QUANTITATIVE DETERMINATION OF PROPILDAZINE IN RAT PLASMA BY GAS–LIQUID CHROMATOGRAPHY

PAOLO VENTURA, MARGHERITA ZANOL, MARCO VISCONTI and GIORGIO PIFFERI[*]

ISF – Italseber Research Laboratories, 20090 Trezzano s/N, Milan (Italy)

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SUMMARY

A gas–liquid chromatographic method for the evaluation of the new anti-hypertensive drug propildazine (ISF 2123) in rat plasma is described. The procedure involves separation of the drug from plasma by cation-exchange chromatography, subsequent acylation of the dried eluate with heptafluorobutyric anhydride and quantitation with electron-capture detection. Propildazine can be determined in concentrations down to *ca.* 0.4 μg/ml.

INTRODUCTION

Propildazine, 3-hydrazino-6-[(2-hydroxypropyl)methylamino]pyridazine di-hydrochloride (I), is a new, potent, peripherally acting vasodilator[1]. The chemistry[2] and pharmacology[2,3] of this drug, as well as its clinical effects on hypertensive patients[4], have been described elsewhere.

The present study is concerned with the quantitative determination of I in rat plasma by gas–liquid chromatography (GLC). Because compound I is unstable in basic media, its direct extraction from aqueous solutions with organic solvents is not possible without partial decomposition. However, the drug was recovered from plasma by means of a strong cation-exchange resin. To achieve good gas chromatographic properties, compound I was then acylated with heptafluorobutyric anhydride (HFBA)[5] at 80° to afford, as described for other 3-hydrazinopyridazines[6,7], the corresponding 1,2,4-triazole[4,3-*b*]pyridazine (II). Derivative II is stable and shows high sensitivity to electron-capture detection.

I   II

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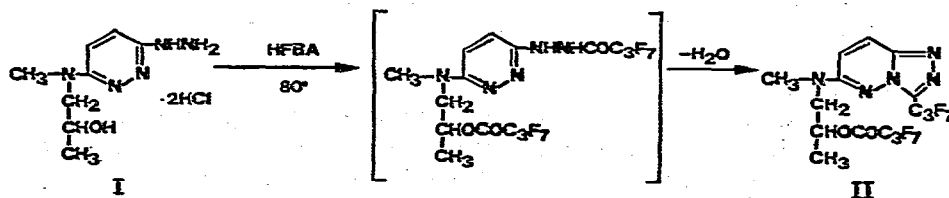
[*] To whom correspondence should be addressed.

This method was applied to the determination of plasma levels of the intact drug in hypertensive rats after intravenous administration of propildazine.

EXPERIMENTAL

Chemicals and reagents

Propildazine was an in-house reference standard; (RS)-2-(isopropylamino)-1-(4-nitrophenyl)ethanol, used as internal standard (IS), was obtained from Selvi (Milan, Italy). Methanol, toluene, benzene, acetone and ethyl acetate were analytical grade solvents (Baker, Deventer, The Netherlands). Ethyl acetate and benzene were distilled before use. The siliconizing agent was DRI-FILM-SC 87 (Pierce, Rockford, Ill., U.S.A.). Hydrochloric acid, trimethylamine (TMA), ammonia, lindane (pesticide standard) and phosphorus pentoxide were purchased from Carlo Erba (Milan, Italy). Heptafluorobutyric anhydride (HFBA) was obtained from Fluka (Buchs, Switzerland). The ion-exchange resin was AG 50 W (Bio-Rad Labs., Richmond, Calif., U.S.A.), sifted to give 20–50 mesh beads and cleaned as follows: 200 ml of resin were placed in a chromatographic column and eluted twice with 1 l of methanol, twice with 1 l of 1 N methanolic HCl and three times with 1 l of methanol to eliminate residual HCl. The resin was stored under methanol and washed with distilled water on the column, immediately before use.

Glassware

Spherical bottles and test-tubes were cleaned ultrasonically with a detergent then rinsed with water, methanol and acetone. They were then dried in an oven and silanized in a 10% toluene solution of DRI-FILM-SC 87. After 2 h, the glassware was dried in air and used for the analyses.

Gas chromatographic conditions

A Hewlett-Packard Model 5710A gas chromatograph equipped with a ^{63}Ni electron-capture detector (ECD) was employed. The column was a Pyrex glass tube (2 m × 3 mm I.D.) packed with 3% OV-17 on Chromosorb W HP (80–100 mesh). The column temperature was programmed to increase over the range 170–230° at a rate of 8°/min, then maintained at 230° for 8 min. The detector temperature was 300° and that of the injector port was 250°. The carrier gas (argon–methane, 9:1) flow-rate was 38 ml/min.

Sample handling

Blood samples were drawn into heparinized test-tubes and immediately centrifuged at 700 g for 10 min. The plasma was rapidly acidified to pH 4 with 1 N HCl. A 50-μl volume of a methanolic solution of IS (40 μg/ml) was added per ml of plasma, centrifuged at 2900 g for 15 min and stored at −20°.

Extraction procedure

Clear plasma samples (1 ml) were percolated through glass columns (5 mm I.D.) packed with 1 ml of the resin pretreated as previously described. After washing with 1 ml of distilled water, 3 ml of 0.25 N HCl and 5 ml of methanol, respectively, the drug and its IS were eluted with 1 ml of 3 N methanolic HCl and 1 ml of methanol and collected in a 3-ml spherical bottle. The solutions were dried under nitrogen at 40°.

Derivatization

The residue was taken up with 100 μl of ethyl acetate, added together with 25 μl of a 0.06 M solution of TMA in benzene and 10 μl of HFBA. The stoppered bottle was allowed to stand in an oil-bath at 80° for 30 min. After cooling to room temperature, 0.5 ml of benzene were added and the excess of HFBA was eliminated by shaking with 0.5 ml of water for 1 min and with 1 ml of 5% aqueous ammonia for 5 min, respectively. The aqueous and organic phases were transferred to a test-tube and the bottle was rinsed with 0.5 ml of benzene. After centrifugation at 270 g for 5 min, 1 μl of the benzene layer was injected into the chromatograph.

Quantitation

External calibration curve. To test-tubes containing amounts of compound I ranging from 0.5 to 15 μg in 1 N methanolic HCl solution were added 50 μl of a methanolic solution of IS (40 μg/ml). The methanol was evaporated to dryness under nitrogen and the residue was derivatized and purified as previously described.

Internal calibration curve. Aliquots (1 ml) of rat plasma (pH 4) containing 0.5–20 μg/ml of I and 2 μg/ml of IS were treated on ion-exchange columns, and the dried eluates were derivatized and purified as described above. In order to evaluate the recovery of the drug, analogous experiments were carried out, but IS (2 μg/ml) was added after column elution.

The external and internal calibration graphs were constructed by plotting the ratios of the peak area of the heptafluorobutyl (HFB) derivative of I to that of the HFB derivative of IS against known amounts of I.

Application

Groups of seven spontaneously hypertensive female rats (SHR-Okamoto-Aoki strain 180–200 g b.w.) were treated intravenously with 5 mg/kg of I and sacrificed after 5, 10, 20 and 45 min, and 1, 2, 4, 8 and 24 h. Blood samples were handled as previously described.

RESULTS AND DISCUSSION

The conditions required to achieve the maximal derivatization of the pure drug and IS were examined by following the reaction by GLC, using lindane (L) as external marker. The optimal temperature was found to be 80° and the best molar ratios of HFBA and I or IS were established as ranging from 2:1 to 20:1. Fig. 1 shows the effect of the reaction time on the formation of the HFB derivative of propildazine and of the IS; the optimal time seems to be 30 min for both compounds. Under these conditions, the ratio between the relative molar response factors of I and of pure II to lindane gave a derivatization yield of ca. 85%. [Compound II was obtained by reaction of I with HFBA (molar ratio 1:2) in a 0.06 M benzene–TMA solution and purified on preparative thin-layer silica gel plates (solvent system, chloroform–methanol, 9:1; $R_F = 0.67$). The structure of II was confirmed by mass spectrometry (M^+ at m/e 571).]

A typical gas chromatogram of the HFB derivatives of IS and I obtained from plasma extracts is compared with that of a blank extract in Fig. 2. The retention times are 3.60 and 9.50 min, respectively.

The external and internal calibration curves for the quantitation of the drug are shown in Fig. 3. Each point is the mean of seven values. The precision of the method was tested on the internal calibration curve by evaluating the standard
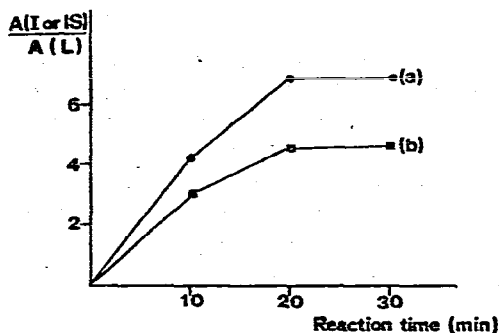
Fig. 1. Effect of the reaction time on the formation of HFB derivatives of propildazine (a) and IS (b). Reaction temperature 80°; molar ratio HFBA: I = 10:1. L = Lindane.
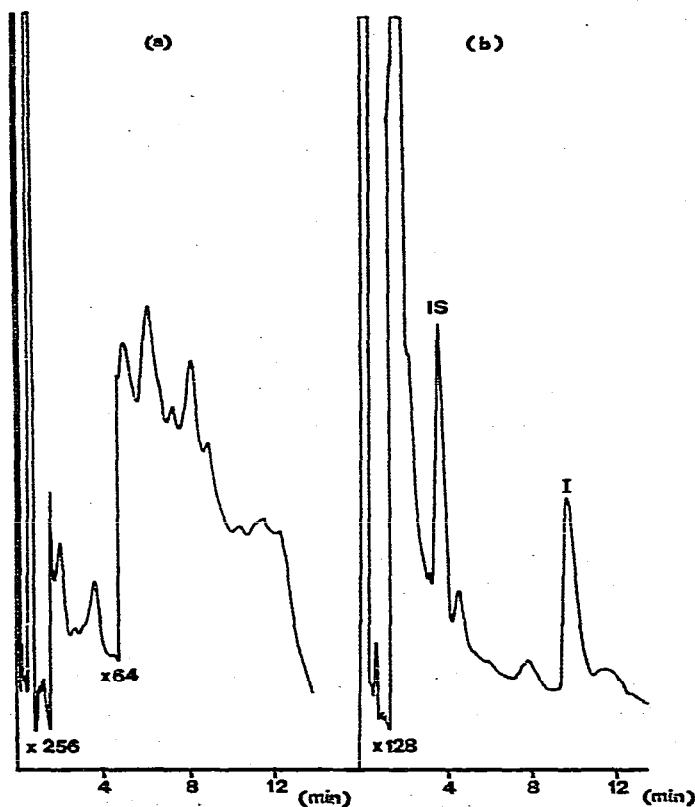

Fig. 2. Gas chromatograms of a blank extract of 1 ml of plasma (a) and of an extract of 1 ml of plasma containing 10 μg/ml of propildazine and 2 μg/ml of IS (b).

deviation and the coefficient of variation (%) of each determination. Table I gives the values obtained. Good linearity was found for drug concentrations in plasma ranging from 0.4 to 20 μg/ml. The minimum detectable amount was 0.03 ng, but interference from endogenous compounds raised the minimum measurable plasma level to 0.4 μg/ml. The recovery of the overall procedure, calculated from the linearity curves, was *ca.* 60%.
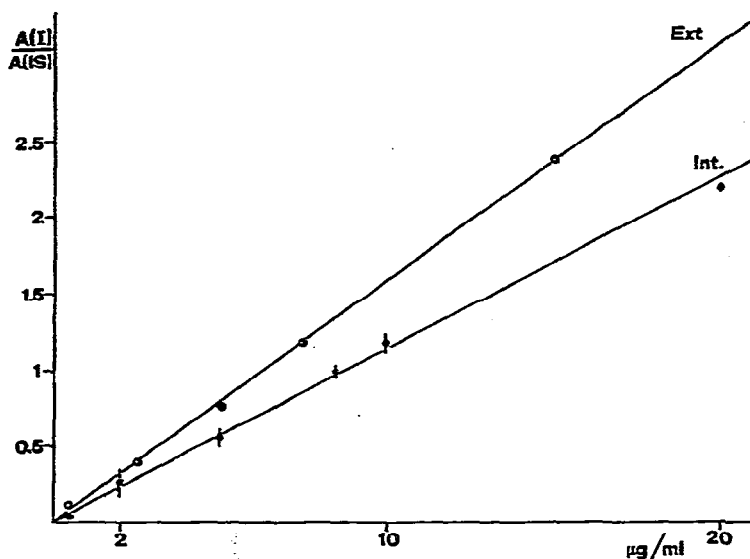
Fig. 3. External (Ext.) and internal (Int.) calibration graphs obtained as described under Experimental.

TABLE I

EVALUATION OF THE PRECISION FROM THE INTERNAL CALIBRATION CURVE

$\bar{y} = \Sigma\ y_j/n$, where $y_i \equiv A_i(\mathrm{I})/A_i(\mathrm{IS})$.

Concentration (µg/ml)	$\bar{y}$ (n=7)	s ($\bar{y}$)	Coefficient of variation (%)
0.5	0.04	0.01	14.29
2.0	0.26	0.02	3.85
5.0	0.56	0.03	2.68
8.5	1.01	0.04	1.98
10.0	1.19	0.06	2.42
20.0	2.22	0.00	0.00

Plasma samples from spontaneously hypertensive female rats were monitored up to 24 h after a single injection of 5 mg/kg of propildazine (see Fig. 4). Each point is the mean of seven values; the coefficient of variation is < 10 %, as can be seen from Table II.

CONCLUSIONS

The high water solubility of I and the instability of its conjugated base require the use of a strong cationic resin as an alternative to liquid–liquid extraction. Under these conditions, basic endogenous compounds are also recovered, limiting the sensitivity of the method. Because of the very low therapeutic doses of propildazine[4], the assay is not sufficiently sensitive for monitoring plasma levels in humans. Further studies will be carried out to attempt to improve the sensitivity of this new method and to confirm its selectivity by excluding possible interference from drug metabolites.
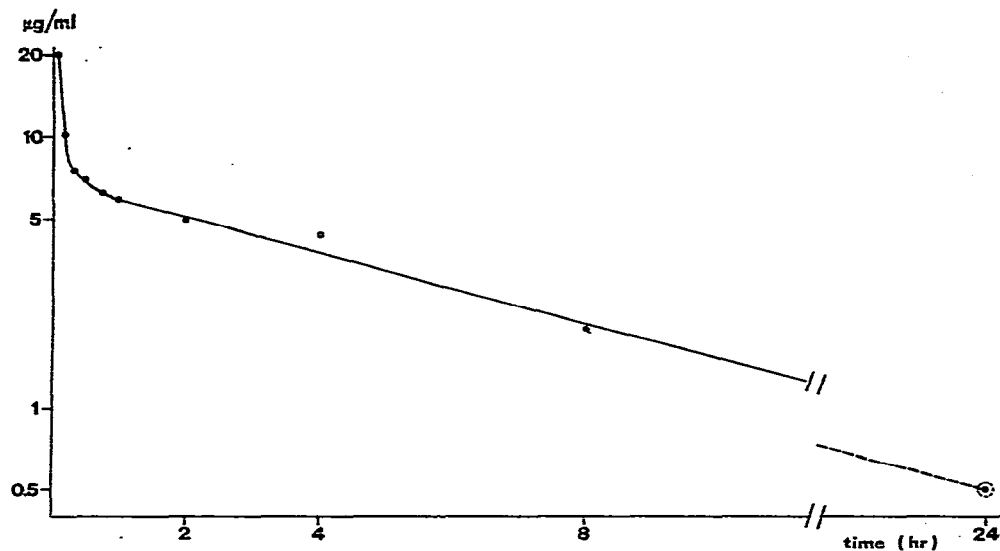
Fig. 4. Profile of propildazine in rat plasma after a single intravenous administration of 5 mg/kg to spontaneously hypertensive female rats.

TABLE II

GLC ESTIMATION OF RAT PLASMA LEVELS OF PROPILDAZINE AT VARIOUS TIMES AFTER INTRAVENOUS ADMINISTRATION OF 5 MG/KG OF THE DRUG

Time	$\bar{y}$ ($\mu g/ml$)	s ($\bar{y}$)	Coefficient of variation (%)
min			
5	19.93	3.42	7.00
10	10.04	0.71	2.89
20	7.42	1.62	8.91
30	7.06	1.72	9.19
45	6.16	0.89	5.45
h			
1	5.93	0.92	6.33
2	4.84	1.11	8.65
4	4.49	1.22	10.03
8	1.92	0.23	5.35
24	n.v.*	—	—

* n.v. = Not valuable.

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