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Note

Gas chromatographic determination of clioquinol (Vioform) in human plasma

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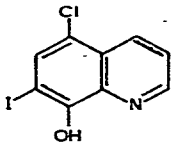
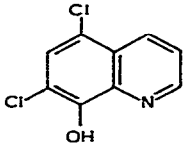
Clioquinol* (5-chloro-8-hydroxy-7-iodoquinoline) is used in the treatment of infections of the gastrointestinal tract and of the skin. Several methods for the



clioquinol



5,7-dichloro-8-hydroxyquinoline
(internal standard)

assay of clioquinol have been described. Clioquinol in pharmaceutical preparations has been determined by the formation of copper complexes followed by iodometric titration [1]. Thin-layer chromatographic determination of clioquinol and/or its conjugate in plasma at concentrations down to 40 ng/ml has been described [2]. Several gas chromatographic (GC) methods have already been published [3—8] but they suffer from certain disadvantages. In particular, methods using the acetyl derivative of clioquinol for electron-capture detection (ECD) [4, 5] require a laborious extraction procedure, and methods using extractive methylation followed by GC—ECD analysis [6, 8] present an instability problem. A high-performance liquid chromatographic method is available [9] for measurement of conjugate levels but it is not applicable to the parent drug. A qualitative method utilizing gas chromatography—mass spectrometry has been published and used to detect a new iodine-containing metabolite [10].

This paper reports a rapid procedure, particularly adapted to a large series of samples, which permits the determination of clioquinol down to 50 ng/ml of

*Vioform®, Ciba-Geigy.

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plasma and which has been used successfully in this laboratory for seven years.

EXPERIMENTAL

Chemicals and reagents

Clioquinol and 5,7-dichloro-8-hydroxyquinoline were supplied by Ciba-Geigy (Basle, Switzerland). All reagents and solvents were of analytical grade. A pH 5 buffer (Titrisol, Merck 9885, E. Merck, Darmstadt, G.F.R.) was prepared by diluting the contents of seven vials with 1000 ml of water. Sodium sulfate (Merck 6649) solution was prepared by diluting 300 g of sodium sulfate with 1000 ml of water. Acetic anhydride (Fluka 45830; Fluka, Buchs, Switzerland) was purified before use by adding 20 g of sodium acetate to 100 ml of acetic anhydride and boiling under reflux for 15 min. Then, the acetic anhydride was distilled off and collected between 135 and 140°C. Pyridine (Fluka 82702) was distilled at 115—116°C with potassium hydroxide pellets and stored over the same reagent. The solution of internal standard contained 400 ng of 5,7-dichloro-8-hydroxyquinoline in 20 μl of methanol—water (1 : 1, v/v).

Equipment

A Hewlett-Packard, Model 5713A, gas chromatograph equipped with a Hewlett-Packard electron-capture detector (Model 18713A) was used. The peak areas were estimated by a Hewlett-Packard electronic integrator. The column was operated at 195°C, the injector at 250°C and the detector at 300°C with an argon—methane (90 : 10) flow-rate of 60 ml/min. The glass column was washed as described previously [11]. The column packing was 3% OV-101 on Chromosorb W HP 80—100 mesh (Applied Science Labs., State College, PA, U.S.A.). The filled-column (2 m × 3 mm I.D.) was conditioned as described previously [11].

Extraction

Twenty microlitres of the internal standard solution were measured into a glass tube, to which 1 ml of plasma, 2 ml of sodium sulfate solution and 5 ml of diethyl ether—dichloromethane (4 : 1, v/v) were then added. The tube was shaken mechanically (Infors shaker) for 25 min at 300 rpm and centrifuged at 4800 *g* for 10 min.

An aliquot of the organic phase was transferred to another tube and 3 ml of pH 5 buffer were added. The tube was stoppered and shaken mechanically for 10 min at 300 rpm, and then centrifuged for 3 min at 2450 *g*. An aliquot of the organic phase was transferred to another tube and dried under a nitrogen stream in a water-bath at 37°C.

Derivatization and chromatography

To the dry residue were added 50 μl of 10% pyridine in toluene and 50 μl of acetic anhydride. The tube was stoppered tightly, agitated and put in a dry heating block (Grant Instruments) at 70°C for 1 h. Excess reagent was removed by evaporation to dryness under a nitrogen stream in a water-bath at 37°C; 1 ml of ethyl acetate was added, and the tube was shaken on a Vortex mixer. A 2-μl portion of the ethyl acetate solution was injected into the gas chromato-

graph. The clioquinol content was calculated from the peak area ratio by reference to a calibration curve. This curve was obtained by extraction of plasma spiked with increasing amounts of clioquinol (from 50 to 10,000 ng/ml) and a constant amount of internal standard (400 ng/ml of plasma).

Study in man

Eight healthy fasted subjects, who had been advised to take no drugs during the week preceding the experiment and none besides clioquinol throughout the duration of the study, received 400 mg of clioquinol as Entero-Vioform powder with 150 ml of water. Blood samples were collected before and 2, 4, 8, 24, 48 and 72 h after the administration. Samples were centrifuged and the plasma was removed and stored at −20°C until analysis.

RESULTS AND DISCUSSION

Precision and recovery

Table I gives the results obtained when the described procedure was applied to spiked plasma samples. The results demonstrate the good reproducibility of the assay down to concentrations of 50 ng of clioquinol per ml of plasma, which may be taken as the sensitivity limit of the method. Lower concentrations could still be detected.

TABLE I

PRECISION AND RECOVERY OF THE DETERMINATION OF CLIOQUINOL APPLIED TO SPIKED HUMAN PLASMA SAMPLES

Amount added (ng/ml)	Number of assays	Amount found (mean, ng/ml)	Standard deviation (±)	Recovery (mean, %)
50	9	50.3	6.0	100.6
100	10	99.4	9.5	99.4
500	6	511	38	102.2
1000	8	1004	58	100.4
5000	8	5024	260	100.5
10000	8	9924	527	99.2
				100.4 ± 1.1

Plasma interference

Fig. 1 shows the chromatograms of an extract of human plasma and of the same extract spiked with 100 ng of clioquinol and 400 ng of internal standard. There is no interference from the normal components of the plasma extract.

Scope and limitations of the described method

Like some already published methods [4, 5, 7], the described assay makes use of the acetyl derivative of clioquinol for GC—ECD, but it is much less laborious, and particularly adapted to large series of samples. It has a sensitivity comparable with that of the already published acetyl-derivative methods. Ex-

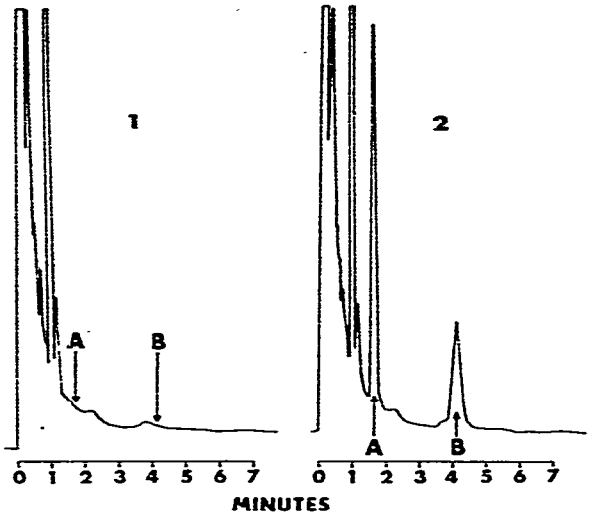

Fig. 1. Examples of chromatograms: (1) human plasma blank (1 ml of plasma); (2) the same plasma spiked with 400 ng/ml internal standard (A), and 100 ng/ml clioquinol (B).

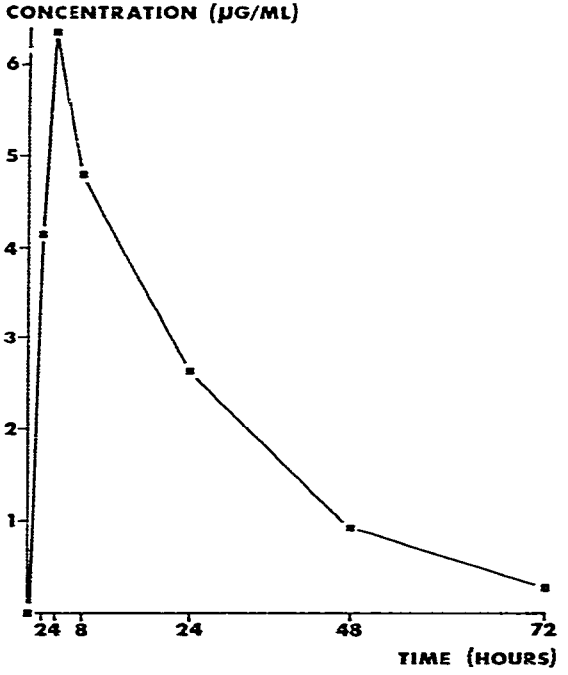

Fig. 2. Average clioquinol plasma concentrations obtained in eight healthy subjects after administration of 400 mg of clioquinol as Entero-Vioform powder.

tractive methylation methods [6, 8] allow determination of about 20 ng of clioquinol per ml of plasma by GC—ECD, but it seems that stability problems were encountered with the methyl derivative and the methylating reagent. The

described method has been used successfully in our laboratory for seven years by different analysts to assay more than 6000 plasma samples with good precision and accuracy.

Study in man

Fig. 2 shows the average curve obtained from the plasma samples of the eight subjects given 400 mg of clioquinol as Entero-Vioform powder. The sensitivity of the method thus appears sufficient to determine clioquinol in bioavailability or pharmacokinetic studies.

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