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Note

Determination of indeloxazine, a new antidepressant agent, in human plasma by gas—liquid chromatography with electron-capture detection

AILEEN G. HAYES and TSUN CHANG*

Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48160 (U.S.A.)

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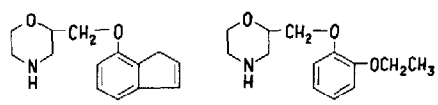
Indeloxazine hydrochloride, (±)2-[(1H-inden-7-yloxy)methyl]morpholine hydrochloride (Fig. 1), is a new potential antidepressant drug structurally related to viloxazine. In various animal models indeloxazine was shown to have a novel psychopharmacological profile different from that of tricyclic antidepressants or viloxazine [1]. As part of the clinical pharmacokinetics and bioavailability studies, a sensitive and specific electron-capture gas—liquid chromatographic assay was developed for the determination of indeloxazine in biological fluids at nanogram levels.



INDELOXAZINE VILOXAZINE

Fig. 1. Chemical structures of indeloxazine and viloxazine.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 5730A gas chromatograph equipped with a 15 mCi ^{63}Ni electron-capture detector, a glass-lined heated injector, and a Model 7672A automatic sampler was used for all analyses. The instrument was fitted with a silanized coiled glass column (1.2 m × 2 mm I.D.) packed with pretested 3% OV-225 on 100—120 mesh Gas-Chrom Q. Argon—methane (95:5) with a preset head pressure of 0.275 MPa and a flow-rate of 50 ml/min was used as the carrier gas. The injection port, column oven, and detector were maintained at 250°C, 200°C, and 300°C, respectively.

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An Autolab IV integrator (Spectra-Physics, Mountain View, CA, U.S.A.) was interfaced with the electrometer and the automatic sampler for unattended operations.

Chemicals

Indeloxazine hydrochloride was obtained from Yamanouchi Pharmaceutical Co. (Tokyo, Japan). The internal standard, viloxazine hydrochloride, was supplied by ICI (Macclesfield, Great Britain); toluene, distilled in glass, from Matheson, Coleman, and Bell (Cincinnati, OH, U.S.A.); heptafluorobutyric anhydride in sealed glass ampoules from Pierce Chemical (Rockford, IL, U.S.A.); pretested 3% OV-225 on 100—120 mesh Gas-Chrom Q from Applied Science Labs. (State College, PA, U.S.A.); argon–methane (95:5) from Matheson Gas Products (East Rutherford, IL, U.S.A.). All other chemicals were of analytical grade.

Reagents

Stock solutions of indeloxazine or viloxazine (internal standard) were prepared by dissolving the appropriate amount of drug in 0.1 *N* hydrochloric acid to make a 1 mg/ml solution as the free base. Aliquots of these solutions were diluted separately with 0.1 *N* hydrochloric acid to yield 200 ng/ml and 50 ng/ml working standards of indeloxazine and 500 ng/ml of the internal standard.

Buffer solution, pH 9.5, was prepared by combining 250 ml saturated ammonium chloride and 105 ml concentrated ammonium hydroxide.

Sample preparation

Aliquots of plasma (0.2—0.5 ml), 0.2 ml of the internal standard (100 ng), 2 ml of ammonium chloride—ammonium hydroxide buffer, and 5 ml of toluene were measured into a set of 16-ml glass-stoppered tubes. The tubes were shaken on a mechanical shaker for 10 min, and centrifuged to separate the phases. A 4-ml aliquot of the toluene layer was back-extracted with 3.5 ml of 0.1 *N* hydrochloric acid in a duplicate set of tubes for 10 min. Following centrifugation 3 ml of the aqueous phase were transferred into another set of tubes containing 0.3 ml 1 *N* sodium hydroxide and 1 ml of ammonium chloride—ammonium hydroxide buffer, and the mixture was extracted with 3.5 ml of toluene as above. Toluene solution (3 ml) was mixed with 50 μl of heptafluorobutyric anhydride in a 13 × 100 mm glass-stoppered tube. The lower one-third of the tube was heated in a 75°C dry heating block for 30 min.

The tube was cooled to room temperature and excess reagent was removed by shaking with 1 ml of water for 1 min, followed by 1 ml of 5% ammonium hydroxide for another minute. After centrifugation, the organic phase was removed and evaporated to dryness at 55°C under nitrogen. The residue was redissolved in 1 ml of toluene (containing 1.5% isoamyl alcohol). The sample was transferred into a 1-ml glass sample vial and capped with a PTFE-faced rubber septum aluminum seal using a hand crimper. The prepared samples were then loaded into the automatic sampler ready for injection (3 μl).

Calibration and precision

Six calibration standards containing 12.5, 25, 50, 100, 150, and 200 ng of indeloxazine in 0.5 ml of blank human plasma were processed daily with each set of unknowns. Calibration curves were constructed by plotting the peak area ratios of indeloxazine to the internal standard versus the amount of indeloxazine in each sample. To determine the precision of the assay procedure, plasma standards were analyzed on three separate days to yield 10—12 replicate values for each of the six concentrations.

Quality control and stability studies

Quality control samples at concentrations of 300 ng/ml, 150 ng/ml, and 75 ng/ml were prepared in pooled blank human plasma, and 0.8-ml portions were stored frozen in disposable glass vials. Samples of 0.5 ml plasma at each concentration were analyzed daily to gauge the reliability of each day's analyses and the stability of indeloxazine in frozen plasma.

RESULTS AND DISCUSSION

Method evaluation

A sensitive and specific gas—liquid chromatographic assay has been developed for indeloxazine from 0.5 ml or less of plasma. The procedure is based on the electron-capture properties of the heptafluorobutyryl derivative which permits detection of low levels of indeloxazine in plasma. Viloxazine serves as an excellent internal standard owing to its structural similarity to indeloxazine and the ease of formation of the heptafluorobutyryl derivative [2]. Fig. 2 shows typical chromatograms obtained from extracts of blank human plasma containing indeloxazine and internal standard, blank human plasma, and a post-dose plasma sample from a human volunteer. The back-extraction

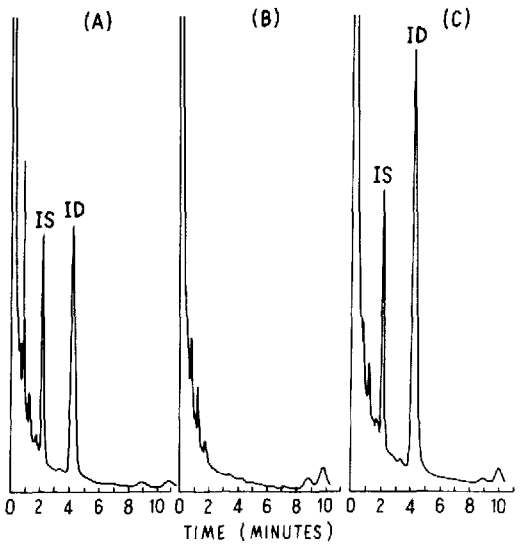

Fig. 2. Chromatograms of an extract of (A) blank human plasma containing indeloxazine (ID) and internal standard (IS); (B) blank human plasma; (C) post-dose plasma sample from a human volunteer.

step was necessary to produce chromatographic tracings free of endogenous interferences. The derivatization reaction was essentially complete within 15 min, but the samples were routinely heated for 30 min to ensure completeness of reaction. The heptafluorobutyryl derivatives appeared to be stable in the toluene solution for several days.

The relationship between the peak area ratio (indeloxazine/internal standard) and the amount of indeloxazine (12.5—200 ng) in 0.5 ml of plasma was linear over the concentration range studied (r = 0.999). The slopes of the calibration curves constructed over a 3-month period showed a relative standard deviation (R.S.D.) of 3.21%.

Precision and accuracy of the assay procedure were determined at six different concentrations ranging from 12.5—200 ng (Table I); the relative standard deviation (R.S.D.) varied from 2.55—6.05%. System reproducibility based on the peak area ratios of six sequential injections of standards had a mean R.S.D. of 1.3%.

TABLE I

PRECISION AND ACCURACY OF THE PROCEDURE APPLIED TO SPIKED HUMAN PLASMA SAMPLES (n = 9)

Amount drug added (ng)	Amount drug found (ng)	Relative standard deviation (%)
12.5	12.40	6.05
25.0	25.27	4.59
50.0	50.02	4.52
100.0	100.23	2.55
150.0	150.17	3.24
200.0	197.56	2.81

The limit of detection was approximately 5 ng/ml using 0.5-ml aliquots of plasma. Extraction recovery from plasma averaged 95%.

Quality control samples containing 300 ng/ml, 150 ng/ml, and 75 ng/ml of indeloxazine were frozen and assayed daily over a 3-month period. The mean values obtained were 302.8 ng/ml, 150.4 ng/ml, and 77.2 ng/ml with a R.S.D. of 2.69%, 3.00%, and 3.84%, respectively, indicating excellent drug stability in frozen plasma.

Human plasma levels

The described method has been successfully applied to clinical bioavailability studies after a single 100-mg oral dose of capsules and enteric coated tablets. The resulting plasma concentration versus time profiles for a representative volunteer are illustrated in Fig. 3. The peak plasma concentrations of 303 ng/ml and 326 ng/ml were reached in 2 h and 4 h, respectively, for the capsule and enteric coated tablet. The apparent disposition half-life was 3.15 h for the capsule and 3.58 h for the enteric coated tablet.

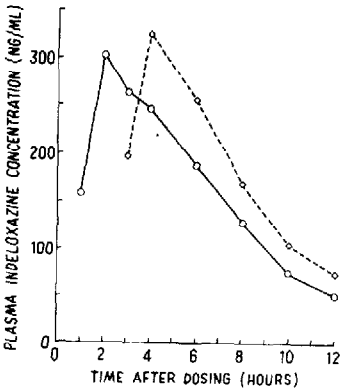

Fig. 3. Plasma indeloxazine concentrations in a normal volunteer following a single 100-mg oral dose of capsule (○——○) or enteric coated tablet (◇ — — — ◇).

The method as presented is suitable for pharmacokinetic and bioavailability studies following a single therapeutic dose.

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