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QUANTITATION OF HEXAPRAZOL IN HUMAN PLASMA AND URINE BY CAPILLARY GAS CHROMATOGRAPHY WITH NITROGEN-SENSITIVE DETECTION

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SUMMARY

A selective and sensitive gas chromatographic assay for hexaprazol, a new antiulcer drug, in human plasma and urine has been developed. The method involves liquid—liquid extraction and capillary gas chromatography with nitrogen-sensitive detection. The limit of quantitation of plasma hexaprazol is ca. 25 ng/ml. The assay procedure permits the measurement of the levels of the unchanged drug following its clinical administration to humans.

INTRODUCTION

Hexaprazol, N-[(N-cyclohexylcarbamoyl)methyl]piperazine monohydrochloride (I, Fig. 1), is a synthetic drug [1, 2] endowed with antiulcer activity [3]. Moreover, this drug acts as a gastric barrier protecting agent and significantly increases the production of gastic mucin [3, 4].

The biotransformation of hexaprazol has been investigated in rat, dog and human urine [5]. Four metabolites were identified: formylhexaprazol (II), acetylhexaprazol (III) and *cis*- and *trans*-hydroxyhexaprazol (IV and V) (Fig. 1).

Pharmacokinetics and bioavailability studies of this drug in humans require a sensitive and selective assay for its determination in biological fluids. This paper describes a procedure that permits the gas chromatographic (GC) determination of the unchanged drug in human plasma and urine using nitrogen-sensitive detection. The method is also selective towards hexaprazol metabolites.

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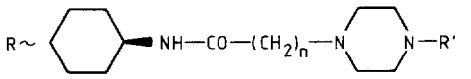

Compound	R	R'	n
I	H	H·HCl	1
II	H	CHO	1
III	H	$COCH_3$	1
IV	*cis*-OH	H	1
V	*trans*-OH	H	1
I.S.	H	H	2

Fig. 1. Chemical structures of hexaprazol (I), and its metabolites (II–V) and the internal standard (I.S.).

EXPERIMENTAL

Chemicals and reagents

Hexaprazol and N-[(N-cyclohexylcarbamoyl)ethyl]piperazine (I.S., internal standard, Fig. 1) were synthesized in our laboratory as reference standards (purity $\geqslant$ 99.8%). All the solvents and reagents were of analytical-reagent grade and were used as such, with the exception of chloroform, which was filtered daily on active, basic aluminium oxide 60 (Merck 1067, Type E; Darmstadt, F.R.G.). All glassware was cleaned by sonication in a 0.3% Extran AP11 (Merck) water solution and then by thorough rinsing with tap-water, distilled water, acetone and ethanol. Test-tubes were silanized with 5% Surfasil (Pierce 42801; Rockford, IL, U.S.A.) in toluene (Merck 8325) and rinsed with toluene and then with absolute ethanol.

Standard solutions

Stock solutions of hexaprazol (1 mg/ml) and of I.S. (1 mg/ml) were prepared by dissolving the compounds in distilled water. The other working standards of hexaprazol (100, 200, 300, 400 and 500 ng/ml of water and 1, 1.5, 2, 3, 5, 10, 20, 40, 60 and 100 μg/ml of water) and of I.S. (500 ng/ml of water and 3, 4 and 40 μg/ml of water) were prepared from these stock standards.

Equipment

A Perkin-Elmer Model Sigma 2 gas chromatograph equipped with a Perkin-Elmer Sigma 10 integrator and a nitrogen–phosphorus detector was used. The column was a 20 m × 0.23 mm I.D. fused-silica capillary column coated with the non-polar chemically bonded phase CP Sil 5CB (Chrompack, Middelburg, The Netherlands). Splitless injection was used with a splitless period of 40 s. The injector and detector temperatures were 250 and 270°C, respectively. The column temperature was raised from 100 to 245°C at 32°C/min following injection and kept at 245°C for 4 min. The pressure of the carrier gas, helium, to the column was 140 kPa. The flow-rates to the septum purge and to the split were 2.5 and 37.5 ml/min, respectively. The detector gas pressures were: hydrogen, 50 kPa; air, 120 kPa. The flow-rate of the make-up gas, nitrogen,

was 30 ml/min. The bead temperature control was adjusted to the best working range (475—650 instrument arbitrary units).

Extraction procedure from plasma

Plasma aliquots (0.5—2 ml) were placed into 15-ml glass centrifuge tubes, and 0.5 ml of I.S. (0.25 μg or 1.5 μg) were added to each tube. The volumes were eventually adjusted to 2.5 ml with distilled water, and then 6 ml of *n*-hexane were added to each tube. The tubes were stoppered with PTFE-lined screw caps, shaken mechanically for 5 min and centrifuged for 10 min at 600 *g*. The organic layer was discarded. The aqueous phase, alkalinized with 1 ml of 1 *M* sodium hydroxide, was extracted twice with 7 ml of chloroform and centrifuged for 10 min at 600 *g*. The combined organic phases were transferred into a clean 25-ml glass centrifuge tube and re-extracted with 5 ml of 0.02 *M* sulphuric acid. Each tube was centrifuged for 10 min at 600 *g* and the organic layer was discarded. The aqueous phase was treated with 0.3 ml of 5 *M* sodium hydroxide and then extracted with 7 ml of chloroform. The organic phase was transferred into a silanized glass tube and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in absolute ethanol (100 or 200 μl); 3 μl of this solution were injected into the chromatograph.

Extraction procedure from urine

Urine aliquots (0.1—1 ml) were transferred into 15-ml glass centrifuge tubes and 0.5 ml of I.S. (2 μg or 20 μg) were added to each tube; the volume of each tube was adjusted to 2 ml with distilled water. The subsequent procedure was as described for plasma. At the end, 3 μl of the residue, redissolved in 0.2 or 1.0 ml of absolute ethanol, were injected into the chromatograph.

Quantitation in plasma and urine

Hexaprazol concentrations in plasma and urine specimens were determined from standard curves obtained using drug-free human biological samples spiked with known amounts of hexaprazol.

Two plasma calibration curves were used, one obtained from lower hexaprazol concentrations (50, 100, 150, 200 or 250 ng) and 250 ng of I.S., the other from 0.25, 0.5, 0.75, 1 or 1.5 μg of hexaprazol and 1.5 μg of I.S.

Two urine calibration curves were prepared using 0.5, 1, 1.5, 2.5 or 5 μg of hexaprazol and 2 μg of I.S. and 5, 10, 20, 30 or 50 μg of hexaprazol and 20 μg of I.S., respectively.

Appropriate calibration curves were obtained daily from the plot of hexaprazol/I.S. peak-height ratios versus the sample concentrations.

Recovery from plasma

Plasma samples (1 ml each) were spiked, in quadruplicate, with 0.25, 0.5 or 1.0 μg of hexaprazol and with 1.5 μg of I.S. The extraction procedure was as previously described. At the end, each plasma sample residue was redissolved in 200 μl of absolute ethanol containing 20 μg/ml compound II. The recoveries of hexaprazol and I.S. were calculated by comparing these plasma samples with reference standards containing the same amounts of hexaprazol, I.S. and compound II.

Recovery from urine

Urine samples (0.5 ml each) were spiked, in quadruplicate, with 10 or 30 μg of hexaprazol and with 20 μg of I.S. The extraction procedure was as previously described. At the end, each urine sample residue was redissolved in 1 ml of absolute ethanol containing 40 μg/ml compound II. The recoveries of hexaprazol and I.S. were calculated by comparing these urine samples with reference standards containing the same amounts of hexaprazol, I.S. and compound II.

Precision

The precision of this method was evaluated by calculating the daily and the inter-day coefficients of variation (C.V.). For both calculations 1 ml of human plasma or urine containing known amounts of hexaprazol and I.S. was used.

To estimate the daily C.V., replicate spiked samples (n = 6) were analysed; concentrations were calculated using the appropriate daily standard curve for each plasma or urine sample. The inter-day C.V. was calculated by analysing, in quadruplicate, spiked plasma or urine samples using the appropriate standard curve obtained daily on three subsequent days.

RESULTS AND DISCUSSION

Representative gas chromatograms obtained from human plasma and urine extracts are shown in Figs. 2 and 3. Hexaprazol and I.S. retention times are 5.2 and 5.8 min, respectively. A total analysis time of ca. 9 min is nevertheless required: hexaprazol metabolites, eventually present, are eluted after the drug and I.S. (retention times, relative to hexaprazol, of metabolites IV, V, II and III are 1.19, 1.23, 1.35 and 1.44, respectively).

The proof that the eluted peak (5.2 min) was hexaprazol was obtained by

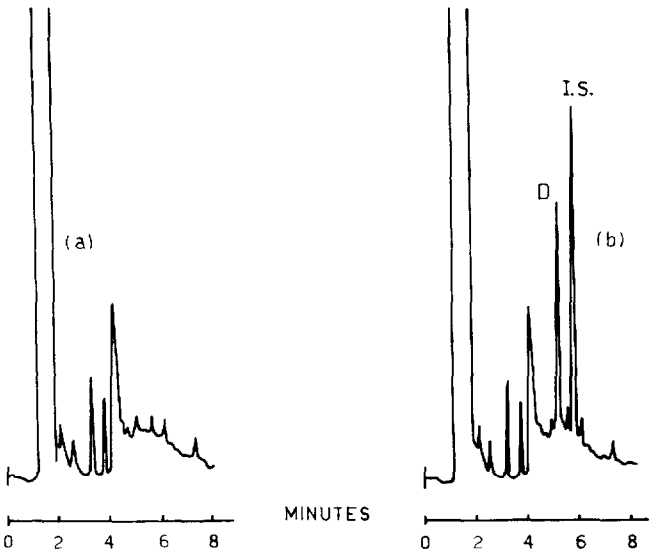

Fig. 2. Chromatograms from human plasma (1 ml). (a) Blank plasma; (b) plasma spiked with hexaprazol (D, 100 ng) and internal standard (I.S., 250 ng).

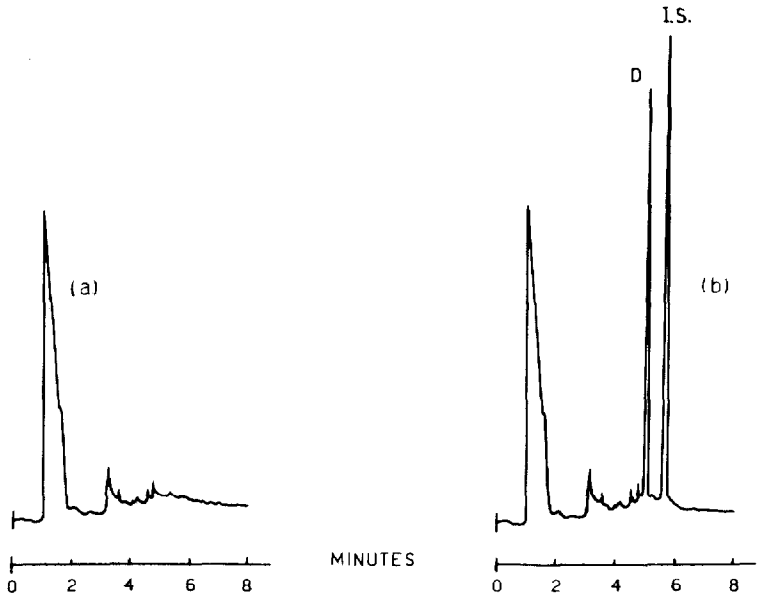

Fig. 3. Chromatograms from human urine (1 ml). (a) Blank urine; (b) urine spiked with hexaprazol (D, 1.0 μg) and internal standard (I.S., 2.0 μg).

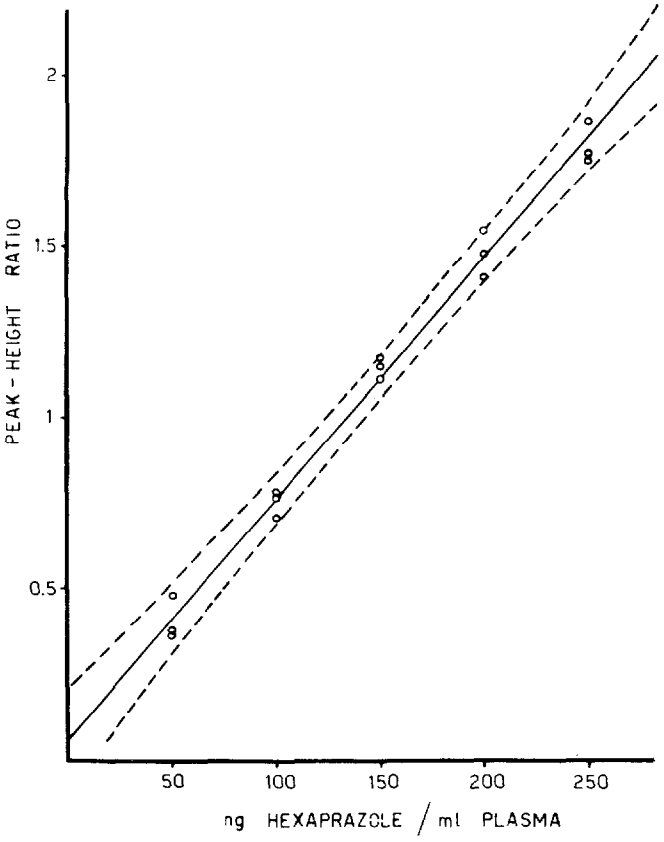

Fig. 4. Calibration line of hexaprazol in human plasma (50—250 ng/ml) with Working—Hotelling 95% confidence region. Equation of calibration line: $y = 0.068 + 7.026x$; $r = 0.9932$.

GC—mass spectrometric analysis. The resulting mass spectrum shows the molecular ion at *m/z* 225 and a fragmentation pattern identical with that previously reported for hexaprazol [5].

The acidic and basic wash-outs in the extraction procedure were required in order to eliminate interference from endogenous nitrogen-containing compounds. Calibration curves, obtained with appropriate concentrations of drug and I.S. in plasma and urine, have been calculated by the method of least squares. An example of a plasma calibration line over a drug concentration range of 50—250 ng/ml is depicted in Fig. 4. The dashed curves represent the 95% [(100 — α)%] confidence bands obtained using the Working—Hotelling method [6]. This confidence region gives the 95% confidence interval for future single observations. The analysis of variance with F test and lack of fit indicates (α = 0.05) the significance of the regression and the adequacy of the linear model [7] (Table I).

The limit of quantitation of hexaprazol in plasma is ca. 25 ng/ml at a signal-to-noise ratio of 10 [8]. The intra- and inter-day C.V. values of plasma and urine samples at different hexaprazol concentrations, representing the variability of the method, are shown in Table II. The increase of inter-day C.V., compared with the intra-day C.V., is ascribable both to the variability due to the

TABLE I

ANALYSIS OF VARIANCE OF THE CALIBRATION LINE

df = degrees of freedom; SS = sum of squares; MS = mean square.

Source	df	SS	MS	F_{ratio} (calculated)	$F_{0.95}$ (tabled)
Regression	1	3.703	3.703	948.210*	4.670*
Residual	13	0.0508	0.0039		
Lack of fit	10	0.036	0.0036	0.9090**	3.710**
Pure error	3	0.012	0.0040		

*948.21 > $F_{(1,13,0.95)}$ = 4.67: regression is significant.
**0.9090 < $F_{(10,3,0.95)}$ = 3.71: lack of fit not significant.

TABLE II

PRECISION OF THE METHOD FOR PLASMA AND URINE

Biological medium	Hexaprazol added (μg/ml)	Coefficient of variation (%)	
		Intra-day*	Inter-day**
Plasma	0.1	4.97	9.71
	0.2	3.91	8.56
	0.5	3.64	5.33
	1.0	2.65	5.95
Urine	1.0	6.16	6.98
	2.5	4.77	6.09

*n = 6.
**n = 4/day for three days.

TABLE III

RECOVERIES OF HEXAPRAZOL AND I.S. FROM PLASMA AND URINE

$n = 4$.

Biological medium	Hexaprazol added (μg)	I.S. added (μg)	Recovery (mean ± S.D.) (%)		Recovery ratio* (mean ± S.D.)
			Hexaprazol	I.S.	
Plasma	0.25	1.5	67.4 ± 6.1	71.4 ± 6.9	0.95 ± 0.06
(1 ml)	0.50	1.5	67.9 ± 4.1	72.9 ± 8.0	0.94 ± 0.06
	1.00	1.5	73.9 ± 3.2	76.6 ± 4.1	0.97 ± 0.04
Urine	10.0	20.0	89.2 ± 3.4	91.0 ± 2.9	0.98 ± 0.05
(0.5 ml)	30.0	20.0	92.4 ± 2.6	90.3 ± 2.8	1.03 ± 0.06

*Hexaprazol-to-I.S. recovery ratio.

multiple standard curves and to sample-to-sample variation, but nevertheless the inter-day C.V. shows good reproducibility between experiments.

Plasma and urine recoveries of hexaprazol and I.S. are reported in Table III. In particular, hexaprazol recoveries, after the double acidic– basic extraction, are sufficiently high: 70% in plasma and 90% in urine. Some variation of drug recovery among different samples reproduces the sample-to-sample variation, but the recovery ratio of the drug to I.S. is quite good.

Examples of the application of this method to the determination of hexaprazol in plasma and urine after clinical administration of the drug in humans are shown in Figs. 5 and 6. Fig. 5 depicts the plasma levels of the drug in one

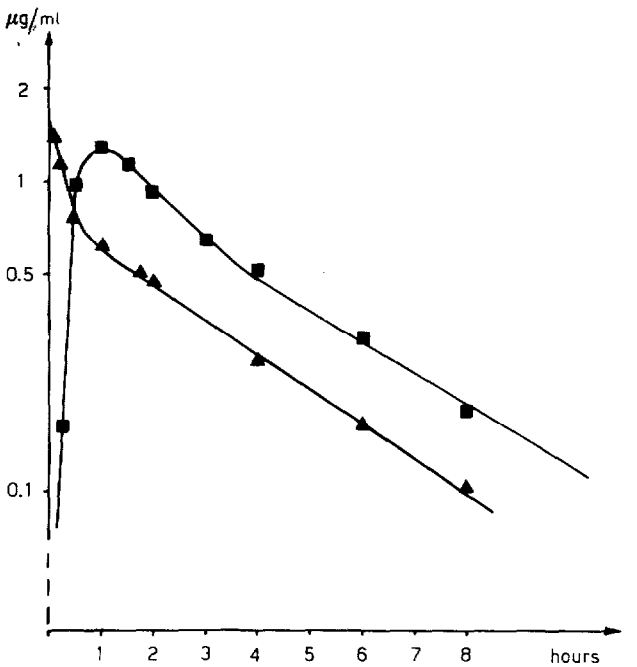

Fig. 5. Plasma levels of hexaprazol base following intravenous or oral administration of 100-mg (▲) or 200-mg (■) doses of the drug, respectively (volunteer F.L.).

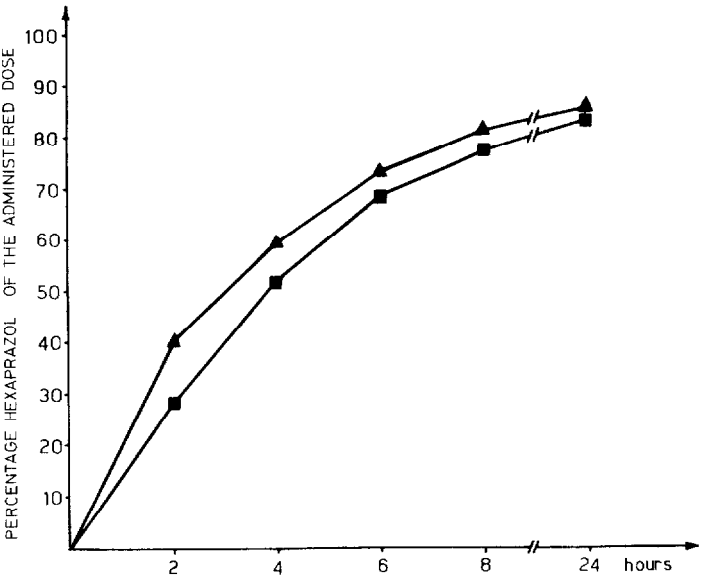

Fig. 6. Cumulative urinary excretion of intact hexaprazol after intravenous (▲) or oral (■) administration of 100-mg or 200-mg doses, respectively (mean of five volunteers).

human volunteer after single intravenous (100 mg) or oral (200 mg) administration. Fig. 6 summarizes the trend of cumulative urinary excretion over 24 h of the intact drug after intravenous (100 mg) or oral (200 mg) administration (mean of five volunteers).

REFERENCES

1 C. Corvi Mora, Ger. Offen., 2 702 537 (1977); Ital. Appl., 76/19 517 (1976).
2 C. Corvi Mora, U.S. Pat., 4 278 796 (1981).
3 R. Scuri, G. Mondani, P.L. Fantini, V. Dalla Valle and B. Valsecchi, Boll. Chim. Farm., 123 (1984) 425.
4 B. Lumachi and R. Scuri, Drugs Exp. Clin. Res., 11 (1985) 693.
5 P. Ventura, M. Schiavi and G. Risoli, Farmaco, Ed. Prat., 39 (1984) 190.
6 J.S. Hunter, J. Ass. Off. Agric. Chem., 64 (1981) 574.
7 N. Draper and H. Smith, Applied Regression Analysis, Wiley, New York, 2nd ed., 1981, Ch. 1.
8 ACS Committee on Environmental Improvement, Anal. Chem., 52 (1980) 2242.