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GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF NOMIFENSINE IN HUMAN PLASMA

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SUMMARY

An analytical method based on solvent extraction, formation of a fluorinated derivative and quantitation by gas–liquid chromatography using an electron capture detector has been developed for the determination of nomifensine in biological fluids.

The specificity (controlled by mass spectrometry) and the sensitivity appear to be satisfactory for drug level measurements in human body fluids. Its relative simplicity in fact permits its use in serial analysis.

INTRODUCTION

Nomifensine is a new antidepressant agent, which, unlike other available antidepressants, has been shown to inhibit strongly dopamine uptake in the striatum of rats[1–4]. In humans, the antidepressive activity of nomifensine appears to be comparable with that of imipramine when administered at the daily dose of 75–100 mg[5,6].

Only radioactive data are presently available on the pharmacokinetics of nomifensine. Knowledge of the pharmacokinetic profile of a drug is today considered essential both for a better understanding of its mechanism of action and for a more rational treatment schedule.

In this paper we report a sensitive and specific gas chromatographic method for the determination of nomifensine in human biological fluids. The method is based on solvent extraction and the formation of a fluorinated derivative. The sensitivity is of the order of 1 ng/ml of plasma.

EXPERIMENTAL

Standards and reagents

Nomifensine was kindly supplied by Hoechst (Frankfurt, G.F.R.). 2-Amino-5-chlorobenzophenone was used as internal marker.

Other reagents were diethyl ether R.P. (Carlo Erba, Milan, Italy), ethyl acetate

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R.P. (Carlo Erba) and heptafluorobutyric anhydride, *puriss.* p.a. (HFBA) (Fluka, Milan, Italy).

Diethyl ether and ethyl acetate were distilled before use. [^{14}C]Nomifensine (spec. act. 186 μCi/mg) also supplied by Hoescht was used for the evaluation of the extraction yield.

Apparatus

A Carlo Erba Fractovap G-I gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) was used.

The chromatographic column was a glass tube, 2 m long and 4 mm I.D., packed with 100–120 mesh Chromosorb Q, coated with 3% OV-17 (Applied Science Labs. State College, Pa., U.S.A.), conditioned for 1 h at 250° (40 ml/min flow-rate of nitrogen), 4 h at 340° (no nitrogen) and 24 h at 275° (40 ml/min flow-rate of nitrogen). The operating conditions were: column temperature, 245°; carrier gas (nitrogen) flow-rate, 40 ml/min; and scavenger gas (nitrogen) flow-rate, 60 ml/min. The ECD was used with a pulse current at an excitation voltage of 50 V and a pulse interval of 30 msec.

For mass spectrometry, an LKB Model 9000 mass spectrometer combined with a gas chromatograph was used. The mass spectrometric conditions were: ionization beam energy, 70 eV: ion source temperature, 290°; accelerating voltage, 3.5 kV; and trap current, 60 μA. A 2 m long glass column packed with 3% OV-17 on 100–120 mesh Gas Chrom Q operated at 200° with a helium gas flow-rate of 20 ml/min was used. On analyzing the samples using the direct insertion probe, the mass spectrometric conditions were the same as above, and the temperature of the probe was 140°.

Determination of standard external calibration curves

Nomifensine was dissolved in methanol (1 μg/ml) and individual aliquots covering the range 10–50 ng (in triplicate) were dried in test tubes under a stream of nitrogen. 50 μl of HFBA (1:8 solution in ethyl acetate) were added to the dry residue. The tubes were capped, shaken in a mixer for about 20 sec, and incubated at 40° for 30 min. Then the solutions were evaporated to dryness under a stream of nitrogen. When the tubes were dry, the nitrogen flow-rate was increased for 10 min. This last step was found to be essential to prevent the presence of interfering peaks from the reaction mixture. Subsequently, 0.3 ml of hexane were twice added to each tube and evaporated under a stream of nitrogen. The derivative was then dissolved in 100 μl of ethyl acetate containing as a marker, 2-amino-5-chloro-benzophenone, and 1 μl of this solution was injected into the gas chromatograph.

Extraction procedure

Increasing amounts of nomifensine from 10 to 400 ng/ml were added to 2 ml of human plasma. The plasma was then treated with phosphate buffer (pH 9.5; 0.5 *M*) to a final volume of 2.4 ml, followed by the addition of 0.1 ml of 2 *N* NaOH. The samples were extracted with 5 ml of diethyl ether while shaking at room temperature for 20 min in a horizontal position. The tubes were centrifuged at 1200 *g* at 4° for 5 min, and 4.5 ml of ether were transferred to a second test tube containing 2.5 ml of 0.5 *N* HCl. After shaking at room temperature for 10 min, the tubes were

centrifuged, whence the organic phase was carefully withdrawn and the acidic aqueous phase quantitatively transferred to another test tube and washed with 5 ml of diethyl ether. After removing the organic phase, 0.7 ml of 2 *N* NaOH were added to the acidic aqueous phase and shaken in a horizontal position with 5 ml of diethyl ether at room temperature for 20 min. After centrifugation, 4.5 ml of ether were transferred to a fourth test tube and evaporated to dryness in a water-bath at 40°. To the dry residue, 50 μl of HFBA (1:8 solution in ethyl acetate) were added and the samples were processed as described above. When performing determinations on samples from human volunteers who received nomifensine (50 mg orally) in a fasting state, 0.5–2 ml of plasma were used. An internal calibration was always carried out in addition to the unknown samples.

RESULTS AND DISCUSSION

The reaction of nomifensine with HFBA, under the conditions used, was completed with a yield of 99%. The nomifensine heptafluorobutyrate is stable over a period of 60 days at 4°.

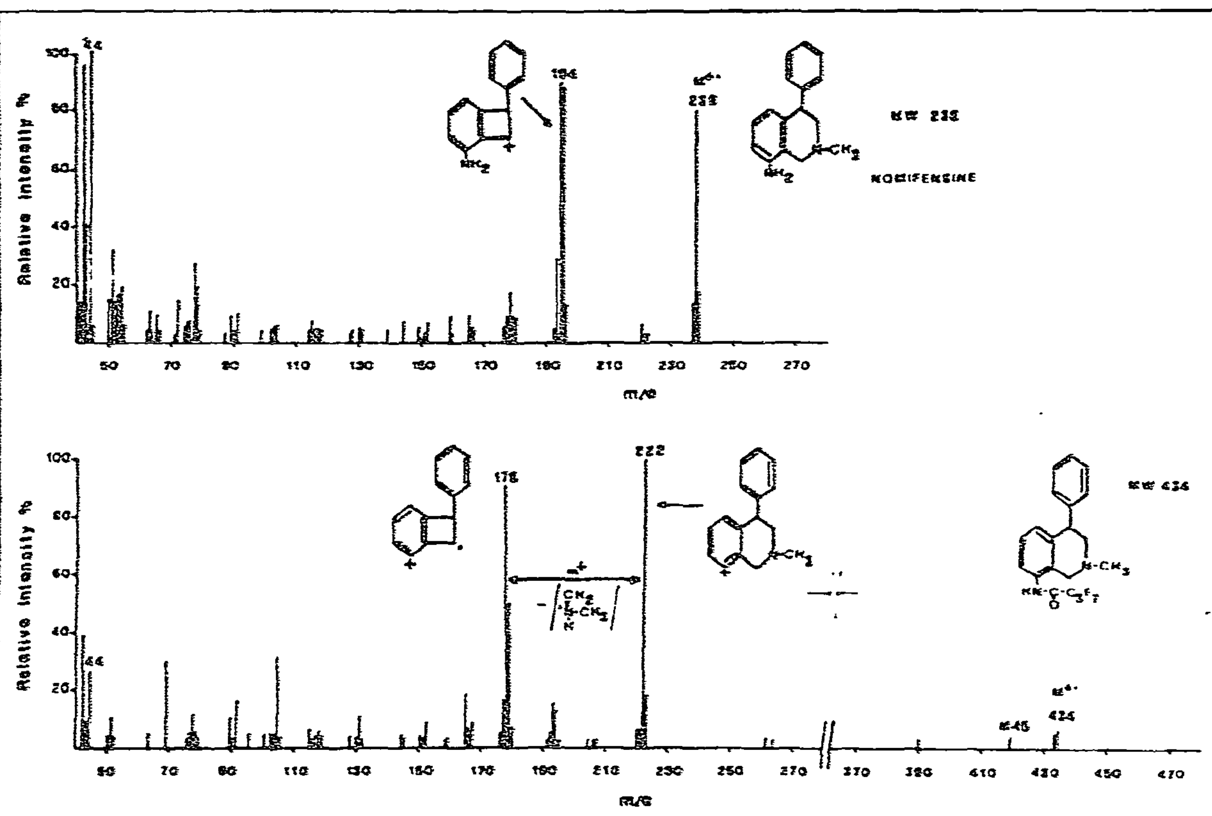

Fig. 1. Mass spectra of nomifensine (top) and nomifensine heptafluorobutyrate (bottom). Nomifensine was analyzed by a direct insertion probe and its heptafluorobutyrate derivative by gas chromatography–mass spectrometry as described in the text.

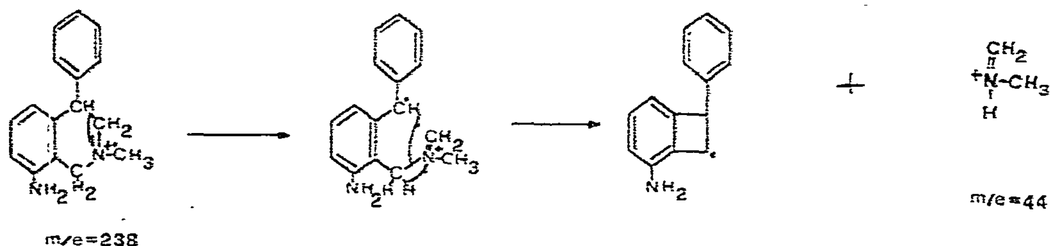

Fig. 2. Possible fragmentation mechanism for the formation of the ion at *m/e* 44 in the mass spectra of nomifensine and its derivative.

Fig. 1 shows the mass spectra of nomifensine and nomifensine heptafluorobutyrate. The mass spectrum of nomifensine shows a molecular ion at *m/e* 238 (relative intensity 80 %). The presence of a base peak at *m/e* 44 is due to the mechanism shown in Fig. 2. Another intense peak (91 %) is present at *m/e* 194 and a possible structure for this ion is suggested in Fig. 1.

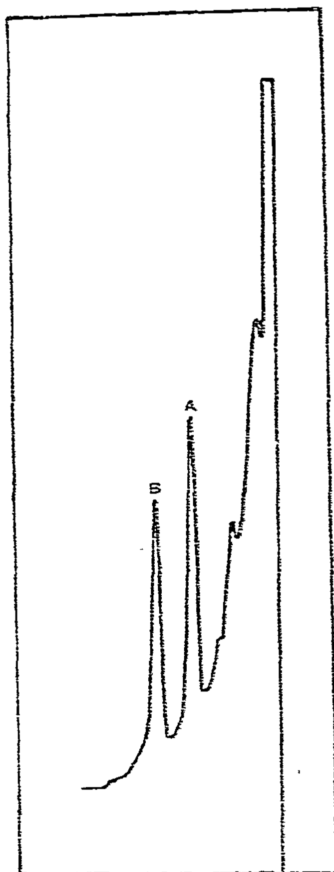

Fig. 3. Gas chromatogram of 300 pg of nomifensine as its heptafluorobutyrate (A) and 1 ng of 2-amino-5-chloro-benzophenone (B) obtained from plasma extract.

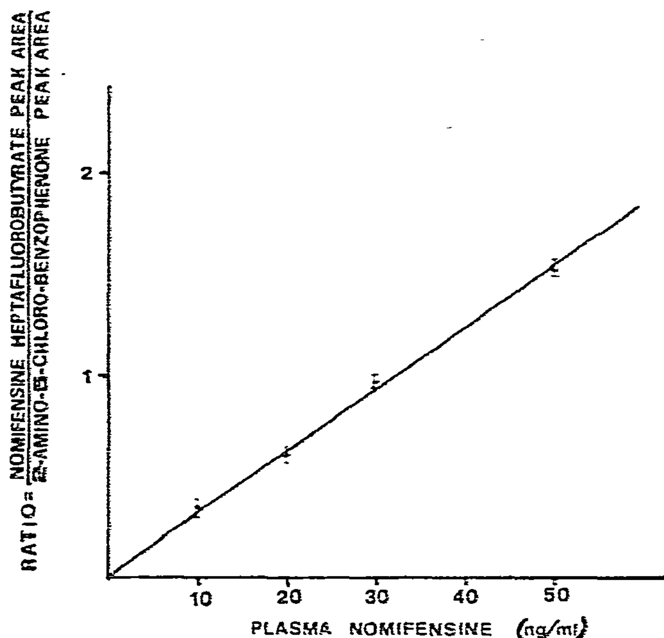

Fig. 4. Standard calibration graph for nomifensine added to the plasma and carried through the analytical procedure.

The mass spectrum of nomifensine heptafluorobutyrate shows a molecular ion at *m/e* 434, suggesting that only one heptafluorobutyrate group is present in the molecule. The base peak at *m/e* 222 arises from the loss of HN–OC–C_3F_7 (*i.e.* 212 a.m.u.). The second most intense peak in the spectrum is present at *m/e* 178, and the structure of this ion is shown in Fig. 1.

A typical gas chromatogram of the heptafluorobutyrate derivative of nomifensine and of the marker obtained with plasma extract is reported in Fig. 3.

The calibration curve obtained by plotting the ratio of the peak area of nomifensine heptafluorobutyrate to that of the marker against known amounts of nomifensine added to the plasma is shown in Fig. 4. A good linear plot was obtained for concentrations in the range 5–50 ng/ml of plasma. The minimum detectable amount

TABLE I

NOMIFENSINE PLASMA LEVELS (ng/ml) IN HEALTHY VOLUNTEERS AFTER A SINGLE ORAL ADMINISTRATION OF 50 mg

Subject	*Time*					
	1	*1.5*	*2*	*4*	*6*	*8*
No. 1 (V.L.)	117	142	130	77	45	33
No. 2 (K.L.)	177	170	110	36	21	12
No. 3 (P.J.)	22	55	75	93	37	15
Mean ± S.E.	105	122	105	68	34	16
	45	34	16	16	7	3

with such a procedure was for an injection of 100 pg, which means that by using 3 ml of plasma and reducing the solvent volume to 30–20 μl in the last step, concentrations as low as 1 ng/ml could be detected.

Other compounds usually encountered in the course of antidepressant therapy, such as benzodiazepine and barbiturates, were found not to interfere. The recovery of the extraction procedure checked by means of [^{14}C]nomifensine was 93 $\pm$ 2%.

Administration of nomifensine (50 mg orally) to three healthy volunteers led to the plasma levels reported in Table I. It appears that nomifensine is absorbed rapidly from the gastrointestinal tract, with peak plasma levels between 1 and 4 h. The apparent rate of disappearance from plasma is also a rapid process ($t_{\frac{1}{2}}$ about 3–4 h) and no measurable concentrations were found after 24 h.

CONCLUSIONS

The method described is capable of detecting nomifensine concentrations in human plasma at levels as low as 1–5 ng/ml. Its specificity tested against other compounds which may be encountered in the course of antidepressant treatment was excellent. Its relative simplicity makes it useful for routine analysis and pharmacokinetic studies.

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