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Note

Determination of dabequin in biological fluids by gas chromatography with a nitrogen-selective detector

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Dabequin [benzo(6)-2-diethylaminoethylaminoquinoline, DQ] is an aminoquinoline derivative with the formula $C_{19}H_{23}N_3$ (Fig. 1), produced in the Soviet Union, which is claimed to have pharmacological activity against *Plasmodium falciparum* [1,2]. At present, the clinical assay of DQ in patients diagnosed as having malaria *P. vivax* [3,4] is carried out at our Institute. DQ is used as its diphosphate in 250-mg capsules (150 mg DQ base).

This paper describes a fast and efficient extraction method with a high DQ recovery from whole blood and urine, by gas chromatography (GC) using a nitrogen-selective detector. Whole blood and urine samples from three *P. vivax* patients were analysed and the DQ concentrations determined.

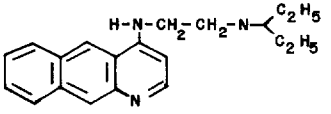

Fig. 1. Structure of dabequin.

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EXPERIMENTAL

Reagents and standards

The methanol used was HPLC grade (Aldrich) and the chloroform (Reactivul, Bucarest, Roumania) was double-distilled. All other chemicals were of analytical grade and were used without further purification.

Dabequin diphosphate was supplied by the Institute of Parasitology and Tropical Medicine (Moscow, U.S.S.R.). The papaverine used as internal standard (I.S.) was supplied by the National Industry of Pharmaceutical Products, Cuba. Glassware and centrifuge tubes were pre-cleaned with 0.1 *M* hydrochloric acid in an ultrasonic bath, followed by a rinse with double-distilled water, and silanized with a 2% solution of dimethyldichlorosilane in 1,1,1-trichloroethane (BDH, Poole, U.K.).

The standard solutions of DQ diphosphate, calculated as DQ base (0.01–10.0 $\mu g\ ml^{-1}$), were prepared in double-distilled water. These solutions and the papaverine base solutions (40 $\mu g\ ml^{-1}$) were prepared in methanol. Sequential dilutions were prepared from the 1 $mg\ ml^{-1}$ and 100 $\mu g\ ml^{-1}$ solutions.

Gas chromatography

A Pye Unicam Model 204 gas chromatograph equipped with a nitrogen-specific detector and a CDP-1 computing integrator was used. The glass column (1.5 m × 2 mm I.D.) was packed with 3% OV-1–OV-17 (1:3) on Chromosorb G (100–120 mesh, AW/DMCS) [3]. The column temperature was 285 °C, and the injection port and detector temperatures were 300 °C. The flow-rate of the carrier gas (argon) was 40 $ml\ min^{-1}$. Under these conditions the retention time of DQ base is 12.4 min and that of I.S. ca. 13.8 min. The resolution was calculated to be $R = 1.33$ for equal concentrations of the two substances.

Samples

Whole blood samples, free of DQ, were supplied by the Ciudad de la Habana Blood Bank. Patients' whole blood samples were taken with potassium oxalate as anticoagulant and frozen for 24 h at −20 °C. Urine specimens used for the spiked extractions were collected from two normal volunteers, who had never taken DQ before. Patients' urine samples were taken during 24 h.

Procedure

Whole blood and urine blanks were made with 1–4 ml of each and analysed by GC. They did not exhibit any interference with DQ base or papaverine peaks in the chromatograms. Three different methods were tested.

Method A. An aliquot of 2.0 ml of whole blood or urine was spiked with 2.0 ml of the corresponding DQ diphosphate standard solution, 0.1 ml of I.S. (40 $\mu g\ ml^{-1}$) and 5 ml of 0.1 *M* sulphuric acid in a 20-ml screw-cap centrifuge

tube. The mixture was extracted with 6 ml of chloroform for 5 min with gentle mixing on a platform shaker. After centrifugation for 10 min at 1500–2000 *g*, the aqueous layer was transferred to another centrifuge tube, and 0.5 ml of 60% potassium hydroxide solution and 6 ml of chloroform were added. After shaking for 5 min and centrifugation, the aqueous phase was discarded and the chloroform layer was evaporated to dryness at 45 °C under a stream of nitrogen. The residue was dissolved in 200 μl of methanol, then transferred to a micro-tube and evaporated to dryness again. This residue was dissolved in 10 μl of methanol, and 2–3 μl were injected into the gas chromatograph. Patients' whole blood and urine samples were prepared the same way.

Method B. For the extraction, a 2.0-ml aliquot of whole blood was spiked with 2.0 ml of DQ diphosphate standard solution, 0.1 ml of I.S. and 1 ml of 0.1 *M* sodium hydroxide in a 20-ml screw-cap centrifuge tube. The mixture was extracted with 6 ml of chloroform for 5 min with gentle mixing on a platform shaker. After centrifugation for 10 min, the aqueous phase was discarded, and 5 ml of 0.1 *M* sulphuric acid were added to the chloroform layer. The mixture was shaken and recentrifuged. The aqueous layer was transferred to another centrifuge tube, and 0.5 ml of 60% potassium hydroxide solution and 8 ml of chloroform were added. After shaking for 5 min and centrifugation, the upper aqueous phase was discarded and the chloroform layer was processed as before.

Method C. An aliquot of 2.0 ml of whole blood was spiked with 2.0 ml of DQ diphosphate standard solution, 0.1 ml of I.S. and 0.5 ml of 60% potassium hydroxide in a 20-ml screw-cap centrifuge tube. The mixture was extracted with 8 ml of chloroform for 5 min with gentle mixing on a platform shaker. After centrifugation for 10 min, the aqueous phase was discarded, and the chloroform layer was treated as in method A.

Linear least-squares regression analysis was employed to fit plots of peak-height ratio (DQ/I.S.) versus DQ concentration. The ABSTAT program for the NEC PC-9801-F computer was used.

RESULTS AND DISCUSSION

The determination of DQ was made using Viala's chloroquine method [5] employing a nitrogen-selective detector. Papaverine was chosen as I.S. because

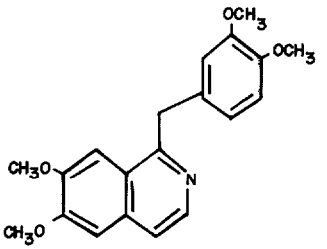

Fig. 2. Structure of papaverine.

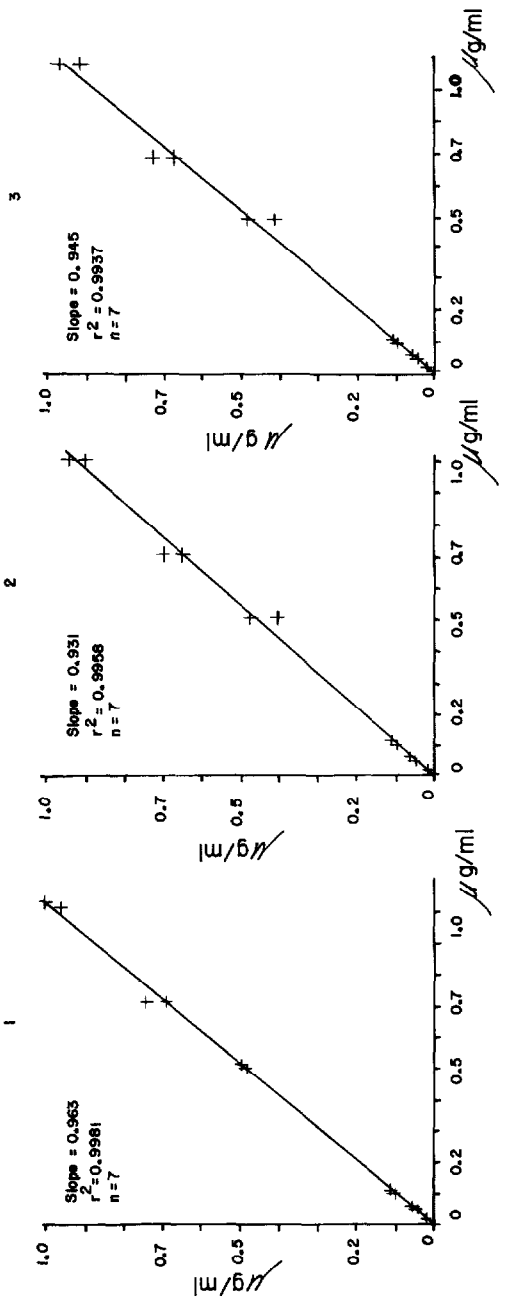

Fig. 3. Comparison of results for (1) methods A and C, (2) methods B and C and (3) methods A and B.

TABLE I

PRECISION OF METHODS A, B AND C, USING WHOLE BLOOD

Within-day precision of spiked samples; $n = 7$.

Added ($\mu g\ ml^{-1}$)	Coefficient of variation (%)		
	Method A	Method B	Method C
1.0	3.8	4.1	2.7
0.5	5.9	6.2	5.8
0.1	8.3	9.0	7.5
0.05	12.0	14.0	11.0
0.01	14.0	20.0	14.0

it is a quinoline alkaloid (Fig. 2), and it is not associated with antimalarial drugs in therapeutic applications. The assay was evaluated using nine standard calibration points in the concentration range 0.01–10.0 $\mu g\ ml^{-1}$ in whole blood and urine.

Standard curves of DQ base in methanol and the DQ diphosphate spiked extractions by method A exhibit an excellent linearity over the concentration range 0.01–10.0 $\mu g\ ml^{-1}$, with coefficients of determination (r^2) consistently at least 0.99; the standard error of estimation was 0.06–0.14. The intercepts did not differ significantly from zero.

The efficiencies of the three extraction methods were determined by spiked extraction of whole blood with 0.01–10.0 $\mu g\ ml^{-1}$ of DQ diphosphate. A comparison of the present methods for the assay of DQ is shown in Fig. 3. The results indicate that the three methods are equivalent.

The limits of determination from spiked whole blood samples, using 2.0 ml of the sample, are for method B ca. 0.05 $\mu g\ ml^{-1}$ and for methods A and C ca. 0.01 $\mu g\ ml^{-1}$, and are satisfactory for the drug concentrations in biological specimens from patients given a therapeutic dose of DQ. The coefficients of variation (C.V.) are presented in Table I.

Within-day reproducibility of the methods was determined at four concentrations (1.0, 0.5, 0.1 and 0.05 $\mu g\ ml^{-1}$) for seven analyses. Inter-assay precision was determined at the same concentration in five replicates. The samples were frozen at $-20°C$ for five weeks. The determinations were carried out each week. The intra-assay C.V. for methods A, B and C were 7.5, 8.3 and 6.8%, respectively, for DQ in whole blood. These values (always less than 15%) demonstrate that the reproducibility of all three methods is good. The inter-assay C.V. for methods A, B and C were 8.9, 9.5 and 8.1%, respectively.

The three extraction methods can thus all be used for DQ determination in

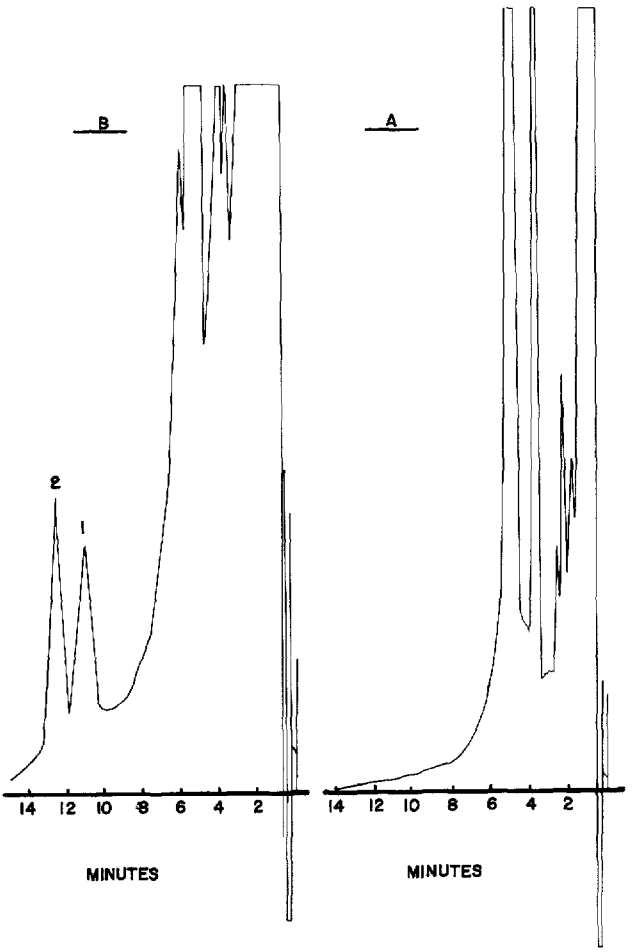

Fig. 4. Gas chromatograms from (A) a patient's urine blank and (B) a urine extract 6 h after the initial dose of DQ, obtained by method A. Peaks: 1 = DQ (0.18 ± 0.03 mg); 2 = papaverine (I.S.; 0.1 ml of 40 μg ml^{-1} solution).

biological fluids, but we propose the use of method A, because it combines the speed and efficiency of method C with the quality of the analysis of method B.

When applied to whole blood and urine samples, method A gives an average recovery (mean of seven determinations for each biological medium) of 98 ± 7 and 95 ± 11%, respectively, for 1.0, 0.7, 0.5, 0.1 and 0.05 μg ml^{-1} DQ.

The usefulness of method A was demonstrated by measuring DQ in whole blood in three patients infested with *P. vivax*, who received orally 14.0, 15.3 and 14.8 mg kg^{-1} DQ free base as the initial dose. The peak concentration in blood occurred at 4–5 h after drug ingestion and ranged between 0.17 and 0.38 μg/ml.

Figs. 4 and 5 show representative chromatograms, obtained by method A, of

Fig. 5. Gas chromatograms from (A) a patient's whole blood blank and (B) a whole blood extract 6 h after the initial dose of DQ, obtained by method A. Peaks: 1=DQ (0.10 ± 0.01 μg ml^{-1}; 2=papaverine (I.S.; 0.1 ml of 40 μg ml^{-1} solution).

a patient's urine and whole blood samples 6 h after the initial dose. The blank samples of each biological fluid did not exhibit any interfering peaks in the chromatograms.

The present analytical method can be applied to pharmacokinetic studies, and is also suitable for the therapeutic control of patients receiving this drug.

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