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Note

Determination of pindolol in human plasma by high-performance liquid chromatography with amperometric detection

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Few methods for the assay of pindolol (Fig. 1), a beta-blocking drug, have been published. The first assay was described by Pacha [1]. This fluorimetric method, after reaction with *o*-phthalaldehyde, lacks specificity and sensitivity because interfering substances can be co-extracted from biological fluids causing serious variations in blank values. More recently, an electron-capture gas—liquid chromatographic (GLC) procedure was proposed [2], but this also shows insufficient specificity depending on reagent impurities. Another electron-capture GLC method, using a wall-coated open tubular column, was described by the same author, but a derivatization step is required which can lead to insufficient specificity [3]. A high-performance liquid chromatographic (HPLC) method with fluorescence detection has been proposed [4] but this involves a time-consuming extraction procedure to eliminate interfering substances and the claimed sensitivity of 2 ng/ml of plasma can rarely be attained in routine use. The lack of sensitivity of the HPLC procedure has been recently confirmed [5]. A simple and highly sensitive HPLC method with amperometric detection is reported in this present paper.

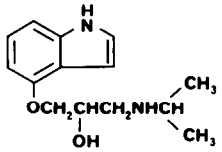

Fig. 1. Structural formula of pindolol.

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EXPERIMENTAL

Reagents

All reagents were of analytical grade. Ethanol was purchased from E. Merck (Darmstadt, F.R.G.); distilled water was HPLC grade; sodium dihydrogen phosphate was obtained from Sigma and perchloric acid from E. Merck; diethyl ether was from E. Merck and pindolol was obtained from Sandoz (Rueil Malmaison, France).

Chromatographic system

A Waters 6000A solvent delivery system fitted with a Rheodyne 7125 sample valve equipped with a 100-μl loop was used in conjunction with a column (15 cm × 3.9 mm I.D.) packed with LiChrospher octadecylsilane (particle size 5 μm) The mobile phase consisted of methanol—0.01 *M* perchloric acid (1:4, v/v) maintained at a flow-rate of 2 ml/min (about 70 bars).

The detector was a Metrohm ELCD system composed of a 641 VA detector, a glassy carbon electrode and an Ag/AgCl reference electrode, fixed at a potential of 1.0 V used at a sensitivity of 50 nA full scale.

Standard solutions and blood sample

The standard stock solution (1 mg/ml) of pindolol was prepared by dissolving the compound in perchloric acid (0.01 *M*); drug-free venous blood was obtained from healthy human subjects. Blood samples were obtained from healthy subjects receiving an oral dose of 15 mg of pindolol. Blood was collected into plastic tubes containing lithium heparin and centrifuged at 2000 *g* for 10 min. Plasma was stored at —20° C until assayed.

Extraction of pindolol and estimation

Plasma (1 ml) was placed into a 15-ml stoppered glass tube, to which were added 0.5 ml of 1 *M* sodium hydroxide and 10 ml of diethyl ether. Pindolol was extracted into the diethyl ether by shaking for 20 min. After centrifugation (2 min at 2000 *g*), 9 ml of the ether layer were transferred to a conical tube containing 200 μl of perchloric acid (0.01 *M*) and pindolol was extracted into the aqueous phase by vortexing the solution for 15 sec. After centrifugation, the aqueous phase was frozen at —20° C and the organic phase was discarded; 100 μl of the acidic phase were injected into the HPLC column. A calibration curve was prepared by treating plasma samples containing known amounts of pindolol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 ng/ml) in the same way as the unknown samples.

RESULTS AND DISCUSSION

Pindolol isolated from plasma gave a retention time of 2.4 min and is well separated from any analytical artefacts (Fig. 2). The pindolol peak height is linearly related to its plasma concentration up to a concentration of 200 ng of pindolol per ml of plasma, as indicated by the high correlation coefficient (r = 0.997) of an eleven-point curve. Variations in the calibration curve from day to day were small, the coefficient of variation of the slope being 6.5%.

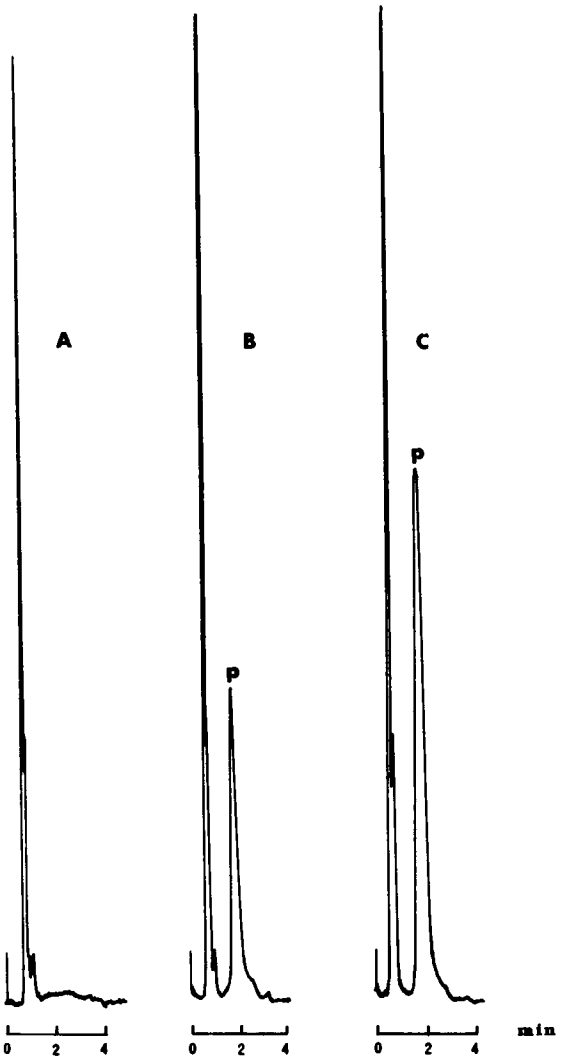

Fig. 2. High-performance liquid chromatograms of extracts from plasma: (A) control drug-free plasma; (B) plasma containing 30 ng/ml pindolol; (C) patient's plasma, 1 h after an oral dose of 15 mg of pindolol (70 ng/ml). p = pindolol.

TABLE I

REPRODUCIBILITY OF THE ASSAY FOR PINDOLOL IN PLASMA

n = 10 at all concentrations.

Pindolol (ng/ml)	Coefficient of variation (%)
1	11.0
10	6.7
80	1.2

Recovery of pindolol from plasma was estimated by comparing the peak height after an injection of pure solution of pindolol with the peak height after the injection of extracted plasma containing the same dose of pindolol. The recovery, at any concentration, was over 95%. The reproducibility of the assay, together with the coefficient of variation, is given in Table I.

The limit of detection of the assay is about 0.5 ng/ml when 1 ml of plasma is used. It did not appear necessary to include an internal standard in the assay.

Plasma samples from patients taking pindolol were analysed. The plasma pindolol concentrations observed in these patients after an oral dose of 15 mg are in agreement with the known pharmacokinetics of this drug and are given in Table II.

TABLE II

PINDOLOL CONCENTRATIONS IN PLASMA OF HEALTHY SUBJECTS AFTER AN ORAL DOSE OF 15 mg OF PINDOLOL

Subject	Pindolol concentration (ng/ml)				
	Time of sampling after dose (h)				
	0	1	3	8	24
1	0	78	40	17	0
2	0	103	67	22	15
3	0	26	15	10	0

In vitro, other drugs were investigated for possible interference: prazosin, chlorothiazide, diltiazem, quinidine, digoxin, propranolol. None of these drugs interfered in the estimation of pindolol.

These results indicate that the HPLC we propose is very sensitive (a three- to four-fold increase in sensitivity over the usual HPLC fluorimetric procedure), and is suitable for use in pharmacokinetic studies and drug monitoring in patients, even for 12-h plasma levels which are frequently less than 2 ng/ml. The method shows good reproducibility, though some care must be taken: it is necessary to clean the working electrode after use to preserve good sensitivity, and it is essential to stabilize the electrode until the response of the detector is constant. In summary, this technique is easy to perform and is inexpensive.

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