma (Fig. 1A). This may be a metabolite, as yet unidentified. Additionally, in a number of urine extracts a minor endogenous component was seen to elute with a retention time between that of the internal standard and primaquine (Fig. 2). This resulted in a marginally reduced level of sensitivity in these samples.

The extraction solvent, hexane—ethyl acetate (9:1) gave optimal recovery of primaquine with minimal extraction of endogenous compounds. Calibration curves were linear in the range 0—200 ng (r = 0.99) and analytical recovery of primaquine was 93% (± 5, n = 6). The minimal detectable quantity of primaquine in plasma which gave a peak three times baseline noise at the highest detector sensitivity (× 0.0025 a.u.f.s.) corresponded to a level of 1 ng/ml.

The intra- and interassay variation of spiked plasma samples were respectively 8.7% (n = 8) and 5% (n = 5) at 25 ng/ml, and 4.2% (n = 10) and 2.7% (n = 5) at 100 ng/ml.

This assay was applied to a study of the pharmacokinetics of primaquine, following 45 mg orally, in man (all values are means ± standard deviation, n = 4). In all cases plasma primaquine levels could be measured throughout the 24 h of the study. Primaquine was rapidly absorbed reaching peak plasma levels of 150.2 ng/ml (± 28.0) at 2.6 h (± 0.7). The plasma elimination half-life was 6.3 h (± 0.9). Urinary excretion of primaquine over 24 h was 459.5 μg (± 315.0) or 1.1% (± 0.7) of the dose. A typical log plasma concentration versus time curve is shown in Fig. 3.

The advantages of this assay over earlier methods are that it is inexpensive, sample treatment is rapid and simple, sensitivity is increased while selectivity is retained and it is capable of measuring primaquine levels after clinically relevant doses. It may also be possible to quantitate the less stable metabolites of primaquine using this assay or a minimally modified procedure.

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REFERENCES

1 T.G. Rajagopalan, B. Anjaneyulu, V.D. Shanbag and R.S. Grewal, J. Chromatogr., 224 (1981) 265.
2 J. Greaves, D.A. Price-Evans, H.M. Gilles and J.D. Baty, Biomed. Mass Spectrom., 6 (1979) 109.
3 J.K. Baker, J.D. McChesney, C.D. Hufford and A.M. Clark, J. Chromatogr., 230 (1982) 69.