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Note

Assay of the antiarrhythmic compound stirocainide in plasma by fused-silica gas–liquid chromatography and nitrogen-selective detection

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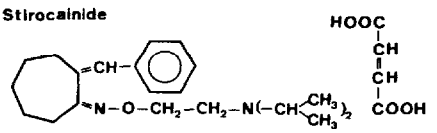
Stirocainide, 2-benzal-1-(2-diisopropylaminoethoxyimino)cycloheptane hydrogen fumarate (Fig. 1), is an antiarrhythmic compound currently under clinical development. Its electrophysiological characteristics as a class I agent resemble partly lidocaine and partly quinidine [1—3]. Its chemical structure is unique within this class of antiarrhythmics. Preliminary pharmacokinetic studies in humans showed that concentrations down to several ng/ml must be assayed precisely in order to establish its pharmacokinetic profile.

Stirocainide



Internal Standard

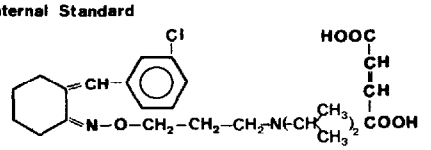


Fig. 1. Structures of stirocainide and of the internal standard.

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EXPERIMENTAL

Chemicals

Stirocainide and the internal standard 2-(*m*-chlorobenzal)-1-(3-diisopropylaminopropoxyimino)cyclohexane hydrogen fumarate (Fig. 1) were synthesized by Egyt Pharmaceutical Works (Budapest, Hungary). Methanol and *n*-hexane, both LiChrosolv, were from Merck (Darmstadt, F.R.G.). $LiAlH_4$ analysed was from Aldrich (Beerse, Belgium). Hexane was washed with sulphuric acid and water and distilled over $LiAlH_4$. Glassware was rinsed with methanol before use. Water was distilled twice in a quartz apparatus.

Equipment

The gas chromatograph F 22 from Perkin-Elmer (Überlingen, F.R.G.) with a nitrogen-selective flame-ionization detector was equipped with a capillary injection system from Gerstel Labormechanik (Mülheim, F.R.G.) in the splitless mode. We used a DB-5 fused-silica column from J&W Scientific (Rancho Cordova, U.S.A.), 60 m × 0.32 mm I.D., and film thickness 0.25 μm. The carrier and make-up gas was helium with flow-rates of 2.3 and 38 ml/min, respectively. The temperatures were oven 230°C, injector 270°C, and detector 300°C. The flow-rates of the fuel gases were 120 ml/min for synthetic air and 1.5 ml/min for hydrogen.

Peak areas were integrated by the data system 3352 B from Hewlett-Packard (Palo Alto, U.S.A.).

Assay

Internal standard, 10—100 ng in 25 μl of methanol, was added to 1 ml of plasma and extracted twice with 3 ml of hexane (10 min, 100 rpm, lab shaker by Braun, Melsungen, F.R.G.). After centrifugation, the organic phase was evaporated at 30°C under nitrogen in conical tubes. The residue was redissolved with 200 μl of methanol and transferred to 0.3-ml conical vials (Wheaton Scientific, Millville, U.S.A.). After evaporation, the residue was redissolved in 20 μl of methanol and 2 μl were injected into the gas chromatograph. Quantification was based on the peak area ratios of analyte to internal standard.

Since stirocainide and the internal standard are light-sensitive, especially in organic solvents, care was taken to protect the samples, extracts, and standard solutions.

RESULTS

In Fig. 2A the chromatogram of a blank extract is shown and in Fig. 2B the chromatogram of a clinical plasma sample containing 8.0 ng/ml stirocainide and 100 ng/ml internal standard. A peak from human plasma close to stirocainide may be used as a crude quality check for the performance of the system. If it cannot be separated anymore, the glass insert of the injector and/or the column have to be cleaned (the column may be rinsed with a few millilitres of hexane and the first centimeters of it are discarded). Usually about 50 plasma samples may be analysed before cleaning becomes necessary.

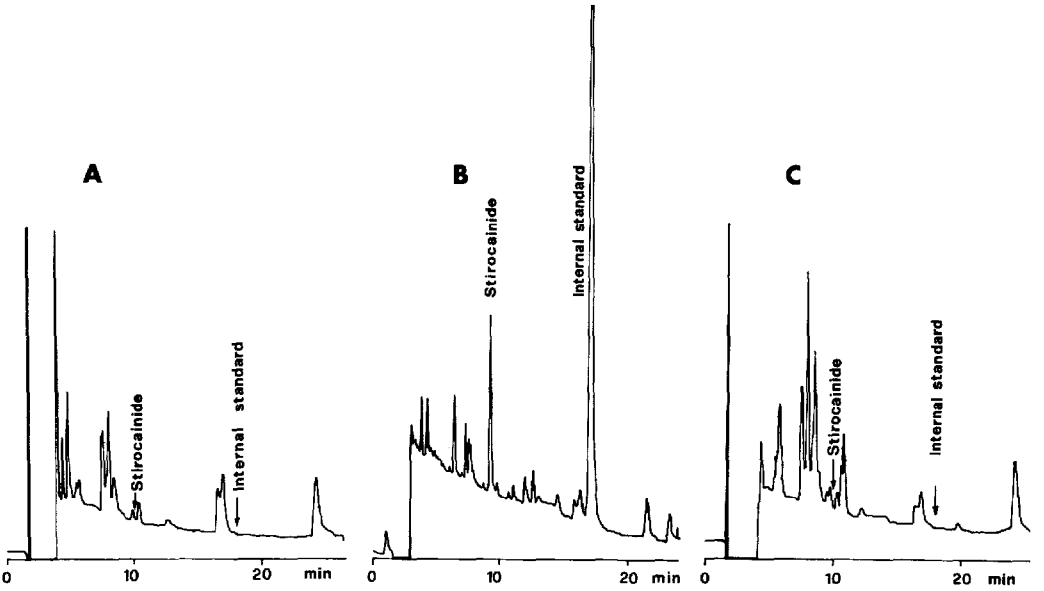

Fig. 2. Gas chromatograms of extracts of human plasma: (A) drug-free plasma; (B) clinical sample containing 8.0 ng/ml stirocainide and 100 ng/ml internal standard; (C) drug-free plasma stored for 5 min in a plastic syringe.

Because of this separation problem, high-quality fused-silica columns have to be used. If the column temperature is increased, stirocainide is no longer separated from the unknown plasma component. Since this component is missing in extracts from rabbit or rat plasma, the assay reported here is at least as sensitive in these species as it is in man. Dog plasma is comparable to man's. Plasticizers represents another problem. In Fig. 2C, the chromatogram of an extract is shown where blank plasma was left in a plastic syringe for 5 min. These syringes and catheters may be used only if the contact time with the sample is kept below 5 min. Otherwise, the amount of plasticizer increases to such an extent that separation of trace amounts of stirocainide will be impossible.

Five calibration curves were analysed on five different days, each in duplicate, in the range 5—80 ng/ml. Linear-regression analysis resulted in the following equation: $Y = 0.514\ (\pm\ 0.437) + 0.948\ (\pm\ 0.057)X$. The correlation coefficient was 0.9986 ± 0.0016.

The coefficients of variation between days calculated from these data are shown in Table I. Also shown are coefficients of variation for analyses on the same day. From these results we conclude that our detection limit is about 2 ng/ml. The extraction yield was $98.4 \pm 9.2\%$ ($n = 4$) for stirocainide (2—25 ng/ml) and $83.8 \pm 9.8\%$) ($n = 4$) for the internal standard (100 ng/ml). Stability of stirocainide was tested by adding 20 μg of stirocainide to 100 ml of heparinized blood at 37°C. Aliquots of 5 ml each were centrifuged immediately and stored at −20°C for up to one month. Approximately 35 ml were left at 37°C for up to 1 h before being centrifuged. Samples analysed immediately had a concentration of 320 ng/ml. All other samples were in the range

TABLE I

REPRODUCIBILITY AND LINEARITY OF THE ASSAY OF STIROCAINIDE

Human plasma was spiked with stirocainide in the concentrations indicated and with 100 ng of internal standard per ml of plasma.

Concentration (ng/ml)		C.V. (%)
Added	Found	
Day-to-day variation*		
5	5.05	9.8
10	9.88	2.6
20	19.04	3.3
40	38.67	5.5
80	76.36	5.8
Within-day variation**		
2	2.66	11.8
5	4.78	5.9
10	12.17	9.9
25	28.01	2.2

*Five calibration curves were run in duplicate on five different days. Each coefficient of variation is based on five duplicate measurements.
**Within-day variation of ten spiked plasma samples.

293—344 ng/ml, except blood left for 1 h before being centrifuged. In this sample, haemolysis occurred and a concentration of 261 ng/ml only was found. In another test for stability human plasma was spiked with 20 ng/ml stirocainide and stored at —20°C. Up to now six samples have been analysed during a period of three months. A mean of 19.5 ng/ml was found (C.V. = 4.7%) with no tendency for declining concentrations. All samples were within the 95% confidence limit of the regression line $Y = 19.2 + 0.01X$. These results show that stirocainide is stable in whole blood at 37°C for approximately 30 min and that it is stable in plasma at —20°C for at least three months.

Currently we are using the assay to study the pharmacokinetics of stirocainide in steady state. Initial results from human volunteers who received 60 mg b.i.d. for 14 days show trough levels of approximately 10 ng/ml and maximum levels of about 50 ng/ml.

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