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Note

Determination of propisomide, a new antiarrhythmic agent, in biological samples by gas chromatography with a thermionic detector

J. NECCIARI*, D. MERY, Y. SALES, D. BERTHET and W. CAUTREELS

Centre de Recherches Clin-Midy, Groupe Sanofi, 34082 Montpellier (France)

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Propisomide, *dl*-2[2-(diisopropylamino)ethyl]-4-methyl-2-(2-pyridyl)-pentamide (CM-7857, Fig. 1), displayed a potent antiarrhythmic activity as demonstrated on a model of myocardial infarction in the conscious dog [1]. Cellular electrophysiology of this compound has shown an efficiency on experimental models of arrhythmia and in patients with rhythm disturbance [2]. Safety and tolerance of propisomide were assessed in man after intravenous and oral administration. The drug appeared to be particularly safe and well tolerated [3]. Tolerance studies were completed by pharmacokinetic studies. Therefore a quantitation technique of the parent drug in plasma and urine was developed.

The chemical structure of propisomide and the use of the thermionic detector for gas chromatographic determination of disopyramide in the literature [4, 5] suggested this method for the determination of propisomide in biological samples.

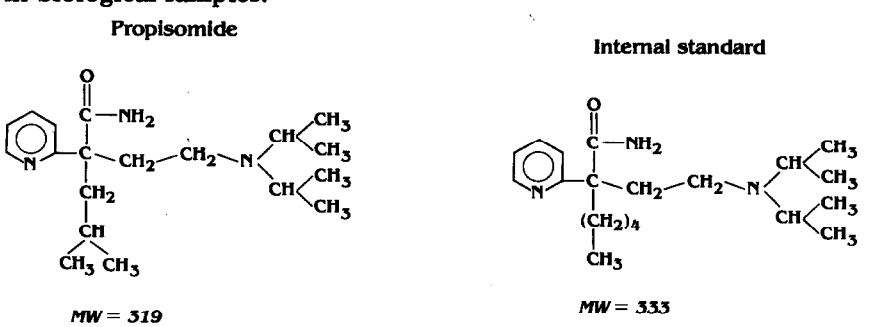

Fig. 1. Structures of propisomide and internal standard.

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EXPERIMENTAL

Reagents

Solutions of propisomide and internal standard (CM-7973, Fig. 1) were prepared in acetonitrile (Uvasol; Merck, Darmstadt, F.R.G.) in the concentration range 1 mg/ml to 1 μg/ml. R.P. Normapur sodium carbonate, R.P. Normapur potassium dihydrogen phosphate and sodium hydroxide (10 *M*) were obtained from Prolabo (Paris, France). Analytical grade acetonitrile, methanol and methylene chloride were obtained from Merck, Carlo Erba (Milan, Italy) and Solvant Documentation Synthese Compagny (Peypin, France), respectively.

Extraction procedure in plasma

Plasma (500 μl) and 25 μl of internal standard solution (0.1 μg/ml) were put into a centrifuge tube (Du Pont de Nemours, Newtown, CT, U.S.A.); 200 μl of a buffer solution pH 12 (0.5 *M* sodium hydroxide, 1 *M* sodium carbonate) and 6 ml of methylene chloride were added and the mixture was shaken for 15 min. After centrifugation (5 min, 12 000 *g*) the organic layer was recovered and 4 ml of a buffer solution pH 2 (0.01 *M* potassium dihydrogen phosphate) were added. After agitation (15 min) and centrifugation (5 min, 12 000 *g*), the aqueous layer was separated and adjusted to alkaline pH by addition of 200 μl of a basic solution (5 *M* sodium hydroxide, 1 *M* sodium carbonate). The mixture was extracted with 6 ml of methylene chloride (15 min) and, after centrifugation (5 min, 12 000 *g*), the organic layer was removed and evaporated to dryness under a dried nitrogen stream. The residue was reconstituted in 50 μl of methanol and 2 μl of this solution were injected.

Extraction procedure in urine

Extraction was realized without clean-up; 200 μl of internal standard solution (10 μg/ml) were added to 1 ml of urine. After addition of 1 ml of buffer solution pH 12 (0.5 *M* sodium hydroxide, 1 *M* sodium carbonate) and 6 ml of methylene chloride, the mixture was shaken for 15 min and then centrifuged (5 min, 12 000 *g*). The organic layer was separated and evaporated to dryness under nitrogen; 500 μl of methanol were added to the residue and 2-μl aliquots were submitted to analysis.

Chromatographic conditions

All analyses were run on a Varian 4700 gas chromatograph (Palo Alto, CA, U.S.A.) with a thermionic detector. The chromatographic separations were performed on a packed column (OV-17, 3% on Chromosorb W AW, 100—120 mesh, 2 m × 18 mm I.D.). Helium was used as the carrier gas at a flow-rate of 30 ml/min. The injector and detector temperatures were 300°C, the oven temperature was 250°C. The combustants for the thermionic detector were hydrogen (1.4 Pa) and air.

Peak areas were obtained using a Varian CDS 401 Vista data station.

Preparation and storage of plasma and urine samples

Blood samples were collected into Vacutainer tubes (Becton Dickinson,

France) containing sodium heparin as anticoagulant. Plasma was separated immediately by centrifugation and stored at −20°C. Urine samples were immediately frozen and stored at −20°C until analysed. The use of heparin as anticoagulant did not change the repartition of propisomide in blood samples — the drug was equally distributed in plasma and red blood cells. Any possible plasticizers in the rubber stopper of Vacutainer tubes did not affect the chromatographic traces.

Calculations

Plasma concentrations were calculated from standard curves established from chromatograms of spiked plasma samples containing known amounts of propisomide (0.2, 0.5, 1, 2.5 and 5 μg/ml).

Urine concentrations were calculated from standard curves established after analysis of spiked urine samples in the concentration range of propisomide (25, 75, 150, 200 and 500 μg/ml).

Standard curves were generated daily by plotting peak area ratios (propisomide/internal standard) versus propisomide concentrations.

Mass spectrometry

Mass spectra of propisomide and internal standard were recorded using a Ribermag R10-10B mass spectrometer coupled on-line to the SIDAR data system (Rueil-Malmaison, France)

Gas chromatographic conditions were the same as described above. Helium was used with a flow-rate of 20 ml/min. The oven temperature, injector temperature and interface temperature were, respectively, 250°C, 280°C and 280°C. Under these conditions the retention time of propisomide and internal standard were, respectively, 4.5 min and 6.7 min. Mass spectra were obtained in the chemical ionization mode with ammonia as reagent gas, ionization energy of 70 eV, ionization current of 200 μA and ion source pressure of 0.1 Torr.

RESULTS

Typical gas chromatograms obtained from spiked human plasma are shown in Fig. 2. No interfering peaks were observed under the chromatographic conditions adopted.

The plasma standard curve was linear over the range 0.2—5 μg/ml, the

TABLE I

PRECISION OF THE GAS CHROMATOGRAPHIC ASSAY AND EXTRACTION EFFICIENCY OF PROPISOMIDE IN PLASMA

Concentration (μg/ml)	Peak area ratios (± S.D., $n = 5$)	C.V. (%)	Extraction efficiency (%)
0.2	0.131 (± 0.009)	6.8	63.6
0.5	0.326 (± 0.024)	7.4	65.5
1	0.680 (± 0.027)	3.9	59.3
2.5	1.564 (± 0.044)	2.8	64.2
5	3.351 (± 0.174)	5.2	67.5

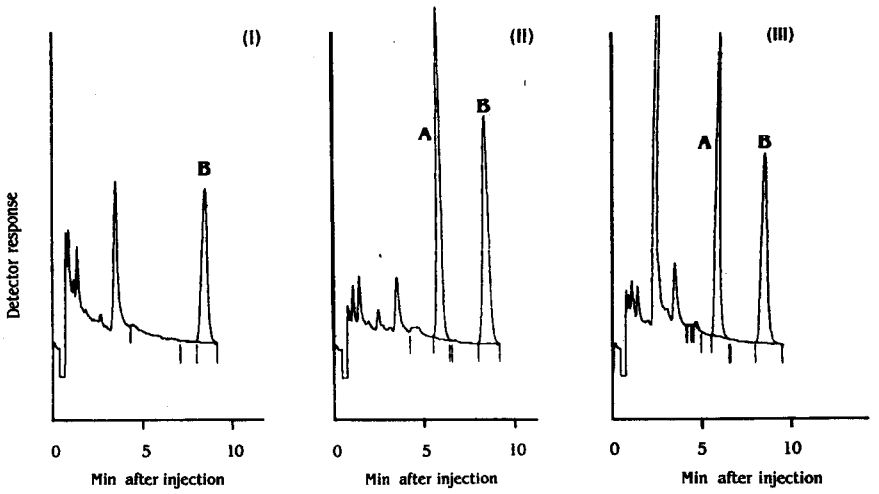

Fig. 2. Chromatograms of extracted human plasma: (I) blank human plasma sample; (II) human plasma sample spiked with 2 μg/ml propisomide; (III) human plasma sample after administration of propisomide to the patient (concentration found: 2.89 μg/ml). Peaks: A = propisomide, B = internal standard.

correlation coefficient was better than 0.998 and the regression curve passed through the origin. The reproducibility of the method was tested by repeated analysis of spiked human plasma samples (range 0.2—5 μg/ml propisomide) daily for five days. The results of the reproducibility test and the extraction recoveries in the same concentration range are presented in Table I. For each concentration, the coefficient of variation is less than 7.5% and the extraction recovery ranges from 59.3% to 67.5%. The losses occurred during clean-up with buffer solution pH 2, the remaining drug was in the aqueous phase (about 10%). The limit of quantitation in plasma was about 25 ng/ml.

The urine standard curve was linear over the range 25—500 μg/ml, the correlation coefficient was better than 0.997 and the regression curve passed through the origin. Reproducibility, tested using the same procedure as for plasma, and the extraction recoveries are reported in Table II. The coefficient of variation is better than 4% and the extraction recovery ranges from 84.3% to 93.5%. The limit of quantitation in urine was about 50 ng/ml.

Mass spectra of the compounds were recorded from a plasma extract obtained from a patient receiving propisomide. The sample was assayed by gas

TABLE II

PRECISION OF THE GAS CHROMATOGRAPHIC ASSAY AND EXTRACTION EFFICIENCY OF PROPISOMIDE IN URINE

Concentration (μg/ml)	Peak area ratios (± S.D., n = 5)	C.V. (%)	Extraction efficiency (%)
25	0.139 ± 0.002	1.3	88.4
50	0.284 ± 0.003	1.1	89.3
100	0.585 ± 0.007	1.2	84.3
200	1.213 ± 0.040	3.3	93.5

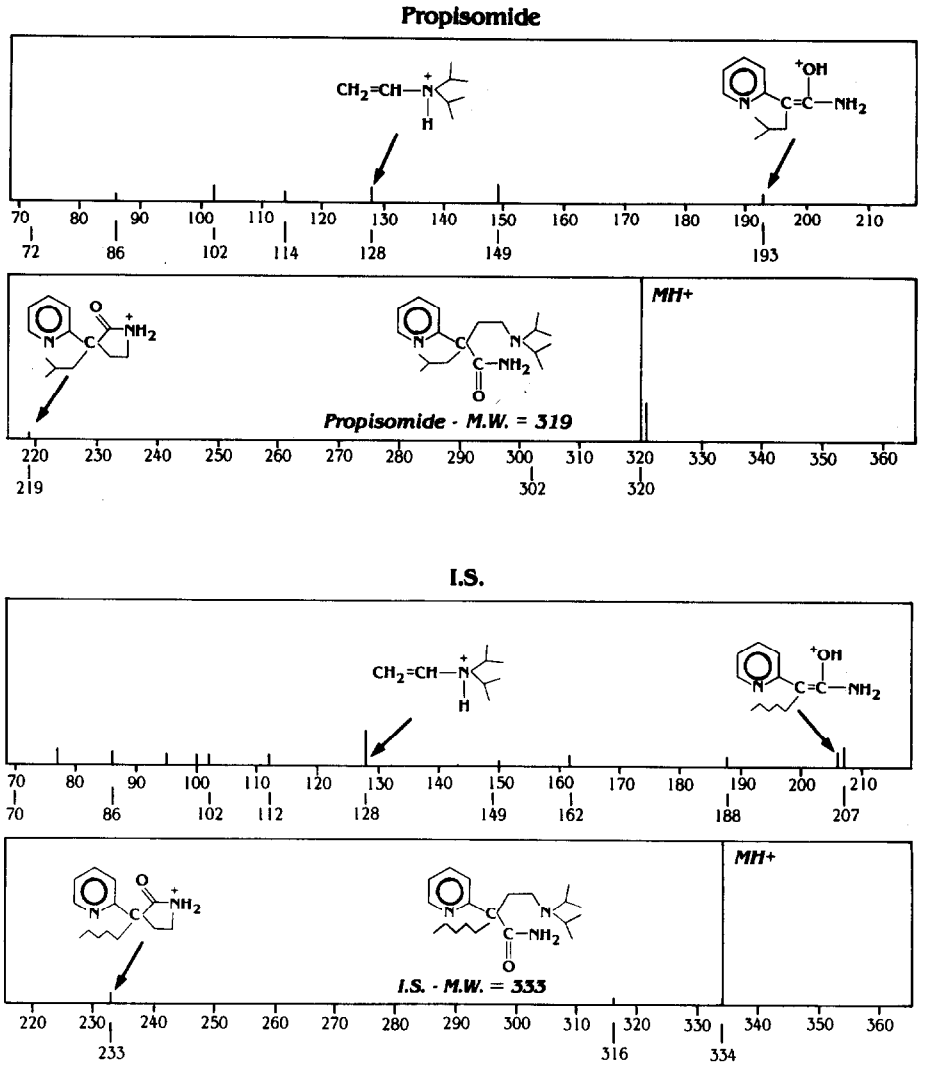

Fig. 3. Mass spectra of propisomide and internal standard (conditions as described in the text).

chromatography with thermionic detection before analysis by gas chromatography—mass spectrometry. Mass spectra of the compounds are shown in Fig. 3. They were completely consistent with mass spectra obtained from the reference compounds. This procedure confirms the specificity of the gas chromatographic method developed.

Application to biological samples

The method described was applied for the determination of plasma levels of propisomide following intravenous administration of 280 mg of the drug during 30 min to cardiac patients and after oral administration of 600 mg of propisomide.

Fig. 4 illustrates the pharmacokinetic profiles of the drug as obtained after

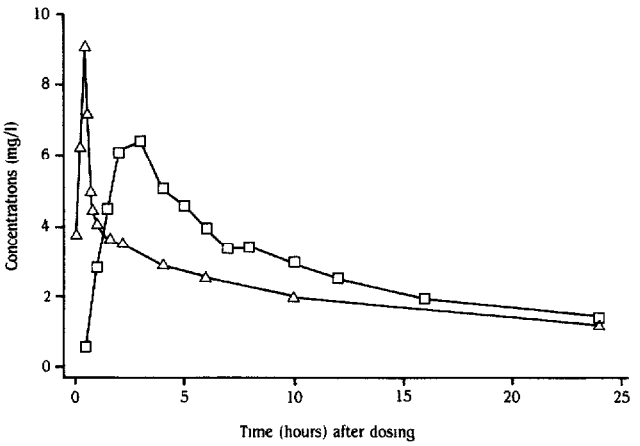

Fig. 4. Concentrations of propisomide in human plasma after oral (□) (600 mg) or intravenous (△) (280 mg for 30 min) administration.

analysis of the plasma samples and confirms the applicability of the method in human pharmacokinetics.

CONCLUSION

A selective, reproducible, and accurate assay was developed for propisomide in plasma and urine. The sensitivity of the method allows its application for the elucidation of human pharmacokinetics after intravenous and oral administration. The method is currently being applied for monitoring the drug in clinical trials. In routine use, the characteristics of the method remain at the level described in this paper.

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