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Note

Determination of low levels of Oxprenolol in blood or plasma by gas–liquid chromatography

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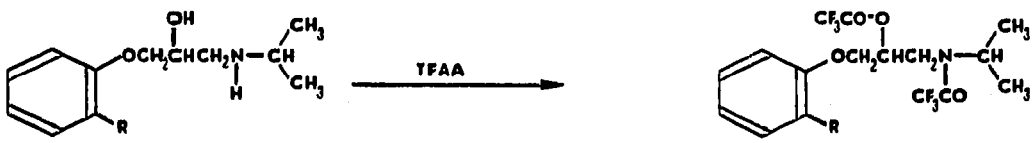
In the last ten years intensive research on adrenergic β-receptor blocking agents has led to the synthesis of a large number of compounds which may be considered as being derived from isopropylaminopropanol[1]. Any study of *in vivo* pharmacokinetics requires a sensitive and specific method of detecting and measuring the unchanged compounds and a GLC method has been developed for Alprenolol (Fig. 1) involving derivatisation with trifluoroacetic anhydride and subsequent quantitisation using an electron capture detector[2]. The method is sensitive (limit 10 ng/ml plasma) and an overall recovery of $80 \pm 15\%$ was obtained. An internal standard for the GLC, dodecachloro-octahydro-1,3,4-methano-2H-cyclobuto(*ed*)pentalene, was added after derivatisation.



Oxprenolol: R = $-OCH_2CH{=}CH_2$

Alprenolol: R = $-CH_2CH{=}CH_2$

Fig. 1. Structure of compounds and derivatives mentioned in the text.

The method presented here, an improvement on the above technique, is used to measure blood or plasma levels of Oxprenolol, 1-(*o*-allyloxyphenoxy)-3-isopropylamino-2-propanol, Ciba 39 089-Ba (see Fig. 1), and compensates for losses by the addition of an internal standard before extraction and derivatisation. Because of the very close similarity to Oxprenolol, Alprenolol was chosen as internal standard and since both react with TFAA to form di-TFA derivatives*, compensation for small changes in the day-to-day performance of the column and detector should be more accurate than by using a compound possessing no structural similarity to the substance under investigation.

* Abbreviations used: TFA= trifluoroacetic acid, TFAA= trifluoroacetic anhydride.

MATERIALS AND METHODS

Gas–liquid chromatography

A Pye series 104 gas–liquid chromatograph equipped with a ^{63}Ni electron capture detector operated at a pulse interval of 150 μsec was used. A glass column, length 2.7 m and I.D. 4 mm, packed with 3% OV-101 (Applied Science Labs., State College, Pa., U.S.A.) on Gas-Chrom Q, 80–100 mesh (Applied Science Labs.) was operated at a temperature of 158°. The injection port and detector temperatures were 210° and 280°, respectively, and the carrier gas (nitrogen) flow-rate was 68 ml/min. All peak areas were integrated using a Model CRS 204 electronic integrator (Infotronics, Boulder, Colo., U.S.A.).

Reagents and materials

Alprenolol was supplied as the hydrochloride by Hässle (Mölndal, Sweden) trifluoroacetic anhydride by Fluka, St. Gallen, Switzerland, and calcium chloride (medium fine granules) by E. Merck, Darmstadt, G.F.R. TFAA and all organic solvents were distilled prior to use.

Extraction procedure

Two millilitres of blood or plasma are shaken in a test-tube with an equal volume of saturated aqueous sodium chloride; 200 ng of Alprenolol hydrochloride dissolved in 100 μl water are added and the sample shaken gently to ensure complete mixing. Then 0.3 ml 1 *N* sodium hydroxide is added and the sample extracted three times with 2-ml portions of methylene chloride–ether (1:4). After centrifugation for 3 min at 4000 rpm (1300 *g*), the combined organic extracts are shaken with 2 ml of 0.1 *N* sulphuric acid and the organic phase discarded. The acid phase is basified with 0.3 ml 1 *N* sodium hydroxide until the pH is between 10 and 11, then extracted twice with 2 ml of the methylene chloride–ether mixture. The organic phase is washed once with 2 ml water to remove the bulk of the alkali present in the organic phase. However, the aqueous phase, after washing, must not be below pH 8 or else the β-blocking agents will be re-extracted into this phase. The organic phase is dried by shaking briefly with a single piece of calcium chloride, and the organic phase evaporated to dryness after separating it from the chloride. The dried extract is then redissolved in 0.5 ml of benzene, 0.1 ml TFAA is added and derivatisation allowed to proceed in a water bath at 35° for 30 min. The sample is then removed, evaporated to dryness and redissolved in 0.5 ml benzene for gas–liquid chromatography.

RESULTS AND DISCUSSION

The overall recoveries were found to be $70 \pm 10\%$, and typical chromatograms are shown in Fig. 2. A calibration curve, prepared by extracting samples containing different concentrations of Oxprenolol and 200 ng Alprenolol, is shown in Fig. 3 together with the 95% confidence intervals. Six samples were analysed at each concentration. To check the accuracy of the method a number of samples was prepared and analysed as unknowns, with one analysis performed per sample. The results of these analyses are presented in Table 1. From the calibration curve it can be seen that the method may be used to measure levels as low as 10 ng/ml and this limit could be

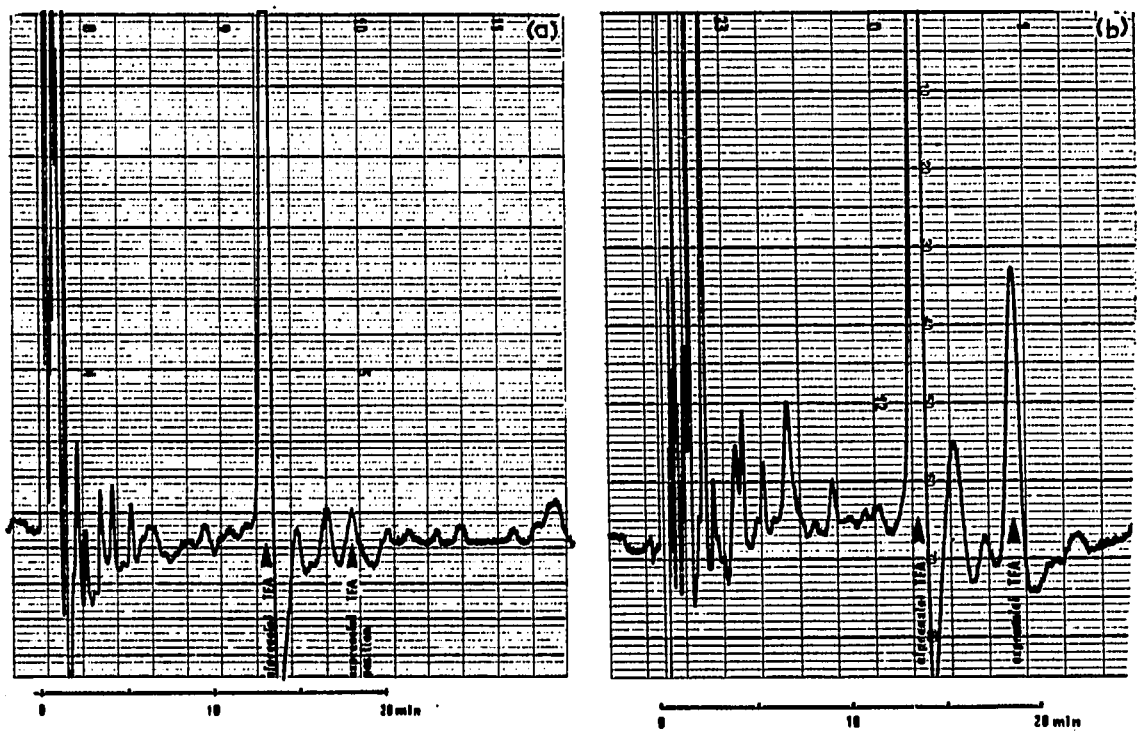


Fig. 2. Typical chromatograms obtained from blood samples containing 200 ng internal standard and (a) no Oxprenolol or (b) 100 ng of Oxprenol.

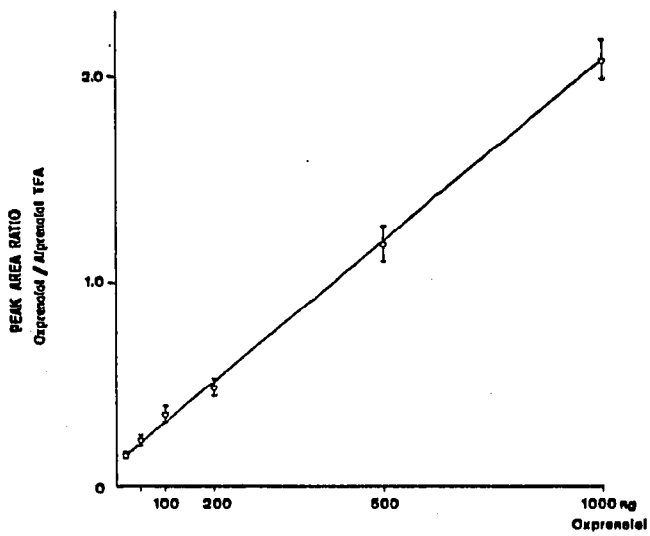


Fig. 3. Calibration curve relating the peak area ratio of the two derivatives to the amount of Oxprenolol present in the original sample. The 95% confidence limits for six samples per concentration are shown (vertical bars).

TABLE I

ANALYSIS OF BLOOD SAMPLES CONTAINING UNKNOWN AMOUNTS OF OXPRENOLOL

Oxprenolol added (ng/ml)	*Found* (ng/ml)*	*Error (%)*
20	20	0
75	60	−20
100	97	− 3
215	200	− 8
350	327	− 7
500	455	− 9

* One analysis per sample

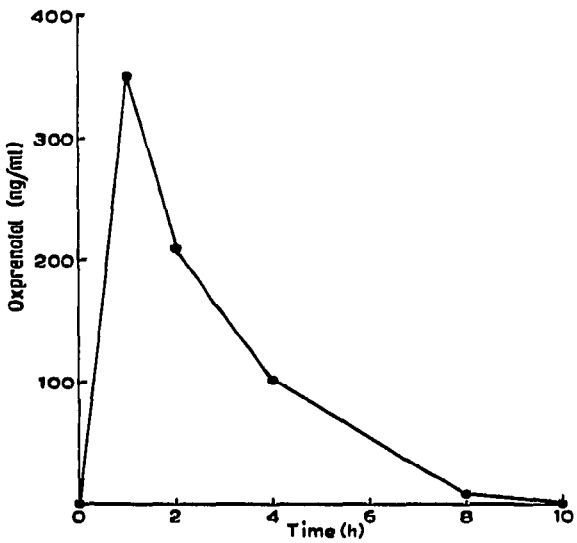


Fig. 4. Blood levels (ng/ml) of Oxprenolol in a volunteer after a single 80 mg oral dose.

extended by starting with more than 2 ml blood or plasma, as the extracts obtained are relatively free from interfering substances.

Blood samples were withdrawn from a volunteer after the oral administration of a single 80 mg dose of Oxprenolol. The blood levels found using the above method are shown in Fig. 4 and it can be seen that the peak concentration appears to be reached within one hour after administration and then decreases rapidly with a half-life of approximately 90 min.

REFERENCES

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2 M. Ervik, *Acta Pharm. Suecica*, 6 (1969) 393.