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Note

Sensitive gas chromatographic method for the determination in blood and urine of SL 75212 [4-(2-cyclopropylmethoxyethyl)-1-phenoxy-3-isopropylaminopropan-2-ol], a new β_1 adrenoceptor blocking agent

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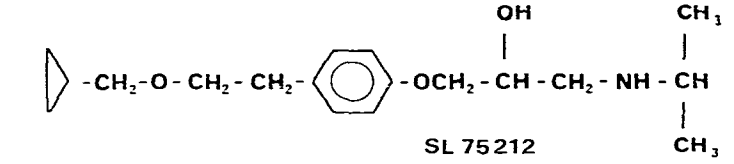
SL 75212 [4-(2-cyclopropylmethoxyethyl)-1-phenoxy-3-isopropylaminopropan-2-ol] is a new β-adrenoceptor blocking agent which has been shown, in animals and in preliminary clinical studies, to possess a very interesting β_1 selective profile[1,2]. The determination of the pharmacokinetic profile of a new therapeutic agent may be important for the better understanding of its mechanism of action and for more efficient therapeutic application. Further, the determination of drug concentrations in body fluids is important for defining possible therapeutic and/or toxic thresholds.

To evaluate the concentration of the drug in human fluids in order to study its clinical pharmacokinetic profile, we have developed a specific and sensitive gas–liquid chromatographic (GLC) assay for SL 75212.

EXPERIMENTAL

Standards and reagents

SL 75212 hydrochloride was synthesized by Dr. Manoury, Chemistry Department, Synthelabo (L.E.R.S.), and [^{14}C]SL 75212 was synthesized by C.E.A. (Saclay, Gif-sur-Yvette, France). Propranolol (I.C.I., Macclesfield, Great Britain) was used as an internal standard. Their structural formulae are shown in Fig. 1.



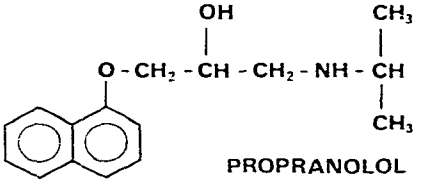


Fig. 1. Structural formulae of SL 75212 and the internal standard, propranolol.

The following reagents (all purchased from Merck, Darmstadt, G.F.R.) were used: diethyl ether, ethyl acetate, sodium hydroxide, 37% hydrochloric acid, *n*-hexane and methanol; heptafluorobutyric anhydride (HFBA) (puriss p.a.) was obtained from Fluka (Buchs, Switzerland).

Gas–liquid chromatographic conditions

Analyses were performed under isothermal conditions on a Perkin-Elmer Model 3920B gas chromatograph equipped with a ^{63}Ni linear electron-capture detector operating at −55 V (pulse current) with a 250 nsec width.

The glass column (2 m × 3 mm I.D.) was packed with Chromosorb W AW DMCS (80–100 mesh) coated with 3% OV-17, (Applied Science Labs, State College, Pa., U.S.A.) and conditioned for 1 h at 270° (40 ml/min argon–methane carrier gas flow), 4 h at 320° (no gas flow) and 24 h at 280° (50 ml/min carrier gas flow). The column temperature was 210°, injection port temperature 280°, interface and detector temperature 300° and carrier gas flow-rate 50 ml/min.

Mass spectrometric conditions

An LKB-2091B gas chromatograph–mass spectrometer with a PDP 11 data system was used. Spectra were obtained under the following conditions in the electron-impact mode: electron energy 70 eV, trap current 100 μA, accelerating voltage 3.5 kV, ion source temperature 250° and molecular separator temperature 250°. For GC, the same column was used as described under *Gas–liquid chromatographic conditions*, except that the helium flow was reduced to 20 ml/min.

Calibration graph and quantitation

Standard solutions of SL 75212 (1 μg/ml) and the internal standard propranolol (1 μg/ml) were prepared in methanol. These solutions were stable for at least 2 months.

Internal standards were prepared by adding 5, 10, 20, 40 and 80 ng of SL 75212 to 1 ml of blank blood and the samples were extracted according to the method described below. The extracts were then derivatized by heating with HFBA at 50° for 15 min, evaporated to dryness under a gentle stream of nitrogen and, when dried, the nitrogen flow was increased for a further 5 min in order to remove compounds derived from the reaction mixture which would appear as interfering peaks on the chromatogram. After dissolution in 200 μl of *n*-hexane, 1 μl of the standard solution of the derivative was injected. The ratio of the peak areas of SL 75212 to internal standard was used to calculate a response factor which was then used by a Perkin-Elmer PEP-2 data system to evaluate the amount of drug in the unknown samples.

Extraction procedure for blood

To 0.5–2 ml of human blood, in 10-ml glass-stoppered test-tubes, were added 20 ng of propranolol (20 μl of the methanolic stock solution) as internal standard, 100 μl of 2 *N* sodium hydroxide solution and distilled water to give a final volume of 3 ml. To this were added 6 ml of freshly distilled diethyl ether. The tubes were gently mixed on a rotating mixer for 15 min and then centrifuged at 4° for 5 min at 800 *g*. The ether phase was transfered to another series of test-tubes containing

2.5 ml of 0.2 *N* hydrochloric acid, mixed for 15 sec on a vortex mixer and centrifuged for 2 min at 800 *g*. The resulting upper ether phase was discarded. A further 5 ml of diethyl ether were added to the aqueous phase and the agitation and the centrifugation repeated. After discarding the ether phase, 300 μl of 2 *N* sodium hydroxide solution were added to the aqueous phase together with 5 ml of diethyl ether and the tubes were mixed on a vortex mixer and centriguged as above. The ether phase was transferred to another series of tubes and evaporated under nitrogen in a water-bath at 40°.

A 200-μl volume of HFBA (1:10 solution in ethyl acetate) was then added to the dry residue and the samples were derivatized as described above.

An internal calibration graph (five points) with various amounts (10–80 ng/ml) of SL 75212 added to the blood was always carried through the procedure with the unknown samples.

Extraction procedure for urine

The same procedure of extraction and derivatization was carried out for measuring SL 75212 in urine. The only difference consisted in the volume of sample, which was always less than 1 ml (0.1–0.5 ml).

RESULTS AND DISCUSSION

The GLC trace obtained from a blood sample to which a known amount of SL 75212 had been added is shown in Fig. 2, together with those obtained from a blood blank and from a volunteer receiving SL 75212 by the oral route. The peaks of SL 75212 and propranolol are well resolved and no interfering peaks from endogenous substances are present.

The GLC–MS analysis confirmed the identity of the gas chromatographic peaks. GLC peaks due to the reaction product of SL 75212 and HFBA showed a molecular ion at *m/e* 699 in the mass spectrum, corresponding to the formation of the diheptafluorobutyrate (Fig. 3).

Another characteristic peak in the spectrum was that at *m/e* 508, corresponding to the loss of a cyclopropylmethoxyethyl-4-phenoxy group. This ion loses a fragment of 43 a.m.u. [$H_3C–\dot{C}H–CH_3$] to give the base peak at *m/e* 465, which in turn loses a fragment of 213 a.m.u. ($\cdot OCOCF_2CF_2CF_3$) to give an ion at *m/e* 252. An ion at *m/e* 55, corresponding to the methylisopropyl group, is also present.

The GLC characteristics of SL 75212 and propranolol are reported in Table I (retention time, height equivalent to a theoretical plate, resolution and symmetry factor).

A standard calibration graph obtained after extraction of SL 75212 from blood is shown in Fig. 4, and the coefficients of variation at different concentrations are reported in Table II.

The absolute recovery of SL 75212, calculated by using ^{14}C-labelled SL 75212, was 86–90%; one extraction was considered to be adequate.

The absolute sensitivity of the electron-capture detector was about 20 pg for SL 75212 and 5 pg for propranolol; this means that it is possible to detect 1 ng/ml of SL 75212 by increasing to 2–4 μl the volume of the final solution injected on to the gas chromatographic column.

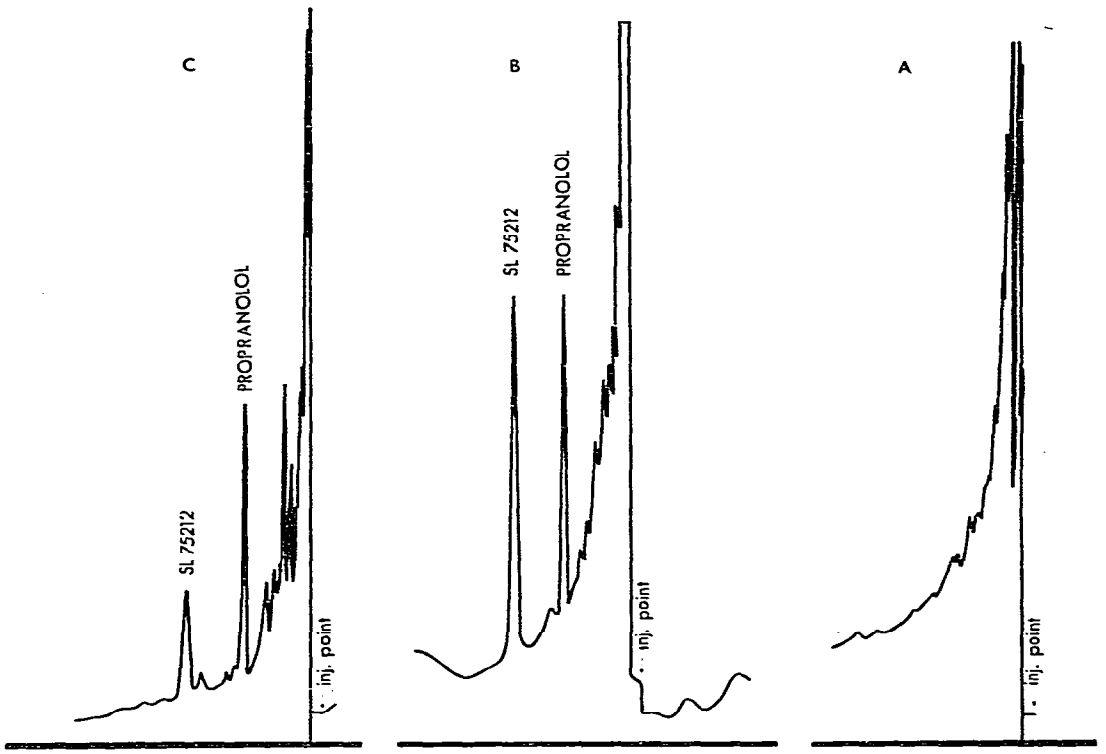


Fig. 2. Gas chromatograms of plasma extracts. (A) Plasma free from drug; (B) plasma to which known amounts of propranolol (20 ng) and SL 75212 (50 ng) have been added; (C) plasma of a volunteer who took 10 mg of SL 75212 orally.

The HFBA derivative of SL 75212 is very stable and the ratio between the amounts of SL 75212 and propranolol did not change during 1 week when the solution was kept at room temperature.

No interfering peaks due to endogenous substances or other cardiovascular drugs were noted. The analysis of blood samples containing guanethidine, clonidine, chlorthalidone, furosemide, lidocaine and quinidine showed that none of these substances interfered with the assay. Other β-blocking agents, such as alprenolol, oxprenolol and metoprolol, gave retention times lower than those of SL 75212 and propranolol.

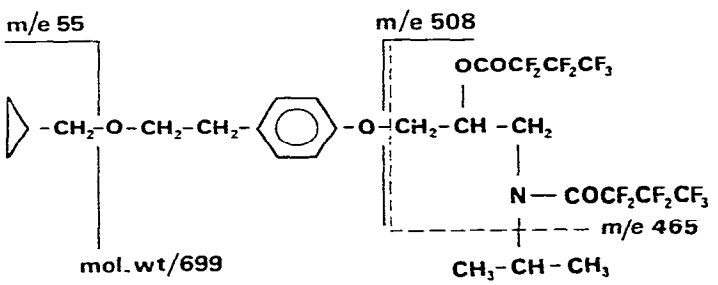


Fig. 3. Fragmentation pattern of the HFBA-derivative of SL 75212.

TABLE I

GAS CHROMATOGRAPHIC CHARACTERISTICS OF SL 75212 AND PROPRANOLOL (INTERNAL STANDARD)

Parameter	*SL 75212*	*Propranolol*
Retention time (min)	7.30	4
Height equivalent to a theoretical plate (mm)	1.37	1.57
Resolution	5.6	8.5
Symmetry factor of peak	0.95	0.96

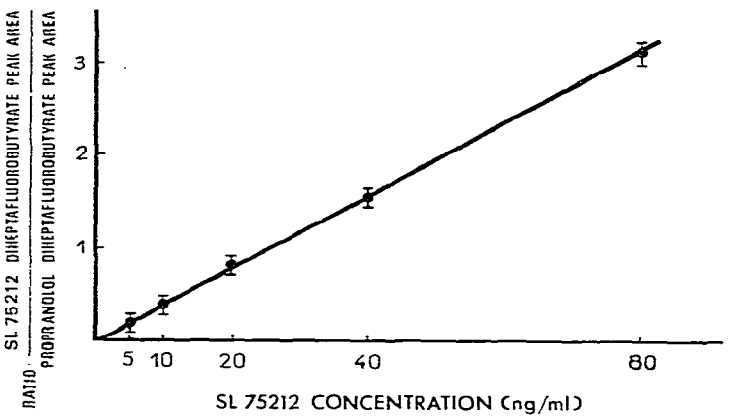


Fig. 4. Standard calibration graph for SL 75212.

TABLE II

ACCURACY OF DETERMINATION OF SL 75212 IN HUMAN BLOOD

Concentration of SL 75212 (ng/ml)	*Mean concentration found ± S.D. (ng/ml)*	*Coefficient of variation (%)*	*Number of determinations*
10	10.8 ± 0.8	7.4	10
20	18.6 ± 0.9	4.8	10
40	40.6 ± 1.2	2.9	10
60	62.4 ± 1.1	1.7	10

TABLE III

SL 75212 BLOOD CONCENTRATIONS IN VOLUNTEERS AT PEAK TIMES AND 24 h AFTER ADMINISTRATION OF A SINGLE DOSE OF THE DRUG ORALLY

Dose (mg)	*Concentration (ng/ml)*	
	2 h	*24 h*
5	10.5	3
5	10.0	5.2
10	16.5	10.5
10	27.0	6.0
20	34.0	13.0
20	49.5	16.0
40	51.5	18.0
40	69.5	19.0

The method described has been applied to the determination of SL 75212 in the blood of volunteers receiving a single dose of the drug by the oral route[3]. The concentration of SL 75212 in blood could be detected even when the smallest doses were given (5 mg). In Table III are reported some values of the concentration of SL 75212 in blood at peak concentration times and 24 h after administration of different doses of the drug. It can be seen that the amount of SL 75212 in blood was directly related to the dose administered.

CONCLUSION

A sensitive and specific method for the determination of SL 75212 has been developed, which provides a relatively simple procedure for all the future pharmacokinetic studies on SL 75212 and also allows the dtermination of blood levels of the drug on a routine basis. The time necessary to prepare 20 samples for GLC injection does not exceed 2 h.

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