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DETERMINATION OF CIANOPRAMINE IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND GAS—LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET, FLUORESCENCE AND ELECTRON-CAPTURE DETECTION

H. HOJABRI and J.D. GLENNON*

Department of Chemistry, University College Cork, Cork (Ireland)

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SUMMARY

New high-performance liquid chromatographic (HPLC) and gas—liquid chromatographic (GLC) methods for the measurement of the antidepressant cianopramine in human plasma are compared for precision, accuracy, selectivity, sensitivity and convenience. Important differences were found with regard to precision, selectivity and sensitivity when ultraviolet, fluorescence and electron-capture detection were used. The mean coefficients of variation for intra-assay variability of cianopramine were 1.0% (HPLC—UV), 1.5% (HPLC—fluorescence) and 5.3% (GLC) over the concentration range studied. The results obtained support the selection of HPLC as the method of choice for the analysis of cianopramine in plasma, based mainly on its merits of sensitivity and convenience, despite the enhanced selectivity of the GLC method.

INTRODUCTION

Separation techniques have played a vital part in the determination of antidepressant drugs at the clinical and quality control levels. Tricyclic antidepressants are by far the most commonly prescribed drugs for the treatment of psychiatric patients suffering from depression. Gas—liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) have been extensively used, employing a vast variety of column packings and mobile phases. The choice of detection in GLC determination of these drugs varies from flame ionization detection (FID), thermionic and nitrogen—phosphorus detection, to mass spectrometric detection [1—8]. In liquid chromatographic determination of tricyclic antidepressants, ultraviolet (UV) detection has mainly been employed using a variety of modes, i.e. adsorption, ion pairing and reversed phase. More frequent use has been made of adsorption chromatography [9—19].

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Cianopramine, a newly synthesized tricyclic antidepressant, resembles imipramine closely (Fig. 1) and is used in this study with imipramine for the comparison of the relative performances of GLC, with electron-capture detection (ECD) and HPLC, with UV and fluorescence detection.

Fig. 1. Chemical structures of cianopramine (I) and imipramine (II).

EXPERIMENTAL

Materials and reagents

Cianopramine, 5-[3(dimethylamino)propyl]-10,11-dihydro-5H-dibenz[*b*,*f*]-azepine-3-carbonitrile hydrochloride (Ro 11-2465) was kindly supplied by its manufacturers Hoffmann-La Roche (Basle, Switzerland) in pure form. The internal standard (for GLC analysis), imipramine hydrochloride (99% purity), was obtained from Sigma (St. Louis, MO, U.S.A.) and nomifensine (for HPLC analysis) was supplied by Hoechst (Middlesex, U.K.). Heptafluorobutyric anhydride was purchased from Pierce (Rockford, IL, U.S.A.). Diethyl ether, methanol, *n*-heptane, ammonia and triethylamine were all analytical-reagent grade (Hopkins and Williams, Essex, U.K.). Methyl chloroformate was obtained from Aldrich (Gillingham, U.K.). HPLC-grade acetonitrile and isopropanol were purchased from Rathburn Chemicals (Walkerburn, U.K.). Plasma was prepared by adding triply distilled water to dry plasma (Blood Transfusion Service Board, Dublin, Ireland).

Gas—liquid chromatography

Preparation of standards. Cianopramine hydrochloride (11.19 mg equivalent to 10 mg cianopramine) was dissolved in 100 ml of methanol to provide a stock solution (100 μg/ml). A series of solutions was made ranging from 3 to 10 μg/ml from the stock solution. Dilution was made using triply distilled water. These working standard solutions were used to spike the plasma samples. Spiking was carried out by addition of 100 μl of these standards to 1-ml aliquots of plasma to yield a concentration range of 300—1000 ng/ml cianopramine 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 methanol. This solution was further diluted with triply distilled water to give an imipramine concentration of 50 μg/ml.

Extraction and derivatization procedure. Plasma samples (1 ml) were spiked with 100 μl cianopramine standard solution and 100 μl of internal standard in precleaned glass tubes and shaken on a vortex mixer for 5 sec. To each tube were added 200 μl of 1 *M* sodium hydroxide. This was followed by the addition of 1.5 ml diethyl ether. Tubes were shaken vigorously for 1 min.

To separate the supernatant, tubes were centrifuged at 700 *g* for 5 min. The separated organic phase was placed in another dry glass tube and evaporated to dryness under a gentle flow of nitrogen. The residue was derivatized according to the previously published procedure [20]. Aliquots (1-μl) of the organic phase were used for GLC analysis.

Apparatus. A Sigma 4 Perkin-Elmer gas chromatograph equipped with a ^{63}Ni electron-capture detector was used. A glass column, 2 m × 2 mm I.D., packed with 3% OV-17 on Chromosorb W HP (80—100 mesh) was used for the analysis. The chromatographic conditions employed were: oven temperature 265°C, detector and injector port temperature 300°C, flow-rate 37 ml/min (oxygen-free nitrogen), make-up carrier gas flow-rate 75 ml/min. A Hewlett-Packard 3390 A reporting integrator was used to record and measure the peak heights.

High-performance liquid chromatography

Preparation of standards. From a stock solution of cianopramine in methanol (100 μg/ml) a series of working standards ranging from 0.5 to 8 μg/ml was prepared by multiple dilution in triply distilled water. To evaluate the efficiency of extraction a separate set of solutions of standard cianopramine in the mobile phase was also prepared. The cianopramine concentration was between 100 to 2000 ng/ml. From a stock solution of nomifensine (100 μg/ml) (internal standard) in methanol a solution containing 10 μg/ml was prepared to be used for the UV detection study.

Extraction procedure. Plasma samples (1 ml) were spiked with 100 μl of the working standard to provide a range of 50—800 ng/ml cianopramine in plasma. To each solution were also added 100 μl of the internal standard. Plasma samples were shaken on a vortex mixer for 5 sec. To each tube were added 600 μl of 1 *M* sodium hydroxide and 2 ml diethyl ether. Aliquots of the organic phase (1.6 ml) were separated after shaking each tube vigorously and centrifuging at 700 *g* for 5 min. This was followed by the addition of another 2 ml of diethyl ether to the plasma sample. This quantity of diethyl ether was later added to the first extract following the same procedure. Evaporation of the diethyl ether was carried out under a gentle flow of nitrogen. The residues were then redissolved in 400 μl of the mobile phase, with 20-μl aliquots used for HPLC analysis.

Apparatus. A Pye-Unicam LC3XP pump, a Pye Unicam LC-UV detector with variable-wavelength detection (190—380 nm), a fixed-volume (20 μl) injection loop system (Rheodyne 7125) and Hewlett-Packard reporting integrator Model 3390 A were used. A Perkin-Elmer LS 3 fluorimeter and a Waters autoinjector 710 B (WISP) with variable injection volume were used for the fluorimetric analyses. A stainless-steel column (25 cm × 4.6 mm I.D.) commercially packed with 5-μm spherical silica Hypersil (Magnus Scientific Instrumentation, Bucks, U.K.) was used for the separation. The mobile phase used was acetonitrile—isopropanol—concentrated ammonia (54:46:0.7) and UV detection was at 235 nm (flow-rate 0.9 ml/min). The detector settings for the fluorimetry were 284 and 450 nm for excitation and emission, respectively (flow-rate 1.5 ml/min).

RESULTS

Gas—liquid chromatography

Cianopramine and the internal standard, imipramine, both have tertiary amine groups and are difficult to chromatograph directly. The drug and the internal standard were derivatized using heptafluorobutyric anhydride as previously reported [20]. Both drugs showed good chromatographic behaviour after being derivatized. Fig. 2 shows typical chromatograms. The retention times for cianopramine and imipramine are 5.70 and 2.10 min, respectively. The calibration curve, constructed by plotting the peak height ratios of cianopramine to imipramine over the concentration range 300—1000 ng/ml in plasma gave a good correlation coefficient of 0.99.

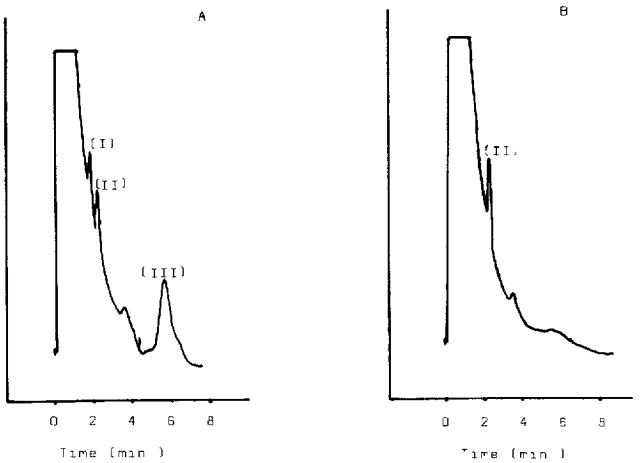

Fig. 2. GLC profiles of (A) extract of spiked plasma containing 300 ng/ml cianopramine (retention time, t_R = 5.7 min) and imipramine (t_R = 2.10 min) and (B) extract of blank plasma (no internal standard or cianopramine present). Peaks: I = internal standard, imipramine; II = endogenous; III = cianopramine.

Selectivity of the method. The closest tricyclic antidepressant in terms of structure is imipramine. The difference between the two retention times of the derivatized forms of these compounds indicates the high selectivity of the method. The isoquinoline-type antidepressant, diclofensine, under similar conditions gives a retention time of 3.38 min.

Precision of the method. Intra-assay variability was determined in the 300—1000 ng/ml concentration range, yielding a mean coefficient of variation (C.V.) of 5.3% for the method. The C.V. was 4.4% at 1000 ng/ml (n = 4) and 8.6% at 300 ng/ml (n = 7).

High-performance liquid chromatography

Cianopramine and nomifensine (internal standard) with pK_a values of 8.7 and 7.0, respectively were both extracted from plasma using diethyl ether as the solvent. The choice of solvent was made by examining the efficiency of extraction for a variety of extracting solvents using FID detection (benzene, ethyl acetate, chloroform, hexane and diethyl ether). Fig. 3 shows

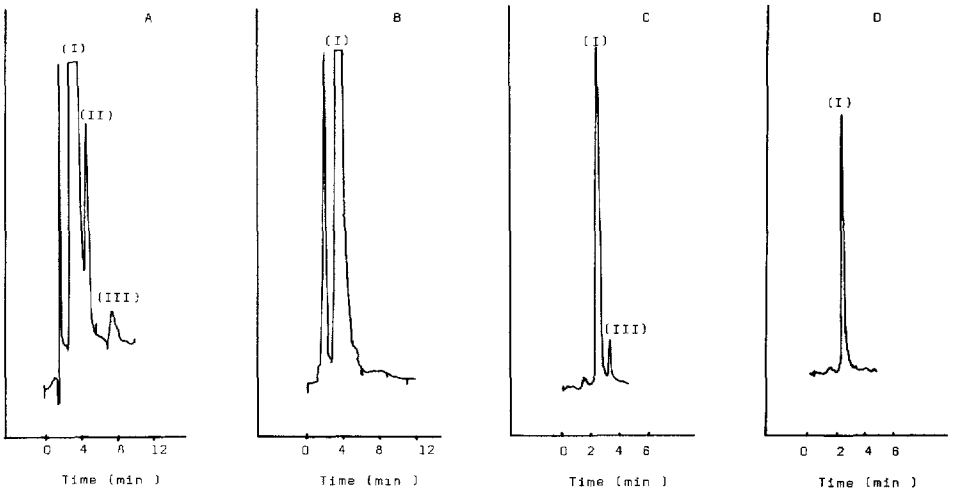

Fig. 3. HPLC profiles of extracts of (A) spiked plasma containing 50 ng/ml cianopramine (retention time, t_R = 7.34 min) and nomifensine (t_R = 4.56 min), (B) blank plasma (no internal standard or cianopramine present), (C) spiked plasma containing 50 ng/ml cianopramine (t_R = 3.40 min) and (D) drug-free plasma. Conditions: A and B, UV detection 235 nm, flow-rate 0.9 ml/min; C and D, fluorescence detection 450 nm, flow-rate 1.5 ml/min. Peaks: I = endogenous; II = internal standard, nomifensine; III = cianopramine.

chromatograms obtained for extracts of blank plasma, and of human plasma spiked with cianopramine and nomifensine, using UV and fluorescence detection.

Limit of detection and quantitation. The limit of detection was taken as the concentration of cianopramine that gives a reading equal to three times the standard deviation of a series of determinations carried out with a solution of a concentration close to the level of the blank. Using the concentration of 50 ng/ml the limit of detection is calculated to be 10 ng/ml with UV detection and 12.5 ng/ml with fluorescence detection for a 20-μl injection. The respective limits of quantitation are 33 and 41 ng/ml, just below the concentration range studied.

Recovery. The overall recovery was calculated by comparing the values obtained for the slope of the extracted standard curve to that of unextracted standards. Using this technique in the concentration range 50—800 ng/ml, the mean overall recovery was 79.6%.

Precision and accuracy of the method. The mean C.V. was taken as a measure of precision of the method (Table I). Values of 1% and 3.5% were obtained for intra-assay and inter-assay variabilities, respectively, for UV detection. The tabulated differences between the mean values of replicate assays of the samples and the true values are a measure of the accuracy of the method. The mean C.V. values for the fluorescence detection were 1.5% (intra-assay) and 2.5% (inter-assay).

Linearity. The plots of peak height ratios of cianopramine to those of the internal standard obtained on different days showed correlation coefficients not less than 0.99. Similar linearity was observed for the fluorimetric analyses.

TABLE I

STATISTICAL EVALUATION OF THE PRECISION FOR THE HPLC ASSAY OF CIANOPRAMINE IN PLASMA ($n \geq 4$)

Concentration added (ng/ml)	Intra-assay		Inter-assay	
	Found (ng/ml)	C.V. (%)	Found (ng/ml)	C.V. (%)
50	46.1 ± 1.5	3.3	43 ± 2.7	6.3
100	110.4 ± 1.5	1.4	105.6 ± 7.0	6.7
200	208.9 ± 0.0	0.0	202.5 ± 4.4	2.2
400	385.6 ± 2.7	0.7	405.8 ± 18.2	4.5
600	587.0 ± 1.5	0.3	586.0 ± 2.3	0.4
800	812.9 ± 2.5	0.3	807.0 ± 7.3	0.9

DISCUSSION

The HPLC and GLC—ECD methods developed in this study are representative of two important approaches used frequently in the analysis of tricyclic antidepressants. The study not only provides new methods of trace-level analysis of the tricyclic antidepressant, cianopramine, but also presents us with the opportunity of comparing the relative merits of a number of detection systems following separation.

The detection and GLC properties of cianopramine are considerably improved by derivatization with heptafluorobutyric anhydride. The derivatization procedure has previously been used for imipramine, amitriptyline, and diclofensine. When compared with HPLC the method shows a higher selectivity, an important attribute for example in multiple drug therapy. However, due to its multistep nature, the method is not very suitable for routine analysis, and under routine rather than rigorous laboratory conditions, the sensitivity was less than anticipated.

Imipramine, amitriptyline, diclofensine, flurazepam, and clobazam were examined for possible interference in the HPLC assay of cianopramine. Only imipramine exhibited similar retention on Hypersil under the conditions employed for the liquid—solid chromatographic separation. From the results obtained, it is evident that the sensitivity of the HPLC method with UV or fluorescence detection of the drug is superior to that achieved by GLC. The method is also preferred in terms of convenience. It can be seen from the chromatograms in Fig. 3A and C that increased selectivity in terms of less endogenous peaks can also be achieved by employing fluorescence rather than UV detection without a significant change in sensitivity. It should be noted that the difference in the illustrated retention times for cianopramine arises from the different flow-rates used. Some improvement in selectivity can also be achieved with UV detection by utilizing the characteristic absorption of cianopramine of 284 nm, albeit with some loss of sensitivity.

In conclusion, it is clear that the newly developed methods are potentially useful in clinical analysis. The results obtained support the selection of HPLC as the method of choice for the analysis of cianopramine in plasma, based mainly on its merits of sensitivity and convenience together with the variety

of detection systems available. Indeed it is likely that even greater sensitivity may be achieved using HPLC—electrochemical detection.

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