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Note

Gas—liquid chromatographic analysis with electron-capture detection of diclofensine in human plasma following derivatization

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Diclofensine (Fig. 1) is a newly synthesised isoquinoline antidepressant. The only commercially available antidepressant with the same basic isoquinoline moiety is nomifensine [1] (Fig. 1). Rapid and high-resolution gas chromatographic (GC) analyses for nomifensine have been developed [2, 3]. Electron-capture detection of a metabolite of nomifensine, 4′-hydroxynomifensine, in biological samples has been studied utilising pentafluoropropionic anhydride as a derivatizing agent [4]. Heptafluorobutyric anhydride was used to derivatize nomifensine in an earlier GC study [5] and very recently in a comparative study of high-performance liquid and gas—liquid chromatography (GLC) [6].

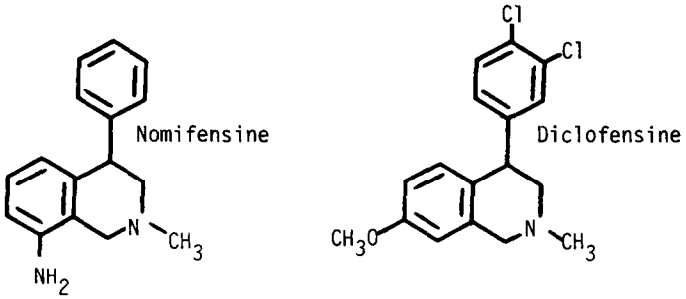

Fig. 1. Chemical structures of nomifensine and diclofensine.

To date no analytical method for the determination of diclofensine has been described. Previous work has concentrated on the antidepressant effect of diclofensine [7—9]. Preliminary investigations in our laboratory have indicated

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that diclofensine does not exhibit sufficient electron-capturing ability to be determined directly. In this paper, we describe a GLC method for diclofensine in plasma which is based on solvent extraction and the formation of a fluorinated derivative of the amine.

MATERIALS AND METHODS

Reagents

Diclofensine [RAC-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-7-methoxy-2-methylisoquinoline hydrochloride] was kindly supplied by its manufacturers (Hoffmann-La Roche, Basle, Switzerland). The internal standard imipramine hydrochloride (purity 99%) was obtained from Sigma (St. Louis, MO, U.S.A.). Heptafluorobutyric anhydride, HFBA (Pierce, Rockford, IL, U.S.A.) was used directly as the derivatizing agent. Methyl chloroformate was obtained from Aldrich (Gillingham, U.K.). Diethyl ether, *n*-heptane and triethylamine were of analytical-reagent grade (Hopkins and Williams, Essex, U.K.). Plasma was prepared by adding triply distilled water to dry plasma (Blood Transfusion Service Board, Dublin, Ireland).

Preparation of standards

Diclofensine hydrochloride (11.12 mg equivalent to 10 mg diclofensine) was dissolved in 100 ml triply distilled water (working standards). Plasma standards were freshly prepared each day by spiking the blank plasma with working standards to yield diclofensine concentrations ranging from 100 to 700 ng/ml in plasma. A stock solution of the internal standard was prepared by dissolving 11.28 mg imipramine hydrochloride (equivalent to 10 mg imipramine) in 100 ml of distilled water to give an imipramine concentration of 100 μg/ml. This solution was further diluted to give a 10 μg/ml solution of imipramine.

Extraction and derivatization procedures

To 1-ml aliquots of plasma were added 100 μl of the working standards together with 100 μl of imipramine hydrochloride, from an internal standard solution containing 10 μg/ml. The plasma was made alkaline (pH 9) with 0.2 ml 1 *M* sodium hydroxide followed by the addition of 1.5 ml diethyl ether. The mixtures were shaken for 30 sec on a vortex mixer and then centrifuged for 5 min at 700 *g*. Aliquots (1.10 ml) of the supernatant were removed and evaporated to dryness under nitrogen in glass tubes. Following reconstitution in 0.5 ml *n*-heptane, sodium carbonate (10 mg) and methylchloroformate (200 μl) were added to each tube. The tubes were then attached to an air condenser and heated at 100°C for 30 min in an oil bath. Following this, the samples were evaporated to dryness under a gentle flow of nitrogen and 0.5-ml quantities of 30% hydrogen bromide in glacial acetic acid added. The tubes were then heated for a further 10 min at 100°C and cooled. Concentrated ammonia (1 ml) was added to each tube. The contents of each tube were shaken with 300 μl of *n*-heptane and 250-μl aliquots of the supernatant were separated out. To each 250-μl aliquot were added 50 μl triethylamine and 5 μl HFBA in a dry glass tube. After 1 h at room temperture, the samples were washed with 2 ml of 0.1 *M* sodium hydroxide and the organic layer separated for analysis by GLC.

Gas—liquid chromatography

A Sigma 4 Perkin-Elmer gas chromatograph with a Ni^{63} electron-capture detector was used, with a 2 m × 2 mm glass column packed with 3% OV-17 on Chromosorb W HP (80—100 mesh). The oven temperature was 265°C with the detector and injection port temperatures maintained at 300°C. The flow-rate was 45 ml/min (oxygen-free nitrogen) with the make up carrier flow-rate at 75 ml/min. The column was conditioned each day by increasing the oven temperature from 100°C to 265°C at a rate of 1°C/min. A Hewlett-Packard 3390 A reporting integrator was used to record and measure the peak height values.

RESULTS AND DISCUSSION

Diclofensine (pK_a = 7) can be extracted from plasma at pH 9 with a number of organic solvents. The efficiency of extraction was determined using flame-ionisation detection by comparing peak heights obtained with a variety of extracting solvents with those obtained by direct injection of a standard diclofensine solution in methanol. The extraction efficiency was found to be 80% with diethyl ether, 50% with chloroform, 72% with toluene and 75% with hexane. The derivatization procedure itself was used for imipramine, amitriptyline and a series of diphenylmethane alkyl tertiary amines [10] and is adapted here for use in the analysis of diclofensine with imipramine conveniently as the internal standard.

Amines are generally difficult to analyse using GC. Although nomifensine can be gas chromatographed as the free base, the chromatographic properties of these compounds are considerably improved by derivative formation. Diclofensine is first demethylated using methyl chloroformate followed by hydrolysis of the urethane to N-desmethyldiclofensine (Fig. 2). The final step involves the derivatization reaction with heptafluorobutyric anhydride in the presence of triethylamine.

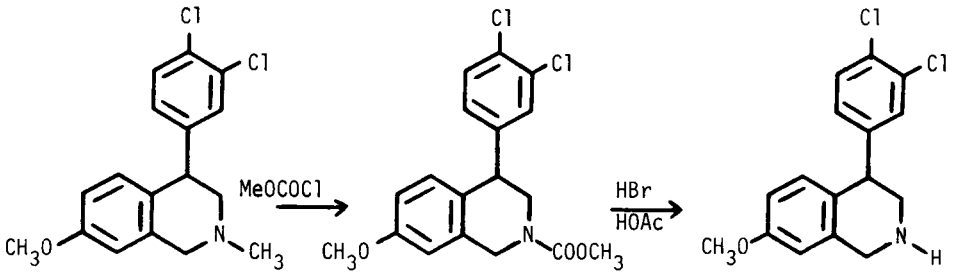


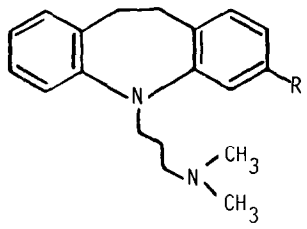
Fig. 2. Demethylation of diclofensine followed by hydrolysis of the urethane to N-desmethyldiclofensine.

A typical chromatogram of diclofensine and the internal standard imipramine is shown in Fig. 3 together with appropriate blank chromatograms. The retention times are 2.78 min for diclofensine and 1.96 min for the internal standard. A plot of peak height ratios of diclofensine to those of the internal standard against diclofensine concentration in plasma is linear over the concentration range 100—700 ng/ml with a correlation coefficient > 0.99.

Fig. 3. Chromatograms of (a) derivatized extract of drug-free plasma; (b) underivatized extract of spiked plasma; (c) derivatized extract of spiked plasma containing 600 ng/ml diclofensine. Peaks: internal standard, imipramine (1), endogeneous (2), diclofensine (3).

Selectivity of the method

With respect to potential interferences in multiple drug therapy, some information on the selectivity of the method is obtained from a comparison of retention volumes. Apart from the internal standard, the tricyclic antidepressant cianopramine (Fig. 4) was also studied by this method. The retention volumes for the antidepressants were considerably different: diclofensine 125 ml, imipramine 88 ml, cianopramine 167 ml. The results indicate that a high selectivity is achieved.



	R
Imipramine	H
Cianopramine	CN

Fig. 4. Structural formulae of imipramine and cianopramine.

Precision of the method

Intra-assay variability was determined in the 100—700 ng/ml concentration range studied, yielding a mean coefficient of variation of 4.8% for the method. The coefficient of variation was 3.3% at 700 ng/ml and 6.2% at 100 ng/ml (n = 4).

Limit of detection

Using the conditions outlined, the limit of detection with 1 ml plasma and 1-μl injection was 70 ng/ml where the signal-to-noise ratio was just greater than 3:1.

CONCLUSION

The derivatization and electron-capture detection of diclofensine is a sensitive and accurate method for the determination of the drug in plasma. Although the levels determined did not reach the expected therapeutic plasma level, the method offers a means of measuring trace amounts of diclofensine. The extraction, separation and determination of spiked plasma samples indicates the potential use of the method in clinical applications. At the time of this study the expected metabolites of this compound were not available. N-Desmethyldiclofensine, a possible metabolite, is an intermediate in the preparation of extracted diclofensine for derivatization. However, such a metabolite could be distinguished by immediate derivatization with heptafluorobutyric anhydride and triethylamine.

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