Journal of Chromatography, 276 (1983) 85—92
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1718

DETERMINATION OF NOMIFENSINE IN HUMAN SERUM

A COMPARISON OF HIGH-PERFORMANCE LIQUID AND GAS—LIQUID CHROMATOGRAPHY

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(First received December 22nd, 1982; revised manuscript received March 1st, 1983)

SUMMARY

High-performance liquid (HPLC) and gas—liquid chromatographic (GLC) methods for the measurement of the antidepressant nomifensine in human serum were developed and compared for precision, accuracy, sensitivity and convenience. No significant difference was found between these two techniques with regard to sensitivity and precision. Both methods can accurately measure serum nomifensine concentrations down to 8 nmol/l. The coefficient of variation (C.V.) for intra-assay variability of nomifensine was 4.8% (HPLC) and 5.5% (GLC) at 150 nmol/l. The HPLC method proved to be both simpler and more selective than the GLC method. The calibration graph was linear over the range 8—1000 nmol/l in the HPLC method, but only up to 150 nmol/l in the GLC method. The selectivity and simplicity of the HPLC method make it useful for both pharmacokinetic studies and therapeutic serum level monitoring of nomifensine. The HPLC method was applied to the analysis of serum samples obtained from four healthy individuals receiving therapeutic dosages of nomifensine.

INTRODUCTION

Nomifensine is a new antidepressant agent with a pharmacological profile different from the classical tricyclic antidepressants [1—3]. A number of methods have been described for the measurement of nomifensine, gas chromatographic procedures having been applied successfully to its analysis in serum [4—6]. This approach, however, requires prior derivatization to a fluorinated derivative and is relatively time consuming.

A radioimmunoassay (RIA) for nomifensine in serum has also been reported [7]. Since a correction for cross-reactivity of the nomifensine conjugate has

0378-4347/83/$03.00

to be made, the accuracy of that method may be suspect. Only one high-performance liquid chromatographic (HPLC) method for nomifensine has been published [8]. The detection limit of that method, about 84 nmol/l, is insufficient for pharmacokinetic studies.

As the currently available techniques for the analysis of nomifensine are either time consuming or lack sensitivity and accuracy, a selective and sensitive HPLC method for the determination of low levels of nomifensine in human serum has been developed. A gas—liquid (GLC) chromatographic method was also developed and then compared for accuracy, reliability and convenience with HPLC.

A study of the pharmacokinetics of nomifensine in healthy individuals after a single dose was made using the HPLC method described.

EXPERIMENTAL

Materials

Nomifensine maleate (M.W. 238.3 as nomifensine base) and *p*-chlorodisopyramide were obtained from Leiras Pharmaceutical Plant (Turku, Finland) and desmethylmaprotiline from Giba-Geigy (Basle, Switzerland). *n*-Hexane, acetonitrile and ethyl acetate were of analytical-reagent grade (E. Merck, Darmstadt, G.F.R.). Heptafluorobutyric anhydride (Pierce, Rockford, IL, U.S.A.) was used to prepare heptafluorobutyrate derivates of nomifensine.

p-Chlorodisopyramide solution in methanol (5 μg/ml) was used as the internal standard in the HPLC method and desmethylmaprotiline solution in ethanol (10 μg/ml) in the GLC method. Serum standards were prepared by spiking fresh human serum to concentrations within the range 12.5—1000 nmol/l.

High-performance liquid chromatography

Extraction procedure. A serum sample (1 ml) was made alkaline with 1 ml of 2 *M* sodium hydroxide solution and 125 μl of internal standard solution (5 μg/ml) were added. This mixture was extracted twice with *n*-hexane (5 ml) and the combined organic extracts were evaporated to dryness at 40°C under a gentle stream of pure nitrogen. The dry residue was dissolved in 100 μl of 0.05 *M* phosphoric acid and 50 μl were injected into the chromatograph.

Chromatographic analysis. Analyses were carried out on a Model SP 740 HPLC system (Spectra-Physics, Santa Clara, CA, U.S.A.) using an HP 1030B UV detector (Hewlett-Packard, Waldbronn, G.F.R.) set at 205 nm. An SP 4100 computing integrator (Spectra-Physics) was used to calculate peak heights. The mobile phase consisted of acetonitrile—0.05 *M* potassium phosphate buffer (26:74, v/v) (pH 3.5) and the flow-rate was 1.6 ml/min. The reversed-phase column was a 10-μm μBondapak C_{18}, 30 cm × 3.9 mm I.D. (Waters Assoc., Milford, MA, U.S.A.).

Gas—liquid chromatography

Extraction procedure. Internal standard (150 μl), 1 *M* sodium hydroxide solution (1 ml) and *n*-hexane (5 ml) were added to the serum sample (1 ml) and the mixture was shaken for 15 min. After centrifugation, the hexane layer

was transferred into a conical tube and 0.5 *M* hydrochloric acid (2.5 ml) was added. After shaking for 15 min the organic phase was carefully withdrawn and the acidic aqueous phase was washed with *n*-hexane (5 ml). The organic phase was removed and 2 *M* sodium hydroxide solution (1 ml) was added to the acidic aqueous phase and shaken with *n*-hexane (5 ml) for 15 min. After centrifugation the hexane layer (4.5 ml) was transferred into a clean test tube and evaporated to dryness at 40°C under a gentle stream of pure nitrogen. To the dry residue 100 μl of heptafluorobutyric anhydride (1:10 solution in ethyl acetate) were added. The tube was shaken in a mixer for about 15 sec, then incubated at 40°C for 30 min. The reaction mixture was taken to dryness under a stream of nitrogen at 40°C and finally reconstituted in 400 μl of ethyl acetate. A 2-μl volume of this solution was taken for GLC.

Chromatographic analysis. Analyses were performed on a Varian Series 2100 gas chromatograph equipped with a ^{63}Ni electron-capture detector (Varian Aerograph, Walnut Creek, CA, U.S.A.). The chromatographic column was a coiled glass tube, 2 m × 2 mm I.D., packed with 3% OV-17 on 80–100 mesh Chromosorb W HP (Applied Science Labs., State College, PA, U.S.A.). The temperatures were column 250°C, injector 270°C and detector 300°C, and the carrier gas (nitrogen) flow-rate was 30 ml/min. Chromatograms were recorded with a laboratory potentiometric recorder.

Pharmacokinetic application of the HPLC method

Four healthy volunteers in a fasting state were given 100 mg nomifensine orally in capsule form. All persons abstained from smoking and drinking alcohol at least 2 days before and during the experiments. They received a standardized breakfast 2 h and a standardized lunch 4 h after the drug administration. During 24 h (after drug administration) twelve blood collections were taken. Serum levels were determined by the HPLC method described above.

RESULTS AND DISCUSSION

High-performance liquid chromatography

Nomifensine and the internal standard, *p*-chlorodisopyramide, show good HPLC characteristics. Nomifensine produced a symmetrical, sharp peak having a retention time of 4.15 min. The internal standard eluted after nomifensine and was well separated from it (Figs. 1a, 2a and 3a).

Fig. 4 shows a typical calibration graph produced by HPLC analysis for known amounts of nomifensine in serum. The response is linear for samples containing 8—1000 nmol/l and the correlation coefficient is $\geqslant 0.998$. The precision of the assay was assessed by multiple analyses of the serum pools and standard control samples. The coefficient of variation (C.V.) for intra-assay variability of nomifensine was 4.8% at 150 nmol/l and 4.0% at 500 nmol/l ($n = 9$).

The detection limit was defined as the signal that was three times higher than the background noise. In this way the detection limit of the HPLC technique was found to be 8 nmol/l, which is sufficient for pharmacokinetic studies and therapeutic serum level monitoring. The HPLC method published earlier [8] is

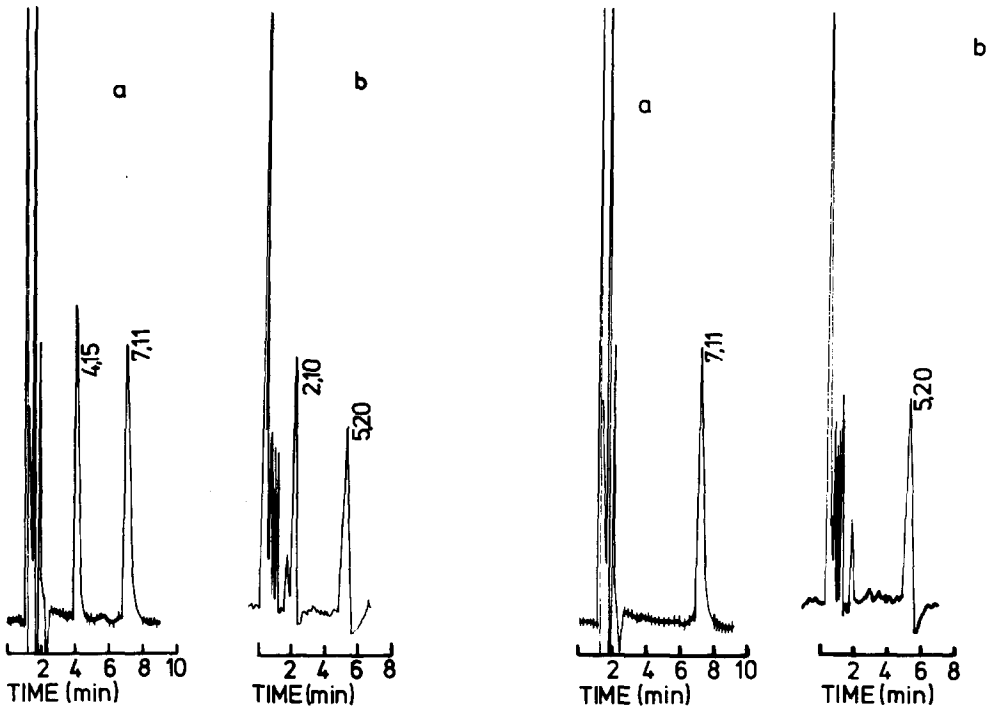

Fig. 1. (a) Liquid chromatogram of the extract from the serum sample of a volunteer 3 h after oral administration of nomifensine. The retention time of nomifensine is 4.15 min and that of the internal standard, *p*-chlorodisopyramide, is 7.11 min. The concentration of nomifensine is 354 nmol/l. (b) Gas chromatogram of the extract from blank serum spiked with 250 nmol/l of nomifensine. The retention times of nomifensine and of the internal standard, desmethylmaprotiline, are 2.10 and 5.20 min, respectively.

Fig. 2. (a) Liquid chromatogram of the extract from the blank serum. The retention time of the internal standard, *p*-chlorodisopyramide, is 7.11 min. (b) Gas chromatogram of the extract from the blank serum. The retention time of the internal standard, desmethylmaprotiline, is 5.20 min.

too insensitive for the determination of low levels of nomifensine, the detection limit being only 84 nmol/l.

The extraction efficiency was tested by determining admixtures of known amounts of nomifensine to serum in the range 25—1000 nmol/l. Comparison of the peak heights for nomifensine extracted from serum samples with those obtained after direct injection of the drug solutions into the chromatograph indicated that the extraction efficiency was 72.1% (S.D. 2.6%; $n = 8$).

Interference by other compounds was also examined. Other commonly prescribed psychotropic drugs were well separated from nomifensine and the internal standard in the column. The capacity factors for these drugs were calculated under the chromatographic conditions described (Table I).

Barbiturates sometimes encountered in the course of antidepressant therapy were found not to interfere. They are not extracted with hexane from alkalinized serum. Endogenous compounds in human serum also did not affect the HPLC analysis of nomifensine.

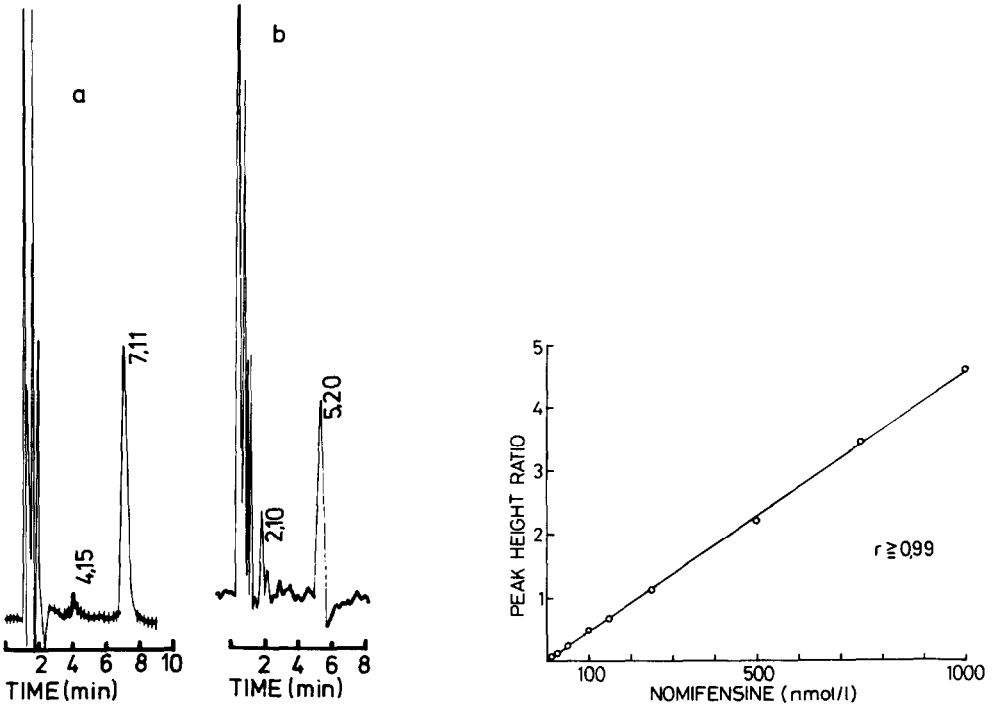

Fig. 3. (a) Liquid chromatogram of the extract from the serum sample of a volunteer 12 h after oral administration of nomifensine. The retention time of nomifensine is 4.15 min and that of the internal standard, *p*-chlorodisopyramide, is 7.11 min. The concentration of nomifensine is 17 nmol/l. (b) Gas chromatogram of the extract from blank serum spiked with 25 nmol/l of nomifensine. The retention time of nomifensine and of the internal standard, desmethylmaprotiline, are 2.10 and 5.20 min, respectively.

Fig. 4. Calibration graph for nomifensine in HPLC. Peak-height ratios of nomifensine to those of the internal standard are plotted against nomifensine concentration in serum. The correlation coefficient ($r \geq 0.998$) indicates linearity over the concentration range studied.

TABLE I

CAPACITY FACTORS OF SOME PSYCHOTROPIC DRUGS

For liquid chromatographic conditions, see the text.

Drug	k'	Drug	k'
Chlordiazepoxide	1.8	Oxazepam	9.2
Nomifensine	2.0	Lorazepam	11.3
Perphenazine	3.5	Desmethylimipramine	11.7
Internal standard	3.8	Desmethylmaprotiline	12.9
Desmethyldoxepin	6.4	Imipramine	13.9
Mianserin	6.7	Nortriptyline	14.8
Doxepin	7.6	Maprotiline	15.3
Haloperidol	8.2	Amitriptyline	17.3

Possible interference of the metabolites of nomifensine in serum after nomifensine administration was considered to be negligible. We noticed that the metabolites are not extractable in our extraction procedure. The assumed metabolites, hydroxynomifensine and hydroxymethoxynomifensine, are extracted at pH 8 [9].

Gas—liquid chromatography

Typical gas chromatograms of nomifensine and the internal standard, desmethylmaprotiline are shown in Figs. 1b, 2b and 3b. The retention times are

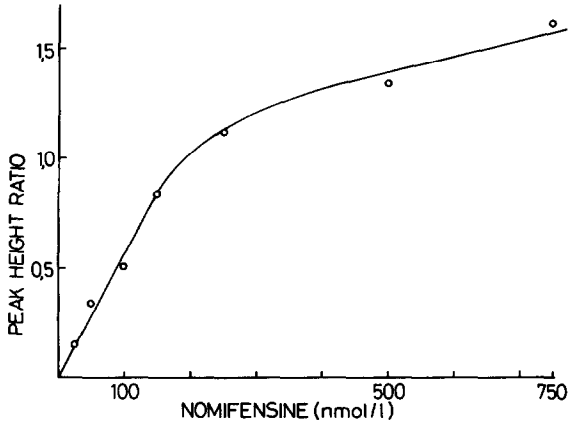

Fig. 5. Calibration graph for nomifensine in GLC. Peak-height ratios of nomifensine to those of the internal standard are plotted against nomifensine concentration in serum. The graph is linear over the concentration range 8—150 nmol/l.

TABLE II

COMPARATIVE PROPERTIES OF HPLC AND GLC FOR THE MEASUREMENT OF NOMIFENSINE IN HUMAN SERUM

Parameter		HPLC	GLC
Extraction of serum samples		One step	Three steps and derivatization
Extraction efficiency:			
at 25—1000 nmol/l (± S.D.)	(n = 8)	72.1 ± 2.6%	65.6 ± 7.7%
at 12.5	(n = 9)	68.2 ± 2.8%	—
at 25	(n = 9)	69.2 ± 5.2%	—
at 50	(n = 9)	74.8 ± 5.5%	—
at 100	(n = 9)	73.5 ± 4.9%	—
Precision (n = 9):			
at 12.5 nmol/l		4.2% (C.V.)	—
at 25		6.7%	—
at 50		5.3%	—
at 100		6.6%	—
at 150		4.8%	5.5% (C.V.)
at 500		4.0%	—
Sensitivity		8 nmol/l	8 nmol/l
Linearity		8—1000 nmol/l	8—150 nmol/l

2.10 min for nomifensine and 5.20 min for the internal standard, which indicates good resolution for these compounds. However, under the GLC conditions described here there is an interfering endogenous compound that elutes just before nomifensine. This makes the GLC assay inaccurate for the measurement of nomifensine at levels below 25 nmol/l in human serum.

The calibration graph for known amounts of nomifensine in serum obtained with the detector used here is linear only in the range 8—150 nmol/l (Fig. 5). There is no difference between HPLC and GLC with regard to precision and sensitivity. The coefficient of variation (C.V.) for intra-assay variability of nomifensine in the GLC method was 5.5% at 150 nmol/l (n = 9) and the detection limit was 8 nmol/l. The sensitivity and precision are similar to those reported by Bailey et al. [4]. The extraction efficiency in the GLC method was checked by comparing the peak heights for nomifensine extracted from serum samples with those of authentic nomifensine in methanolic solutions. The recovery was 65.6% (S.D. 7.7%; n = 7).

The comparative properties of HPLC and GLC for nomifensine analysis are shown in Table II.

Pharmacokinetic application

The serum nomifensine levels obtained after the administration of a single dose of 100 mg to four healthy individuals demonstrate that the HPLC method has sufficient sensitivity for pharmacokinetic studies (Fig. 6). Nomifensine was absorbed rapidly from the gastrointestinal tract. The peak serum concentrations of 512—1005 nmol/l were attained after 1—2 h. The elimination half-life varied between 3.6 and 3.8 h and only very low concen-

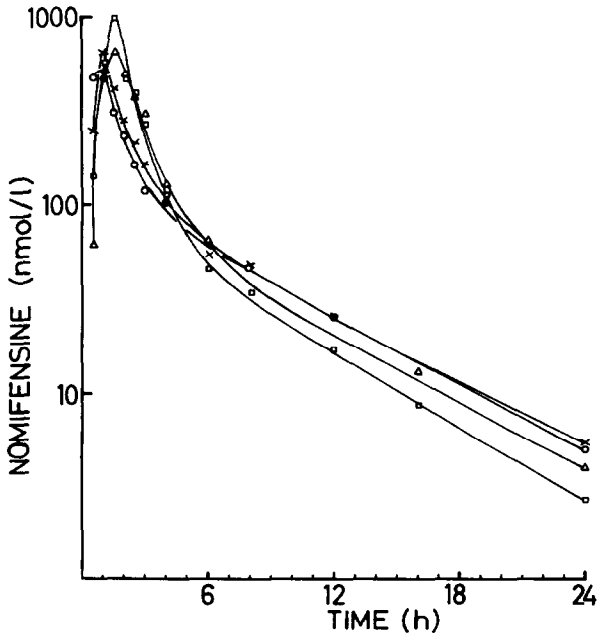

Fig. 6. Serum concentrations of nomifensine after oral administration of a single dose (100 mg) of nomifensine to four healthy volunteers.

trations were found after 24 h. These results are similar to those published earlier [10, 11].

In conclusion, the HPLC assay described here provides an efficient and accurate method for the analysis of nomifensine in human serum. We are currently using the method for pharmacokinetic studies on nomifensine after intravenous administration in dogs. The method has also been applied successfully in a study of steady-state levels in psychiatric patients. Because of the simple extraction step and the short chromatographic run time the method is useful for routine monitoring of nomifensine levels.

ACKNOWLEDGEMENT

The authors thank Mrs. Ulla Heikonen for valuable technical assistance.

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