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## Note

# Determination of oxprenolol in plasma by column liquid chromatography

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Oxprenolol, (±)-1-(*o*-allyloxyphenoxy)-3-isopropylaminopropan-2-ol, is a $\beta$-adrenergic blocking agent frequently used in the treatment of hypertension in pregnancy, cardiac arrhythmias and angina pectoris. Owing to its low therapeutic levels, a sensitive method for assaying this drug in plasma is required. Several methods, including gas chromatoghraphy [1–7], thin-layer chromatography with fluorescence detection [8] and high-performance liquid chromatography (HPLC) [9–14], have been reported for the assay of oxprenolol in biological fluids. We report here a simple, rapid, sensitive, accurate and reproducible method for the determination of this drug in plasma.

## EXPERIMENTAL

### *Materials*

Acetonitrile (Romil Chemicals, Shepshed, U.K.) and dichloromethane (Fisons Scientific Apparatus, Loughborough, U.K.) were of HPLC grade. Orthophosphoric acid (85%, density 1.71 g/ml), potassium dihydrogenphosphate, sodium hydroxide and triethylamine were of analytical-reagent grade (Merck, Darmstadt, F.R.G.). Oxprenolol hydrochloride and propranolol hydrochloride were donated by Ciba-Geigy (Basle, Switzerland) and Indian Explosives (Madras, India), respectively, and were used as received.

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*Preparation of mobile phase and standard solutions*

The mobile phase was prepared by adding 85% orthophosphoric acid dropwise to 1 l of 0.03 *M* potassium dihydrogenphosphate so as to adjust the pH to 2.2. This phosphate buffer (500 ml) was mixed with an equal volume of acetonitrile, then triethylamine (200 μl) was added and mixed well. The final pH of the mobile phase was 3.0. The solution was filtered through a 0.5-μm Millipore filter and degassed in an ultrasonic bath before use. Stock solutions (100 μg/ml) of hydrochlorides of propranolol (internal standard) and oxprenolol were prepared in distilled water. Working standard solutions (500 ng/ml) were prepared daily.

*Extraction procedure*

Solid-phase extraction was employed using Extrelut-3 columns (E. Merck). A 1-ml volume of drug-free plasma, known amounts of drug and 100 μl of internal standard solution were added successively to the Extrelut-3 column. Then 1 *M* sodium hydroxide solution (ca. 2.6 ml) was added to make up the volume to 3.75 ml and the column was kept aside for impregnation (ca. 15 min). The drug was eluted from the Extrelut-3 column with dichloromethane (3 × 5 ml). The organic phase was evaporated on a water-bath at 35°C under a stream of nitrogen, the residue was dissolved in 200 μl of the mobile phase and 150 μl of the solution were injected into the chromatographic system.

*Apparatus*

The liquid chromatograph consisted of a Model M-45 pump (Waters Assoc., Milford, MA, U.S.A.) and a Uvikon LCD-725 UV detector (Kontron, Zurich, Switzerland) operated at range 0.01 and time constant 2; the wavelength was set at 275 nm. Chromatograms were recorded with an HP 3390-A recording integrator (Hewlett-Packard, Palo Alto, CA, U.S.A.). A Rheodyne injector with a 150-μl loop was connected to Spherisorb 3 ODS (particle size 3 μm) column (15 cm × 4.6 mm I.D.) (HPLC Technology, Macclesfield, U.K.). The mobile phase was pumped at a flow-rate of 1 ml/min and the operating pressure was ca. 136 bar.

*Calibration and reproducibility*

Calibration graphs were obtained by adding known amounts of drug (equivalent to 17.6–282.2 ng of oxprenolol base) and internal standard to blank plasma, extracting as described above and injecting. To check the reproducibility of the procedure, five different plasma calibration graphs were constructed on five different days; the mean peak-area ratios of drug to internal standard, standard deviations (S.D.) and coefficients of variation (C.V.) are given in Table I.

*Pharmacokinetic investigation*

In order to check the utility of this technique in pharmacokinetic studies, rapid release (RR) and sustained release (SR) dosage forms containing 80 mg of oxprenolol hydrochloride were administered separately to a dog together with food, and blood samples were drawn at regular intervals for a period of 24 h.

TABLE I

MEAN PEAK-AREA RATIOS OF DRUG TO INTERNAL STANDARD, STANDARD DEVIATIONS AND COEFFICIENTS OF VARIATION WHEN DIFFERENT AMOUNTS OF OXPRENOLOL WERE ADDED TO CONTROL PLASMA ON FIVE DIFFERENT DAYS

| Amount of oxprenolol (ng) | Mean peak-area ratio | S.D. | C.V. (%) |
|---|---|---|---|
| 0 | 0 | – | – |
| 17.64 | 0.1714 | 0.0150 | 8.75 |
| 35.28 | 0.4235 | 0.038 | 9.16 |
| 70.56 | 0.8479 | 0.0609 | 7.18 |
| 141.12 | 1.7734 | 0.1194 | 6 73 |
| 282.24 | 3.3379 | 0.3441 | 10.30 |

## RESULTS AND DISCUSSION

Chromatograms obtained from drug-free plasma, plasma containing added drug and plasma from a dog having received 80 mg of oxprenolol hydrochloride orally are shown in Fig. 1. Under these conditions, the calibration graph of oxprenolol (with propranolol as internal standard) was linear within the range studied. The slope, intercept and correlation coefficient of the regression line were 0.0119,

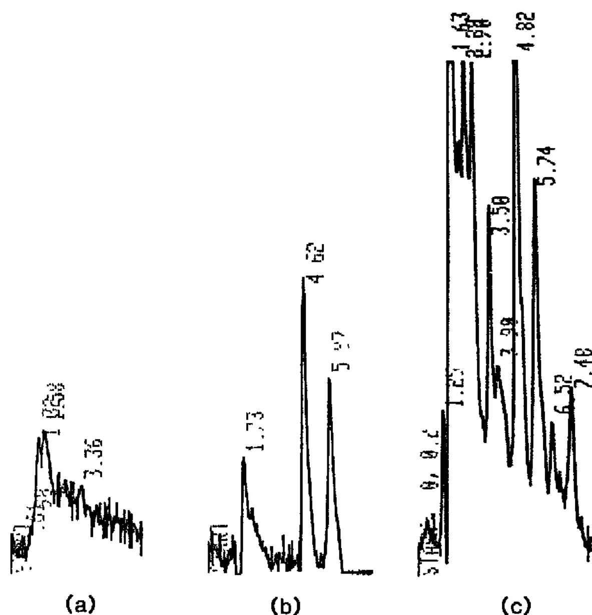


Fig. 1. Representative chromatograms of (a) drug-free plasma, (b) plasma containing 120 ng of oxprenolol (peak with retention time of 4.6 min) and 50 ng of propranolol (peak with retention time of 5.8 min) and (c) dog plasma when 80 mg of oxprenolol hydrochloride was administered orally (peaks having retention times of 4.8 and 5.7 min correspond to oxprenolol and propranolol, respectively). The amounts of oxprenolol detected in (b) and (c) were 115 and 188 ng, respectively.

TABLE II

PLASMA CONCENTRATIONS OF OXPRENOLOL AT DIFFERENT TIMES WHEN RR AND SR DOSAGE FORMS CONTAINING 80 mg OF OXPRENOLOL HYDROCHLORIDE WERE ADMINISTERED TO A DOG

| Time (h) | Plasma concentration (ng/ml) | |
|---|---|---|
| | RR | SR |
| 0.0 | 0.0 | 0.0 |
| 0.5 | 54.76 | 49.90 |
| 1.0 | 360.01 | 144.24 |
| 1.5 | 397.74 | 159.52 |
| 2.0 | 210.83 | 162.52 |
| 2.5 | 198.20 | 164.79 |
| 3.0 | 185.90 | 243.51 |
| 4.0 | 162.48 | 247.38 |
| 6.0 | 131.78 | 285.21 |
| 8.0 | 57.36 | 222.90 |
| 12.0 | 37.50 | 144.24 |
| 24 0 | 10.06 | 54.55 |

0.0026 and 0.999, respectively. The mean within-day coefficient of variation was 7% and the mean between-day coefficient of variation was 10%. The mean absolute recoveries were 91 ± 5%. By injecting 150 μl of the extract, plasma containing down to 10 ng/ml oxprenolol can be easily analysed by this method. The coefficient of variation varied from 8.8% at 17.6 ng/ml to 10.3% at 282 ng/ml.

As can be seen from the chromatograms, no endogenous substances were found to interfere with the drug peaks. Metoprolol and alprenolol also did not interfere. Chromatograms a and b in Fig. 1 were identical when human or dog plasma was used.

This method can also be employed for determining propranolol in plasma using oxprenolol as an internal standard. The limit of detection was 5 ng/ml. This simple method, utilizing a commonly available UV detector, has been successfully used to determine oxprenolol in dog plasma after administering RR and SR formulations containing 80 mg of oxprenolol hydrochloride, as shown in Table II. Clinically, it can also be utilized to determine oxprenolol routinely in the plasma of patients receiving this drug.

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