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Note

Fluorimetric determination of oxprenolol in plasma by direct evaluation of thin-layer chromatograms

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Oxprenolol, 2-hydroxy-3-(*o*-allyloxyphenoxy-)propylisopropylamine, a beta-adrenergic blocking agent, is used frequently in the treatment of angina pectoris and arterial hypertension. Methods for the determination of oxprenolol by gas—liquid chromatography was described by Nambara et al. [1], and by Jack and Riess [2], the latter method being modified by Degen and Riess [3]. Oxprenolol is determined as its N,O-bis(trifluoro)acetyl derivative. All these methods need several extraction steps to obtain extracts free from interfering substances. They are therefore relatively time-consuming. The limit of detection is 10 ng/ml if 2 ml of plasma are extracted [2, 3].

Fluorimetric methods exist for several other beta-blocking drugs such as atenolol [4, 5], bufuralol [6], propranolol [7—11] and sotalol [12]. The spontaneous fluorescence of these drugs is measured either in the cuvette without further purification or following chromatographic separation on thin-layer plates or by high-performance liquid chromatography. Separation from interfering substances extracted from plasma by thin-layer chromatography (TLC) usually avoids a re-extraction step. Therefore, measurement of spontaneous fluorescence of drugs following TLC separation is a specific and less time-consuming technique.

Oxprenolol does not show intensive fluorescence on TLC plates either in the visible region or in the ultraviolet. Therefore, derivatisation of oxprenolol to a fluorescing compound is necessary. 1-Ethoxy-4-(dichloro-*s*-triazinyl)naphthalene (EDTN) was selected as derivatising agent. Chayen et al. [13] have described an assay of corticosteroids, and Stopher [14] reported the estimation of tolamolol using EDTN as the fluorescence reagent.

EXPERIMENTAL

Instrument

A chromatogram spectrophotometer KM 3 from Carl Zeiss with a Perkin-Elmer recorder Model 56 was used.

Standard and reagents

Oxprenolol hydrochloride and metoprolol tartrate (internal standard) were obtained from Ciba-Geigy (Basle, Switzerland). EDTN was supplied by BDH (Poole, Great Britain). Solvents (analytical grade) and TLC plates (silica gel 60 without fluorescence indicator) were obtained from Merck (Darmstadt, G.F.R.).

Method

Extraction. To 1 ml of plasma 10 μl of a methanolic solution containing 100 ng of metoprolol tartrate as internal standard are added; then 0.5 ml of 1 *N* NaOH, 250 mg NaCl and 5 ml of dichloromethane—diethyl ether (1:4) are added. After shaking for 15 min in a horizontal position the tubes are centrifuged briefly to separate the layers. The organic phase is transferred to another tube and evaporated to dryness at 50° under a gentle stream of nitrogen.

Derivatisation and chromatography. A 40 μl volume of a solution of 1 mg of EDTN in 5 ml of ethyl acetate is added to the solid residue. The stoppered tube is heated to 50° for 15 min. After cooling to room temperature the reaction mixture is taken up with a 100 μl Hamilton syringe. The whole solution is applied with a Linomat III (Camag, Switzerland) onto a TLC plate taking care to keep the spot size less than 4 mm in diameter. Together with a series of plasma samples with unknown amounts of oxprenolol, three standards are spotted per plate. The standards are plasma samples spiked with definite identical amounts of oxprenolol hydrochloride. These standards are carried through analysis together with the other samples.

The chromatograms are developed in an unlined glass tank containing the solvent system chloroform—ethyl acetate (95:5). The solvent (freshly prepared each day) is allowed to ascend about 15 cm above the origin. The R_F values for the derivatives of oxprenolol and the internal standard are 0.62 and 0.41, respectively.

After drying in the air the plate is dipped into a solution of 2% paraffin in cyclohexane. The fluorescence is measured after another drying period of at least 15 min.

Measurement and evaluation. The measurements are performed using the monochromator sample mode of the scanner. Fluorescence of the spots due to the derivatives of oxprenolol and metoprolol is measured with the 365 nm line of a medium pressure lamp St 41 and a 436 monochromatic filter. The slit is 0.5 × 6 mm. The plates are scanned at 100 mm/min.

The peak height ratios oxprenolol/metoprolol are calculated. The amount of oxprenolol in the plasma samples is calculated from the peak height ratios of the standards (mean of three determinations).

Reaction conditions

Oxprenolol hydrochloride and metoprolol tartrate (1 μg of each) were derivatised with EDTN solutions containing 1, 4 and 20 mg of EDTN per 10 ml of ethyl acetate. Three series of samples with 0, 100, 500 and 1000 ng of oxprenolol hydrochloride and 300 ng of metoprolol tartrate (both dissolved in methanol) were reacted with 40 μl of EDTN solution (2 mg per 10 ml) at 35, 40, 50 and 70° for 15 min. Another series of samples with 1 μg of both oxprenolol hydrochloride and metoprolol tartrate was heated to 50° for 10, 15, 30 and 60 min. All further analysis steps were carried out as described above.

Standard curves

Standard curve A. To samples containing 0, 1, 3, 5, 7, 10, 30, 70, 100, 300, 700 and 1000 ng of oxprenolol hydrochloride, 100 ng of metoprolol tartrate were added. After the addition of 40 μl of EDTN solution the samples were derivatised, chromatographed and measured as described above.

Standard Curve B. Blank plasma was spiked with 0, 5, 10, 30, 70, 100, 300, 700 and 1000 ng of oxprenolol hydrochloride; 100 ng of metoprolol tartrate were added as internal standard to each sample. The plasma samples were analysed as described above.

Recovery from plasma

Blank plasma was spiked with 500 ng of oxprenolol hydrochloride per ml. Extraction and concentration was carried out as described above; 1000 ng metoprolol tartrate were added to the solid residue. Standards were prepared by adding 1000 ng of metoprolol tartrate to 500 ng of oxprenolol hydrochloride. The further steps of analysis were performed as described above. Recovery of oxprenolol was calculated by comparing the peak height ratios of the plasma samples to the peak height ratios of the pure standards (mean of four determinations).

Reproducibility studies

Reproducibility studies were performed at three concentrations by performing five replicate analyses of blank plasma which had been spiked such that the concentrations of oxprenolol hydrochloride were 30 ng/ml in the first series and 100 and 300 ng/ml in series 2 and 3. To each sample were added 100 ng of metoprolol tartrate as internal standard. Standards were prepared by spiking three blank plasma samples with 50 ng (series 1) or 200 ng (series 2 and 3) of oxprenolol hydrochloride.

Drug interference studies

A number of other drugs were tested for potential interference in the assay. Aliquots of stock solutions of the compounds were heated with EDTN, dissolved in ethyl acetate and chromatographed.

RESULTS

The reaction of oxprenolol, base or salt, with EDTN leads to a single blue

fluorescent product. The same reaction occurs with metoprolol. The fluorescence spectrum of the derivative of oxprenolol on a thin-layer plate is shown in Fig. 1.

The reaction of oxprenolol and metoprolol was carried out with varying amounts of EDTN, between 10 and 60 min and 35 to 70°. In all samples the same relative peak size was obtained, which suggests that the reaction remained constant within this range of conditions. The TLC separation of the EDTN derivatives of oxprenolol and the internal standard, extracted from spiked plasma samples, is demonstrated in Fig. 2. The concentrations are 0 and 30 ng of oxprenolol hydrochloride and 50 ng of metoprolol tartrate. No interference from normal plasma constituents was observed. The minimum detectable amount of oxprenolol is 5 ng.

The linearity of standard curves of pure substances as well as of oxprenolol from spiked plasma samples is excellent ($r>0.9992$ in any experiment) from 10 to 1000 ng oxprenolol (based on the hydrochloride salt). Both curves pass through the origin. It is therefore sufficient to determine only one point on the calibration curve (mean value of three determinations) and to connect this point to zero. The content of unknown samples may be determined by calculation or graphically.

As no radioactive oxprenolol was available, recovery of oxprenolol from plasma could not be determined directly. Our recovery experiments were based upon the assumption that oxprenolol and oxprenolol hydrochloride are derivatised to the same extent with EDTN. Under these conditions recovery from plasma was calculated to be 81.8%. Reproducibility studies were performed at three concentrations in the therapeutic range. The standard deviation was independent of the oxprenolol concentration in the plasma sample. It was calculated to be 4.1%.

A number of other basic drugs were tested for potential interference in the

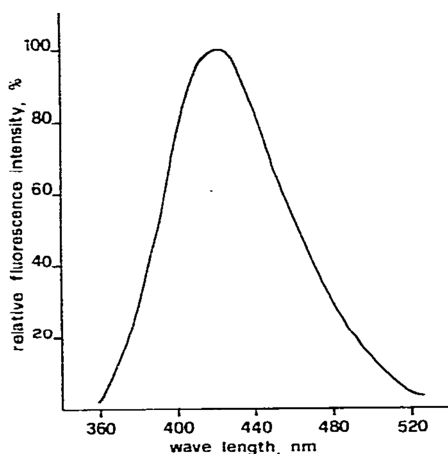

Fig. 1. Emission spectrum of oxprenolol derivatised with EDTN, chromatographed on silica gel 60 plates with the solvent system chloroform—ethyl acetate (95:5). After drying the plate was dipped into a solution of 2% paraffin in cyclohexane. Excitation was with the 313 nm line of a medium-pressure lamp.

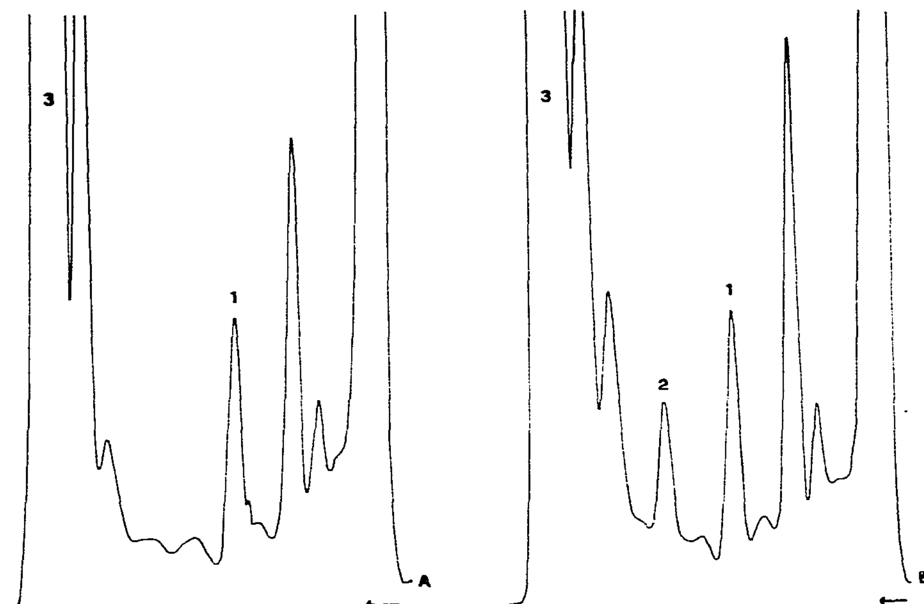

Fig. 2. Scans of chromatograms of 1 ml of blank plasma (A) and 1 ml of blank plasma spiked with 30 ng of oxprenolol hydrochloride (B). Both samples were spiked with 50 ng of metoprolol tartrate. The plasma samples were analysed as described in the text. 1, Derivative of metoprolol; 2, derivative of oxprenolol; 3, excessive EDTN.

assay but none of the compounds investigated in this experiment (the cardioactive agents atenolol, propranolol, digitoxin and chinidin) were found to interfere with the analysis of oxprenolol. Other compounds that were also shown not to impair the usefulness of the assay were guanethidine, hydralazin, triamteren and diazepam.

DISCUSSION

The specificity and sensitivity of the described method is satisfactory for the measurement of blood levels during chronic therapy and for pharmacokinetic measurements where extremely low plasma levels may occur. Its advantage over the gas chromatographic methods described previously is the linearity over a wide range, which includes the therapeutic range of plasma levels. Therefore, it is not necessary to produce a complete calibration graph, but it is sufficient to determine just one point on the curve. The method requires also only 1 ml of plasma for each determination.

Another advantage is that this method is less time-consuming than the gas chromatographic ones, because purification of the plasma extract by re-extraction is avoided. The coefficient of variation of this method is higher than that of the gas chromatographic method described by Degen and Riess [3], 4.1% instead of 3.4%, but the precision is sufficient for the determination of the drug in biological material.

Using EDTN as fluorescence reagent overcomes the disadvantages of using

dimethylaminonaphthalene sulphonyl (Dns) chloride; namely, the requirement of another extraction step after the reaction, the long reaction time (more than 12 h) and the instability of the Dns-amide. Fluorescence of the EDTN derivatives is stable for several days on the thin-layer plate after fixation of the fluorescence by dipping into a paraffin solution. The limit of detection of oxprenolol (5 ng) is slightly higher than the limit of detection Stopher [14] reported for tolamolol (2 ng). Extraction of oxprenolol with dichloromethane—diethyl ether leads to extracts free of interfering substances. Another clean-up by dissolving the dry residue in methanol and shaking with heptane, as described by Stopher, is not necessary in the oxprenolol assay.

In the same way, determination of metoprolol in plasma is possible using oxprenolol as internal standard.

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