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Note

Quantification of oxprenolol in biological fluids using high-performance liquid chromatography

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Oxprenolol, a β-adrenergic blocking drug, has recently been reported to be effective in the control of hypertension in human pregnancy [1]. It was shown to be equivalent to α-methyldopa in the control of maternal hypertension with improved placental and foetal growth. The disposition of oxprenolol in the maternal-placental—foetal system is under investigation in this laboratory. Unlike propranolol whose metabolite, 4-hydroxypropranolol, has been reported to be pharmacologically active [2], oxprenolol has no known active metabolite and so a method for the quantification of oxprenolol only was developed.

Several gas chromatographic (GC) methods have been reported for the quantification of oxprenolol in biological fluids [3—5]. These methods involve lengthy and laborious extractions followed by tedious and expensive derivatization procedures. Trifluoroacetic anhydride was the derivatizing agent used in all the methods reported and the di-trifluoroacetyl derivative of oxprenolol was detected using electron-capture detection following GC separation. The assay time for the methods reported by Jack and Riess [3] and Degen and Riess [4] was over 2 hours per sample. When Walle's method [5] was used with an additional back extraction step, the total analysis time was more than an hour for each sample.

This paper describes a simple and rapid high-performance liquid chromato-

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graphic (HPLC) assay for the quantification of oxprenolol in blood, plasma, urine or breast milk. HPLC offers advantages that (a) no derivatization steps are required prior to quantification and (b) oxprenolol is detected directly.

EXPERIMENTAL

Reagents and materials

Oxprenolol hydrochloride and alprenolol hydrochloride were gifts from Ciba-Geigy (Sydney, Australia) and Astra Chemicals (Sydney, Australia), respectively. The methanol (Ajax, Sydney, Australia) used for the chromatography was analytical grade and the water was glass distilled. The 0.005 *M* solution of 1-octylsulphonic acid, adjusted to about pH 3.5 with glacial acetic acid was prepared from a commercial reagent (PIC-B8, Waters Assoc., Milford, Mass., U.S.A.). The dichloromethane used for extraction was analytical grade and the diethyl ether (anaesthetic grade) was freshly distilled in glass every morning. All other reagents were analytical grade and were used without further purification.

All the glassware was cleaned with a chromic acid mixture and washed with distilled water. The extraction and evaporation tubes were then silylated with Siliclad (Clay Adams, Parisppany, N.J., U.S.A.), washed with distilled water and dried.

Drug extraction from biological samples

Aliquots of biological samples (3 ml of plasma or urine, 1 ml of blood diluted with 2 ml of water or 2 ml of expressed breast milk diluted with 1 ml of water) were pipetted into a 15-ml glass centrifuge tube fitted with a PTFE-lined screw cap and containing an aqueous solution of the internal standard (10 μg of alprenolol hydrochloride per 100 μl). After the addition of 300 mg of sodium chloride and 200 μl of 5 *M* aqueous sodium hydroxide, the mixture

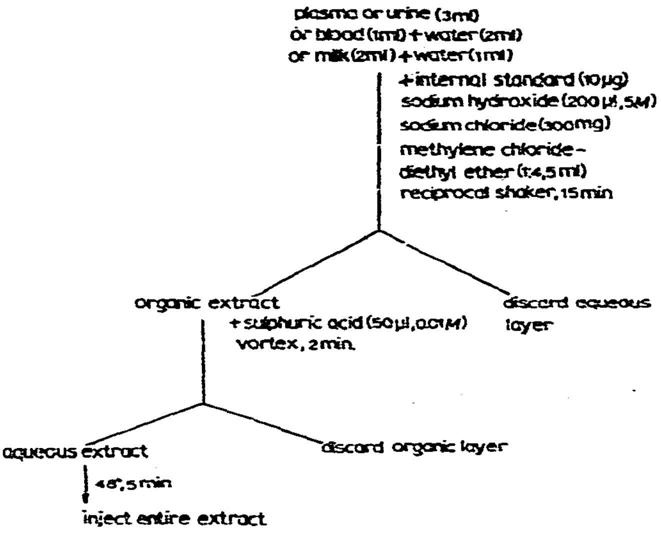

Fig. 1. Schematic diagram for the extraction of oxprenolol from biological fluids.

was extracted with 5 ml of methylene chloride—diethylether (1:4). To minimize emulsion formation, this extraction was done with gentle shaking on a reciprocal shaker for 15 min. After centrifugation at 1500 g for 5 min, the organic extract was transferred to an evaporation tube, a 7-ml glass tube with a 50-μl capillary at the base. After the addition of 50 μl of 0.01 M sulphuric acid, the evaporation tube was stoppered and shaken on a vortex mixer for 2 min. After centrifugation at 1500 g for 5 min the organic phase was aspirated and discarded. The aqueous phase left in the evaporation tube was then heated in a water bath at 48° for 5 min to evaporate off any residual organic solvent. The entire aqueous extract was then injected into the liquid chromatograph. Fig. 1 gives a schematic outline of the extraction procedure.

Chromatography

The HPLC system consisted of a Varian Aerograph Model 8500 high-performance liquid chromatograph, a Valco Valve injection port operated with a 50-μl loop, and a Spectra-Physics Model 770 variable-wavelength UV detector operated at 275 nm. The column (250 × 4.6 mm I.D.) was stainless-steel tubing packed with octadecylsilane-bonded silica gel having an average particle size of 10 μm (Whatman PXS10-25 ODS-2). The output from the detector was connected to a variable input potentiometric recorder (Linear Instruments, Model 355) operating at 5 mV full-scale deflection. Analyses were performed using a mobile phase of 0.005 M 1-octylsulphonic acid (1 vial of PIC-B8 per liter of solvent) in an aqueous solution of 67% (v/v) methanol. The flow-rate was 60 ml/h at an inlet pressure of 2000 p.s.i. The column was water-jacketed and the operating temperature was maintained at 30°.

Calibration and reproducibility

Known quantities of oxprenolol hydrochloride (equivalent to 30—630 ng of the oxprenolol base) were added to blank plasma samples. The samples were then assayed for oxprenolol. Calibration curves were constructed by plotting the peak height ratios between oxprenolol and the internal standard versus the amount of oxprenolol base added. To check the reproducibility of the analytical procedure, three different plasma calibration curves were constructed on three different days using human plasma samples from six different sources: a healthy male, a healthy female and four healthy pregnant women near term. Calibration curves using human urine, blood and expressed breast milk were similarly prepared using samples from at least two different sources.

RESULTS AND DISCUSSION

Under the chromatographic conditions described above, the retention times of oxprenolol and alprenolol were 6 and 8.1 min, respectively. Fig. 2 shows chromatograms of the extract of 3 ml of blank human plasma (Fig. 2a) and 3 ml of blank human plasma from another source to which were added oxprenolol hydrochloride equivalent to 569 ng of oxprenolol base (peak 1) and 10 μg of alprenolol hydrochloride (peak 2) (Fig. 2b). Similar chromatograms were obtained with extracts of urine, diluted blood and diluted human breast milk.

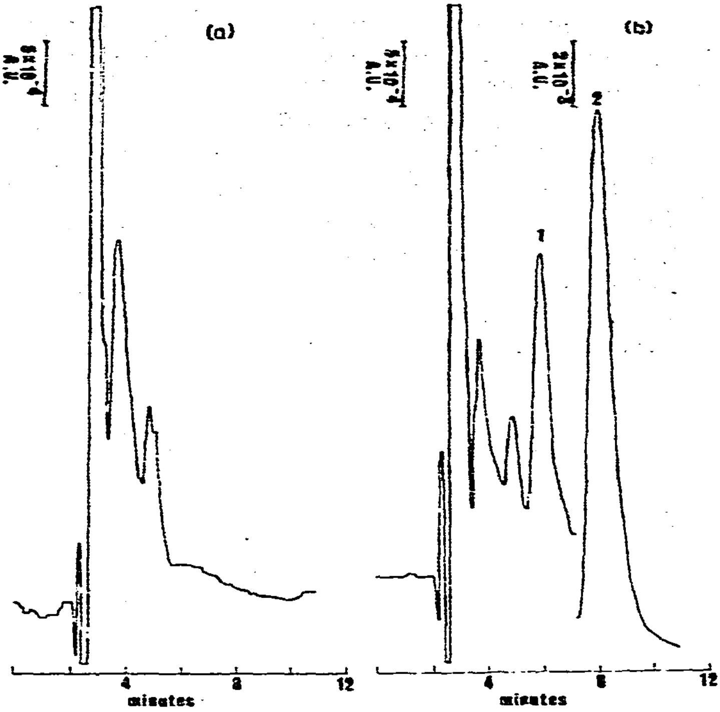

Fig. 2. Chromatograms of (a) blank human plasma and (b) blank human plasma from a different source to which were added oxprenolol hydrochloride equivalent to 569 ng of oxprenolol base (peak 1) and 10 µg of alprenolol hydrochloride (peak 2). Chromatographic conditions are given in text.

There was no endogenous peak interfering with the oxprenolol or alprenolol peaks in any case.

A summary of the calibration data is presented in Fig. 3. The calibration curves are linear and pass through the origin. The method allows reliable quantification of 30 ng of oxprenolol in plasma and thus provided a lower sensitivity limit of 10 ng/ml for a 3-ml sample.

Both benzene [4] and a mixture of dichloromethane—diethyl ether in the ratio of 1:4 [2, 3] have been used as the extraction solvent for quantification of oxprenolol. The extraction of oxprenolol from biological fluids using these two solvents and also chloroform, dichloromethane and diethyl ether have now been investigated. The dichloromethane—diethyl ether (1:4) mixture was found to be most suitable as it extracted the least number of endogenous compounds and it gave adequate recoveries for both oxprenolol and alprenolol from all biological fluids studied (Table I). The back extraction into a small volume of aqueous sulphuric acid removes any neutral endogenous compounds that may interfere with the analysis. Also, this step provides adequate concentration of the sample, making time-consuming evaporation steps unnecessary. However, the residual amount of diethyl ether present in the aqueous extract has to be removed prior to injection as it produces a negative signal which interferes with the oxprenolol peak.

TABLE I

RECOVERIES OF OXPRENOLOL AND ALPRENOLOL FROM VARIOUS BIOLOGICAL FLUIDS

Biological fluid	Oxprenolol (average percentage recovery)	Alprenolol (average percentage recovery)
Plasma	83	96
Urine	71	99
Blood	71	85
Milk	63	62

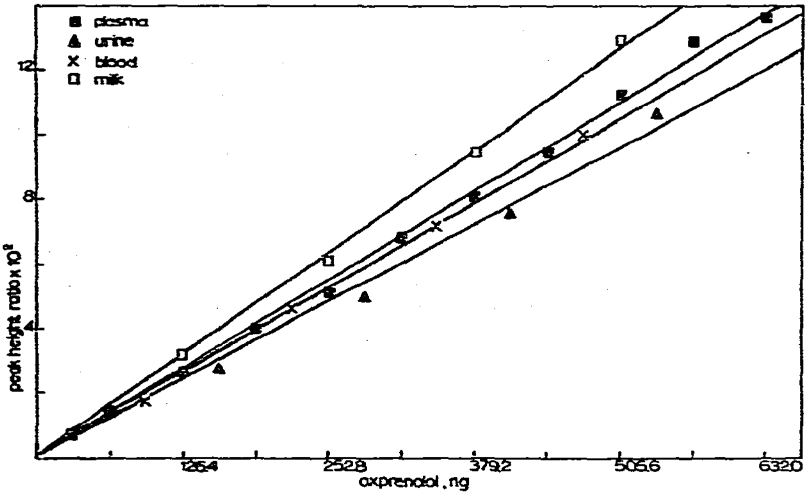

Fig. 3. Calibration curves for oxprenolol in various biological fluids.

Oxprenolol, being a strongly basic compound (pK_a = 9.2) is poorly retained on conventional reversed-phase HPLC systems. In our hands these systems resulted in skewed peaks and they also showed poor separation efficiency. The instability of silica gel at high pH (pH >8) does not allow ion-suppression by increasing pH.

An ion-pairing approach was tried when developing the chromatographic system. Alkylsulphonic acids were used as counter-ions to form neutral complexes with the protonated oxprenolol. The complex, being more lipophilic than oxprenolol, is better retained on a reversed-phase column and therefore gives better peak shape and improved separation. The lipophilicity and thus the retention characteristics of the ion-pair complex are determined by the length of the alkyl chain. Alkylsulphonic acids with pentyl, hexyl, heptyl and octyl side chains were investigated as counter-ions. Symmetrical peak shape as well as resolution of oxprenolol from alprenolol were achieved with all agents used. However, octylsulphonic acid gave the best resolution from peaks related to endogenous substance. When pentylsulphonic acid was used, an endogenous compound with a retention time between oxprenolol and alprenolol appeared thus interfering with both peaks. On changing to alkylsulphonic acids with longer alkyl chains and increasing the methanol concentration to retain the retention of oxprenolol at approximately 6 min, the endogenous peak shifted towards the solvent front. With hexylsulphonic acid, it was completely resolved from the alprenolol peak but merged with the tail end of the oxprenolol peak. With heptylsulphonic acid, the order of elution of the endogenous peak and the oxprenolol peak reversed and the endogenous peak merged with the leading edge of the oxprenolol peak. With octylsulphonic acid, the endogenous compound was resolved from both oxprenolol and alprenolol. The methanol concentration was 67% (v/v) and the octylsulphonic acid was used at a concentration of 0.005 *M*. At the concentration of oxprenolol studied, there was no improvement of the chromatogram observed on doubling the octylsulphonic acid concentration.

Reproducible quantification of oxprenolol was achieved by using alprenolol as the internal standard. Alprenolol was chosen for its close similarity in chemical structure. Other β-blockers, metoprolol and propranolol, were also tried but alprenolol was found to be most satisfactory. The similar chemical and physical properties of alprenolol resulted in acceptable linearity of the calibration data in all biological fluids studied.

The HPLC method is rapid and accurate for the quantification of oxprenolol concentrations in plasma, blood, urine or breast milk. The sample preparation procedure is relatively simple and requires no evaporation or derivatization steps. The total analysis time for a single sample is less than 45 min. Other antihypertensive drugs, namely hydralazine, α-methyldopa and the thiazide diuretics, commonly used with oxprenolol, do not interfere with the analysis.

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