Determination of Oxamniquine in Serum

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Abstract □ A method for the analysis of oxamniquine in serum (or plasma), sensitive to 10 ng/ml, was developed. Oxamniquine and a close structural analog as the internal standard were extracted from serum with ether. After derivatization with *N,O*-bis(trimethylsilyl)acetamide, oxamniquine was determined as its trimethylsilyl ether derivative by GLC using an electron-capture detector. The method was developed to study serum concentration profiles of different dosage forms of oxamniquine.

Keyphrases □ Oxamniquine—GLC analysis, human serum or plasma □ GLC—analysis, oxamniquine, human serum or plasma □ Antischistosomals—oxamniquine, GLC analysis, human serum or plasma

Oxamniquine[1], 6-hydroxymethyl-2-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline (I), is a potent schistosomicide, active principally against *Schistosoma mansoni*. Extensive clinical trials with this drug in South America and in Africa have shown that oxamniquine is curative after a single intramuscular dose (1–6) and is also effective when given orally (7).

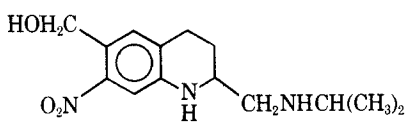
An assay was developed so that serum and plasma concentration profiles of the drug could be determined after both intramuscular and oral administrations of different dosage forms to humans and experimental animals.

EXPERIMENTAL

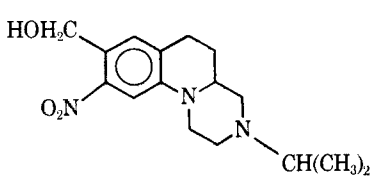
Reagents—The following were used: ether[2]; ethyl acetate[2], redistilled from molecular sieve 4A; pyridine[2], dried over potassium hydroxide; *N,O*-bis(trimethylsilyl)acetamide[3]; citrate–phosphate buffer, pH 5.0, prepared from stock solutions by mixing 51.5 ml of 0.2 *M* Na_2HPO_4 with 48.5 ml of 0.1 *M* citric acid; and 8-hydroxymethyl-3-isopropyl-9-nitro-2,3,4,4a,5,6-hexahydro-1*H*-pyrazine[1,2-*a*]quinoline (II), synthesized according to Baxter and Richards (8).

GLC—A gas-liquid chromatograph[4] fitted with a glass column, 1.85 m in length × 1.0 mm i.d., and a ^{63}Ni-electron-capture detector was used. The stationary phase was dimethyl silicone (OV-101), 5%, on Gas Chrom Q, 80–100 mesh. Operating temperatures were: injector, 250°; oven, 260°; and detector, 290°. The carrier gas was oxygen-free nitrogen with a flow rate of 40 ml/min. Retention times were: oxamniquine, 5 min; and internal standard (II), 7 min.

Standard Curve—Solutions of I and II were prepared daily at a concentration of 50 μg/ml by dissolving 2.5 mg of the compounds in methanol (5.0 ml) and adjusting the volume to 50 ml with distilled water.



I



II

A standard calibration curve was prepared daily by spiking blank serum samples with varying amounts of oxamniquine together with a fixed amount of internal standard. To 1.0 ml of control samples of serum in 10-ml stoppered tubes, 50, 100, 200, 300, and 400 ng of oxamniquine were added (*i.e.*, 1, 2, 4, 6, and 8 μl of the stock solution) using a 10-μl syringe[5]. A constant amount of internal standard, 250 ng, was then added

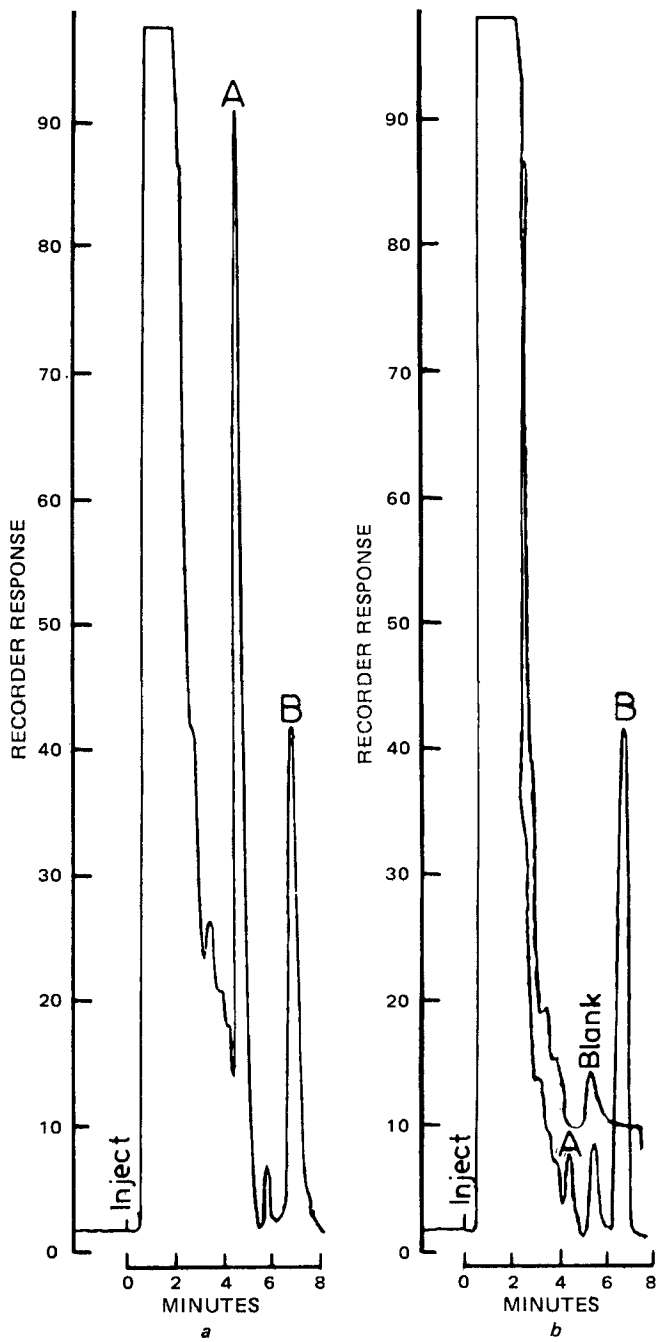


Figure 1—*Chromatograms of oxamniquine (A) and internal standard (B) showing* (a) *a high (440 ng/ml) and* (b) *a low (30 ng/ml) concentration of oxamniquine in human serum and a superimposed blank serum sample chromatogram.*

[1] Mansil, Pfizer.
[2] Analar.
[3] Phase Separations Ltd.
[4] Varian 1400.
[5] Hamilton.

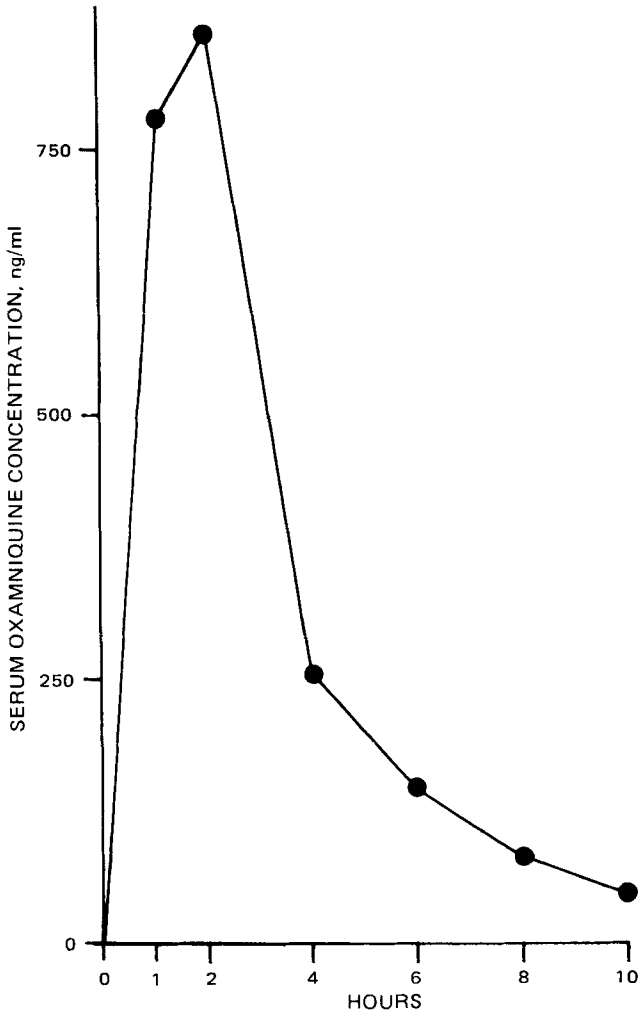


Figure 2—*Serum concentrations of oxamniquine in a human volunteer following administration of a single oral dose of oxamniquine (1000 mg).*

to each of the spiked serum samples containing different amounts of oxamniquine. Sodium hydroxide, 1 *M* (1.0 ml), and ether (4.0 ml) were added and the samples were extracted by mixing for 30 sec using a vortex mixer.

The phases were separated by centrifugation, and the organic layer was carefully removed to a second stoppered tube. A second ether extraction was carried out, and the second extract was added to the first. The combined organic extracts were extracted with citrate–phosphate buffer (5.0 ml) and, after separation of the phases by centrifugation, the organic layer was removed and discarded. Sodium hydroxide, 1 *M* (1.0 ml), was added, and the aqueous phase was then extracted twice with 4.0-ml portions of ether as described.

The extracts were combined in a clean centrifuge tube and placed in a water bath at ambient temperature, and the solvent was evaporated to dryness under a stream of nitrogen. Dry pyridine (50 μl) was added to redissolve the extract. *N,O*-Bis(trimethylsilyl)acetamide (10 μl) was then added, and the tube was stoppered and allowed to stand for 5 min at room temperature. The derivatized material was then diluted with ethyl acetate (0.5 ml) and, after mixing, an accurately measured aliquot (2 μl) was injected onto the column.

The peak heights due to oxamniquine and the internal standard were measured, and peak height ratios (oxamniquine–internal standard) were calculated. The relationship between peak height ratio and oxamniquine concentration was linear for serum concentrations up to 500 ng/ml. The actual mean recovery of oxamniquine in ether extracts of serum determined by comparison of peak height ratios using the described method with those obtained by direct injection of pure sample mixtures was 92 ± 4% (*SEM*).

Sample Analysis—Test serum samples or suitably diluted samples (1.0 ml) to be assayed were transferred to chemically clean stoppered tubes and spiked with 250 ng of the internal standard. These samples were analyzed as already described. The typical sample chromatograms obtained, showing high (440 ng/ml) and low (30 ng/ml) concentrations of oxamniquine in human serum, and a blank sample chromatogram are shown in Fig. 1. A typical serum concentration *versus* time curve in a healthy human volunteer following administration of a single oral dose of oxamniquine (1000 mg) is shown in Fig. 2.

Accuracy and Reproducibility—The accuracy of the assay over the 50–500-ng/ml concentration range was determined. Eight spiked serum samples of concentrations unknown to the operator were assayed. The spiked concentrations (nanograms per milliliter) together with the assayed values (in parentheses) were 49 (50), 98 (97), 123 (128), 148 (168), 197 (202), 246 (240), 395 (404), and 450 (470).

Six replicate assays were carried out on a serum sample spiked at a concentration of 115 ng/ml. The mean value found was 114.5 ± 4.0 ng/ml (*SEM*).

Identity of Oxamniquine Derivative—After derivatization of oxamniquine with *N,O*-bis(trimethylsilyl)acetamide under the described conditions, only one peak was seen on the gas–liquid chromatogram. Combined GLC–mass spectrometry indicated a mass of 351, showing that only a monotrimethylsilyl derivative is formed under the conditions employed. A prominent M − 89 fragment ion peak in the mass spectrum confirmed that the trimethylsilyl group is attached to oxygen (9).

DISCUSSION

A method for the assay of oxamniquine was first developed in these laboratories to determine serum concentrations in toxicity trial animals. The method employed was essentially the same as that described here but used a flame-ionization detector. With this method, drug concentrations down to 750 ng/ml could be determined. However, the method was not sufficiently sensitive to determine with precision the serum concentrations achieved at therapeutic dose levels in clinical trials.

The presence of the nitro group in the oxamniquine molecule suggested that an increase in sensitivity might be gained by employing an electron-capture detector. This change increased the sensitivity of detection some 75-fold, and concentrations of oxamniquine in serum or plasma can be readily determined down to 10 ng/ml. This sensitivity makes the method suitable for the determination of serum concentration profiles of oxamniquine in patients receiving therapeutic doses where maximum plasma concentrations of drug ranging up to approximately 1 μg/ml are found.

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