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Note

Determination of clofazimine in human plasma by thin-layer chromatography

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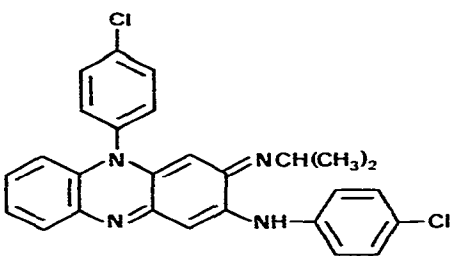
Clofazimine, 3-(*p*-chloroanilino)-10-(*p*-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine (I), is effective in the treatment of leprosy.



I

To date, Barry et al. [1] and Dill et al. [2] have reported, respectively, a spectrophotometric and a fluorometric method of assay for clofazimine in biological fluids and tissue homogenates. The sensitivity of these methods is not sufficient for the determination of plasma clofazimine levels after a single oral dose in man. Gidoh et al. [3] have published a high-performance liquid chromatographic method for the simultaneous analysis of the principal antileprosy drugs and their metabolites in human serum. Meanwhile, we have developed a densitometric method for determining clofazimine in plasma. This method was applied to a healthy volunteer given single oral doses of 200 mg and 400 mg of Lamprene®.

EXPERIMENTAL

Reagents

All solvents and reagents were of analytical grade (Merck, Darmstadt,

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G.F.R.) and were used without further purification. The clofazimine (active substance of Lamprene) originated from Ciba-Geigy Ltd., Basle, Switzerland; acetic acid, citric acid, sodium acetate and buffers pH 4, 6, 8, 10 and 13 (Titrisol®) were from Merck.

Procedure

Plasma samples (1—3 ml, depending on the concentration of clofazimine) are acidified with 2 ml of acetate buffer (according to Walpole, 1 *M*, pH 5) and then extracted with 6 ml of toluene for 15 min using a mechanical horizontal shaker. After brief centrifugation, 5 ml of the organic phase are removed and evaporated to dryness under a stream of nitrogen at 40°C. The residues are dissolved in 100 μl of toluene and aliquots are applied on to the thin-layer chromatographic (TLC) plate.

Thin-layer chromatography

All TLC separations are carried out using 20 × 10 cm precoated HPTLC silica gel 60 plates (Merck) predeveloped in chloroform—methanol (1:1) prior to use. Concentrated extracts (30 μl) are applied to the plate using a Linomat III applicator (Camag, Muttenz, Switzerland), 19 samples being applied to one plate, including biological, calibration and control samples. The plates are developed in toluene—acetic acid—water (50:50:4) using a solvent-saturated, paper-lined tank, and then allowed to stand 30 min at room temperature. R_F value of clofazimine is 0.36.

In situ quantitation of the TLC spots is performed with a Camag scanner coupled to a W+W recorder. Measurements are made in the reflectance mode

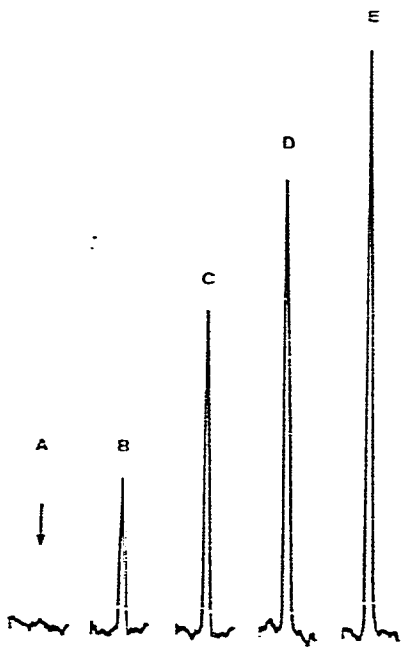

Fig. 1. Typical densitograms of (A) extract of a blank plasma sample and extract of plasma samples spiked with clofazimine (B) 23 ng/ml, (C) 46 ng/ml, (D) 70 ng/ml and (E) 93 ng/ml.

for visible absorption. A mercury light source is used with the monochromator set at 545 ± 15 nm.

Densitograms obtained for blank plasma and for plasma spiked with various amounts of clofazimine are shown in Fig. 1.

RESULTS AND DISCUSSION

Extraction

The pH dependence of the extractability of clofazimine was evaluated by adjusting spiked plasma samples to pH values ranging from 2 to 13 using acetic acid, citric acid or buffers (Fig. 2). Extraction of the biological samples with toluene resulted in good recovery with a low background.

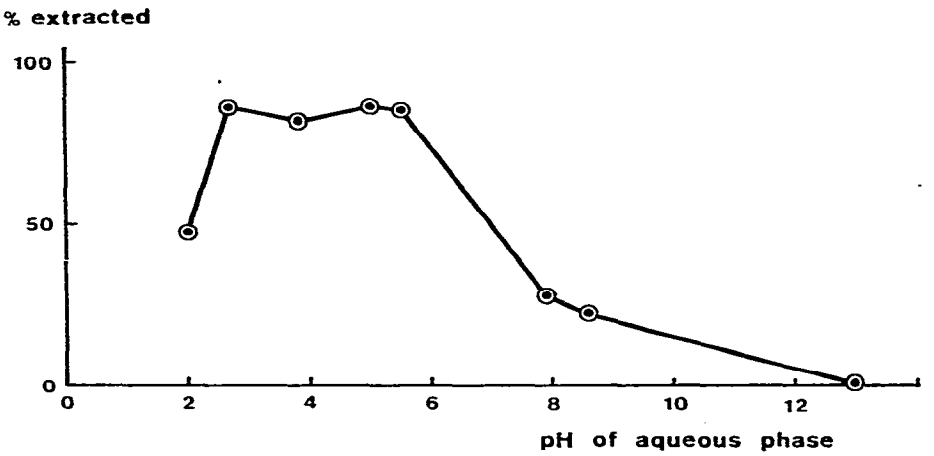

Fig. 2. pH Dependence of extractability of clofazimine with toluene from spiked plasma samples.

Calibration curves

Calibration curves were prepared with spiked plasma samples processed as described above. An almost linear relationship between the peak height and the amount of clofazimine on the plate was observed between 2 and 20 ng/spot. For a calibration range from 2 to 30 ng/spot or higher, calibration curves of the type $y = a + bx + cx^2$ (y corresponding to the peak height and x to the amount of clofazimine in the sample) were employed (Fig. 3).

Accuracy and precision

The method was tested by analysing samples spiked with various amounts of clofazimine unknown to the analyst. The mean values found differed from the initial concentrations by between −3.3% and +15.4% (Table I).

No interference from dapsone or rifampicin was observed.

Application

The method was employed for the determination of plasma clofazimine levels in a healthy volunteer following single oral doses of 200 mg and 400 mg

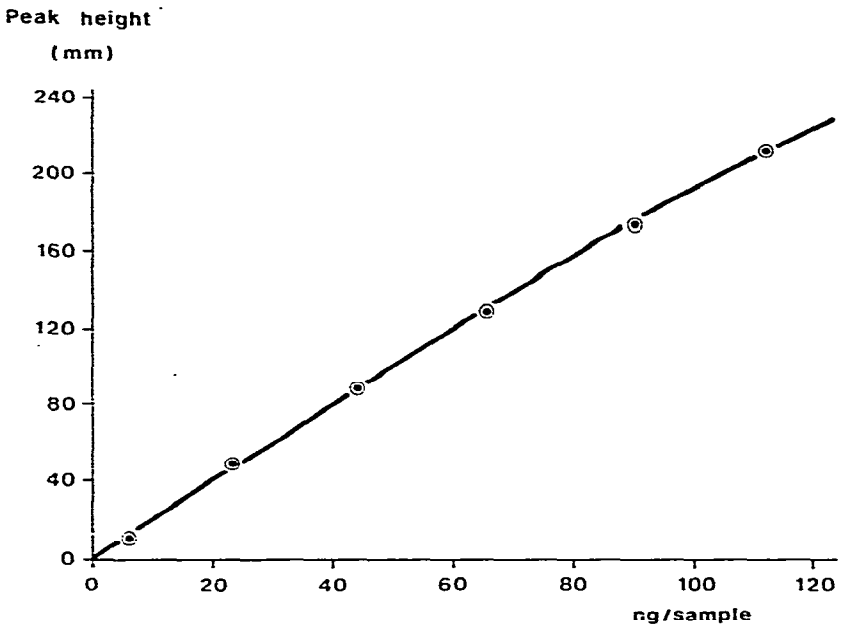

Fig. 3. Calibration curve for the entire analytical method.

TABLE I

COMPARISON BETWEEN INITIAL AND FOUND PLASMA CONCENTRATIONS OF CLOFAZIMINE IN SPIKED SAMPLES

Clofazimine concentration in plasma (ng/g)			
Initial	Found (mean)	No. of determinations	Percentage deviation of mean from initial value
13	15	2	+15.4
33	34	2	+ 3.0
40	41	8	+ 2.5
51	51	2	0.0
60	58	12	— 3.3
84	96	2	+ 2.4
100	100	4	0.0

of Lamprene (2 and 4 capsules of 100 mg of Lamprene) after an overnight fast (Fig. 4). A peak clofazimine concentration of 70 ng/g was reached 8 h after administration of 200 mg of Lamprene and one of 162 ng/g 4 h after the 400-mg dose. Following the distribution phase a slow elimination phase with an apparent elimination half-life of 8 days was observed, clofazimine concentrations still above the limit of detection of 5 ng/g being measured for up to 264 h after ingestion.

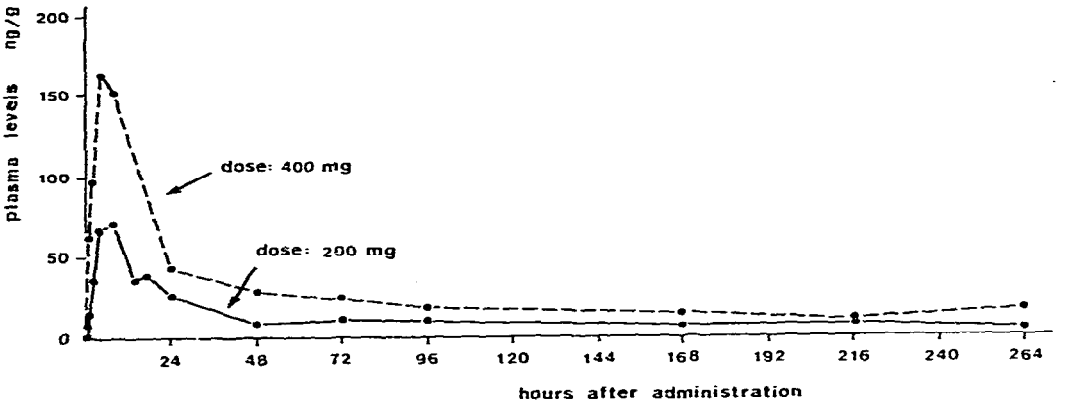

Fig. 4. Plasma levels of clofazimine in a healthy volunteer following single oral doses of 200 mg and 400 mg of Lamprene after an overnight fast.

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