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Note

Gas chromatographic determination of oxprenolol in human plasma

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Various methods for the assay of oxprenolol in biological fluids are available. A direct evaluation in plasma by thin-layer chromatography and fluorimetric determination has been described [1]. A high-performance liquid chromatographic assay for quantitation in blood, plasma, urine and breast milk has also been reported [2]. Several gas chromatographic (GC) methods have been employed [3—6]. We recently published a GC assay for another β-blocking agent, metoprolol, in plasma, using oxprenolol as internal standard [7]. This method was applied for the determination of oxprenolol in plasma. It differs from the previous GC methods [3—6] in the use of: silanized glassware for the acylation reaction, to avoid adsorption; potassium hydroxide—tripotassium phosphate as buffer instead of sodium hydroxide, to improve the reproducibility of the extraction; heptafluorobutyric anhydride instead of trifluoroacetic anhydride, to improve the stability of the derivatives.

EXPERIMENTAL

Chemicals and reagents

Oxprenolol and the internal standard, propranolol, were supplied by Ciba-Geigy (Basle, Switzerland). Alkaline buffer (pH > 13) was prepared by dissolving 30 g of tripotassium phosphate trihydrate (ref. 5102, E. Merck, Darmstadt, F.R.G.) and 16.8 g of potassium hydroxide (Merck 5033) in distilled water and making the volume up to 100 ml. All reagents and solvents were of analytical grade: diethyl ether (Pestipur quality, S.D.S., Valdonne, France), methylene chloride (Merck 6050) hydrochloric acid (Merck 318), hexane (Pestipur quality, S.D.S.). Pyridine (ref. 82702, Fluka, Buchs, Switzerland) was distilled at 115—116°C over potassium hydroxide pellets and stored over the same reagent. Heptafluorobutyric anhydride was purchased from Ventron (ref. PCR, 1300-3, Ventron, Karlsruhe, F.R.G.); potassium dihydrogen phosphate (Merck 4873) was used as a saturated solution.

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Instrumentation

The GC determinations were carried out with a gas chromatograph equipped with an electron-capture detector (Hewlett-Packard, Model 5713 A). The glass column used was 2 m × 3 mm I.D. and packed with 3% SE-30 on 80—100 mesh Gas-Chrom Q. The column temperature was 187°C. The carrier gas (argon—methane, 90:10) flow-rate was 60 ml/min. The column was conditioned as described previously [8]. The glassware was cleaned in a washing machine, and the tubes used for the acylation reaction were then silanized by immersion in a solution containing 1 ml of hexamethyldisilazane, 1 ml of trimethylchlorosilane, 1 ml of pyridine and 100 ml of toluene for 15 min. They were subsequently rinsed with methanol and dried at 100°C.

Calibration

One hundred microlitres of suitable oxprenolol hydrochloride aqueous solutions and 100 μl (1692 pmol) of propranolol hydrochloride aqueous solution were added to 1 ml of plasma to obtain calibration samples in the range of concentrations 33.2—3317 nmol oxprenolol per litre of plasma (10—1000 ng/ml). The peak areas were obtained from a Perkin—Elmer Data System (Sigma 10) connected to the chromatograph. The calibration curves were constructed from the ln—ln plot (a linear plot could also be made) of the peak area ratios against the plasma concentrations. Their equations were calculated by the least-squares method. A fresh calibration curve has to be prepared every month and checked once a week by injecting the derivative extracts kept at +4°C.

Extraction procedure

One millilitre of plasma spiked with 100 μl of the internal standard solution (1692 pmol of propranolol hydrochloride), 1 ml of alkaline buffer, and 4 ml of methylene chloride—diethyl ether (1:4) were shaken for 15 min at 300 rpm, then centrifuged for 2 min. The organic phase was shaken with 2 ml of 0.1 *M* hydrochloric acid for 10 min at 300 rpm and centrifuged. The organic phase was discarded and 1 ml of the alkaline buffer and 4 ml of methylene chloride—diethyl ether (1:4) were added and the mixture shaken for 10 min at 300 rpm. After centrifugation, the organic phase was transferred to a silanized tube and evaporated to dryness under a nitrogen stream. Two microlitres of pyridine, 1 ml of hexane and 10 μl of heptafluorobutyric anhydride were successively added. This order of addition — catalyst, solvent, acylation reagent — appeared to result in a more reproducible analytical yield. The mixture was shaken for about 15 sec. After 1 h at room temperature, 1 ml of potassium dihydrogen phosphate saturated solution was added, and the mixture was shaken for 15 min at 300 rpm. Three microlitres of the organic phase were injected into the gas chromatograph.

RESULTS

Plasma interferences

Fig. 1 shows the chromatograms corresponding to an extract of 1 ml of blank plasma and to an extract of the same plasma containing 332 nmol/l oxprenolol (100 ng/ml) and 1692 nmol/l propranolol (500 ng/ml). The metab-

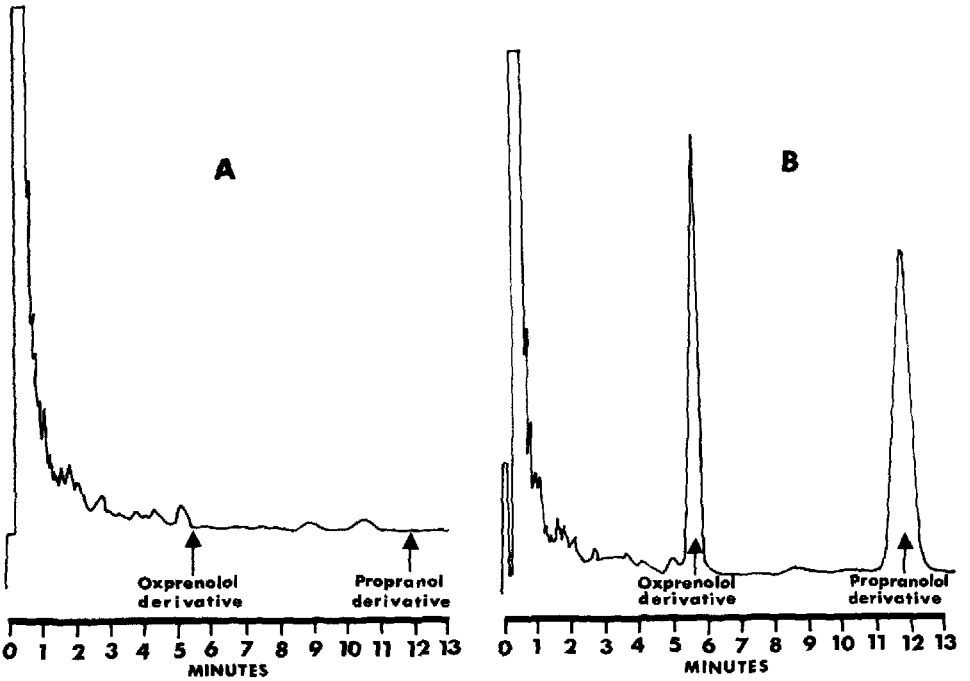

Fig. 1. Chromatograms of: (A) human plasma blank (1 ml plasma); (B) extract of 332 nmol/l oxprenolol and 1692 nmol/l propranolol in plasma.

olism of oxprenolol in man has not yet been fully elucidated, in so far as the presence of metabolites in plasma has not been established [9—15]. Nevertheless, the urinary metabolites found in man and rat [9—15] cannot interfere with the oxprenolol assay as they will not be extracted or derivatized by the described procedure.

Reproducibility and recovery

The results are summarized in Table I. A good reproducibility (coefficient of variation, C.V. = 4.7%) was obtained for the concentration 33.2 nmol/l (10 ng/l), which can be taken as the limit of quantitation. The mean overall recovery was 101.0% (6.5).

TABLE I

PRECISION AND RECOVERY OF THE DETERMINATION OF OXPRENOLOL IN SPIKED PLASMA SAMPLES

Amount added (nmol/l)	Mean amount found (n=6) (nmol/l)	Coefficient of variation (C.V.) (%)	Mean recovery (%)
33.2	33.3	4.7	101
82.9	79.1	2.7	95
332	362	6.4	109
829	818	4.9	99
1658	1680	5.2	101

Mean overall recovery (C.V.) = 101.0% (6.5)

TABLE II

STORAGE STABILITY OF OXPRENOLOL IN HUMAN PLASMA OVER TWELVE MONTHS AT −20° C

Duration of storage at −20° C (months)	Amount of oxprenolol added to plasma (nmol/l)	
	166	1658
	Amount of oxprenolol found (average of two assays) (nmol/l)	
0	164	1655
1	158	
3	158	1667
5	158	1710
9	176	1615
12	181	1665

Stability of oxprenolol in plasma

Spiked plasma samples were kept at −20°C and analysed regularly. Oxprenolol is stable for up to twelve months under these conditions (Table II).

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