Journal of Chromatography, 378 (1986) 163—171
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3075

DETERMINATION OF PINDOLOL IN HUMAN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

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(First received October 25th, 1985; revised manuscript received December 21st, 1985)

SUMMARY

This paper presents a rapid, simple and economical method for assaying pindolol concentrations in plasma and urine by high-performance liquid chromatography using ultraviolet detection. It is sensitive enough for use in single-dose pharmacokinetic studies and may also be used to determine pindolol concentrations in the plasma from patients taking the drug, provided that the patient is not taking any of the drugs which interfere with the method. Drugs which were found to interfere with the pindolol peak are quinidine, *n*-acetylprocainamide and lidocaine. Disopyramide, oxprenolol and levobunolol interfered with the internal standard peak.

0378-4347/86/$03.50

INTRODUCTION

Pindolol is a non-selective β-blocker which has intrinsic sympathomimetic activity and no membrane stabilizing properties [1]. Quantitation of pindolol plasma and urinary concentrations are critical when performing pharmacokinetic and pharmacodynamic studies.

Previously reported techniques for measuring pindolol concentrations in biological fluids include gas chromatography (GC) with electron-capture detection [2, 3], high-performance liquid chromatography (HPLC) with fluorescence [4] and amperometric [5] detection and spectrofluorometric methods with [6] and without [7] reaction with *o*-phthaldehyde. Most of these methods involve tedious sample clean-up procedures or specialized instrumentation.

This paper presents a simple, rapid and economical method for determining pindolol concentrations in plasma and urine samples by HPLC using ultraviolet detection. The method presented here is sensitive enough for use in single-dose pharmacokinetic studies.

EXPERIMENTAL

Apparatus

A Model 2010 high-performance liquid chromatograph equipped with a Model 2050 variable-wavelength ultraviolet detector and fitted with a Rheodyne Model 7125 sample injection valve (Varian Assoc., Walnut Creek, CA, U.S.A.) equipped with a 100-μl loop was used. Analyses were performed on an alkylnitrile column (Micropak CN-10), 30 cm × 4 mm, 10 μm particle size, from Varian Assoc. This normal-phase column, packed in hexane, was converted to a reversed-phase system by rinsing with 100 ml of methylene chloride, followed by 100 ml of acetonitrile—water (50:50) and finally by the mobile phase for pindolol determination. Detector output was recorded at 1 mV with a Beckman recorder (Beckman Instruments, Fullerton, CA, U.S.A.).

Extractions were performed in 13 × 100 mm polypropylene test tubes (Kew Scientific, Columbus, OH, U.S.A.). Other equipment included a reciprocating shaker, vortex mixer, high-speed centrifuge and 16 × 95 mm polypropylene test tubes (Kew Scientific) for preparation and storage of standards and samples.

Reagents

Anhydrous diethyl ether (analytical grade from Mallinckrodt, Paris, KY, U.S.A.) was dried with anhydrous sodium sulfate (analytical grade from Baker, Phillipsburgh, NJ, U.S.A.). Methanol, HPLC grade, was purchased from Mallinckrodt. Carbonate buffer, pH 9.5, consisted of 5.3 g of sodium bicarbonate and 4.2 g of sodium carbonate in 100 ml of water. Phosphate buffer, pH 3.5, consisted of 1.36 g of monobasic potassium phosphate and 100 μl of concentrated phosphoric acid in 1 l of water. Sulfuric acid, analytical grade, was purchased from Baker. The mobile phase consisted of 0.01 *M* monobasic potassium phosphate in water (1.36 g/l), adjusted to pH 2.6 with concentrated phosphoric acid (approximately 0.5 ml). Water used for the preparation of solutions was triple-distilled and stored in glass.

Drug-free plasma was prepared by placing pooled donor plasma (Red Cross, Columbus, OH, U.S.A.) in dialyzer tubing (size No. 36, VWR Scientific, Columbus, OH, U.S.A.) and dialyzing it against a slurry of activated charcoal powder in phosphate buffer for 48 h at 4°C, with constant stirring. The phosphate buffer for stripping plasma consisted of 1.9 g of monobasic potassium phosphate, 8.1 g of dibasic sodium phosphate and 4.11 g sodium chloride per liter of triple-distilled water, adjusted to pH 7.4 with 2 *M* sodium hydroxide.

Formula 963 liquid scintillation cocktail was purchased from New England Nuclear (Boston, MA, U.S.A.). [^{14}C]Pindolol was obtained from Sandoz (Basel, Switzerland).

Drug standards

Pindolol was obtained from Sandoz Pharmaceuticals (East Hanover, NJ, U.S.A.). Alprenolol hydrochloride was obtained from Hassle (Mölndal, Sweden). Stock solutions of pindolol (0.1 mg/ml) and alprenolol (0.87 mg/ml) were prepared by dissolving the compounds in methanol. The pindolol stock solution was stored in a foil-wrapped, glass volumetric flask at 4°C. Stock alprenolol was stored in a polypropylene test tube at 4°C.

The alprenolol stock solution was diluted with water to a concentration of 2.2 μg/ml for use as the internal standard.

Extraction and chromatography conditions

A 0.5-ml volume of plasma or urine (diluted 1:10) was placed in a polypropylene tube, then 0.5 ml of carbonate buffer and 50 μl of the aqueous internal standard solution (109 ng alprenolol) were added. After briefly vortexing the mixture, 3 ml of diethyl ether were added and the mixture was shaken for 10 min. After centrifugation for 4 min at 250 *g* (1500 rpm), the ether layer was transferred to another polypropylene tube containing 100 μl of dilute sulfuric acid (pH 2.2). The mixture was vortexed for 1 min, centrifuged for 4 min at 250 *g* (1500 rpm) and 20—75 μl of the acidic phase were injected onto the HPLC column at a range of 0.0025 absorbance units full scale (a.u.f.s.).

The mobile phase flow-rate was 2.0 ml/min (100 bar). The wavelength of the detector was set at 220 nm. The recorder chart-speed was 0.25 cm/min. All analyses were performed at ambient temperature.

Quantitation

Plasma standards were prepared by serial dilution of the stock pindolol solution to give final concentrations of 3.125, 6.25, 12.5, 25, 50, 75 and 150 ng/ml. The standards were kept frozen in polypropylene tubes, wrapped in foil, and were thawed and extracted daily according to the above extraction procedure. Peak heights were used to quantitate detector response and the peak-height ratio of pindolol to the internal standard was calculated. Two standard curves covering the ranges 3.125—25 and 25—150 ng/ml were used. Drug concentrations of unknown samples having a peak-height ratio higher than that for the 25 ng/ml standard were calculated from the high standard curve. All other unknown sample concentrations were calculated from the low standard curve.

Evaluation of extraction conditions

Choice of extraction tubes. Polypropylene and glass extraction tubes were spiked with drug-free plasma, drug-free phosphate buffer (pH 3.5), plasma and phosphate buffer containing 200 ng/ml unlabeled pindolol plus 3000 dpm of [^{14}C] pindolol (specific activity $2.01 \cdot 10^8$ dpm/mg) to test for binding of pindolol to the tubes. After shaking for 5 min, the solutions were transferred to scintillation vials containing 10 ml of Formula 963 counting cocktail. Control vials contained drug-free plasma and drug-free phosphate buffer plus 3000 dpm of [^{14}C] pindolol in 10 ml of counting cocktail. All vials were counted for 10 min on a Beckman Model LS8100 liquid scintillation counter. Percentage recovery of the label from each tube was calculated by comparing the dpm of the extract to that of the control.

Stability of pindolol in acid medium. Volumes of 50 µl containing pindolol in dilute sulfuric acid (pH 2.2) at a concentration of 200 ng/ml were injected onto the column at 20-min intervals for 2 h at a range of 0.005 a.u.f.s. Peak heights were measured and compared to the peak height for time zero.

Extraction recovery. The extraction efficiency of pindolol and alprenolol from stripped donor plasma and drug-free urine (diluted 1:10 with water) was determined at pindolol concentrations of 50 and 200 ng/ml and alprenolol concentrations of 218 and 873 ng/ml. Only high concentrations were used for urine. Reference solutions were prepared in triple-distilled water and were directly injected onto the HPLC column. Plasma and urine solutions were extracted according to the previously mentioned extraction procedure, except that two thirds of the ether layer was quantitatively transferred, and peak heights were measured. Extraction efficiency (%) was calculated as follows:

$$\text{efficiency} = \frac{(\text{peak height of extract})(3/2)}{\text{peak height of reference}} \times 100$$

Precision

Low and high plasma quality controls were prepared to contain 12.5 and 100 ng/ml pindolol, respectively. Within-day precision was determined by analyzing each quality control ten times on the same day. Aliquots (0.5 ml) of each quality control were stored at −20° C in polypropylene tubes, wrapped in foil. Day-to-day precision was determined by analyzing one aliquot of each quality control per day for ten days over a period of two months. Day-to-day precision was also evaluated by variations in the slope of the standard curve.

Drug interferences

Fifteen drugs were tested for potential interference with pindolol and the internal standard by comparing the retention times of the drugs after direct injection onto the HPLC column.

Application

Serum concentrations of pindolol at various times following the oral administration of 10 mg Viskin® (Sandoz) to a healthy volunteer were measured and fitted to a one-compartment open model [8]. Absorption was assumed to be more rapid than elimination [9]. Pindolol concentrations in

urine collected for 24 h following administration were quantitated, and the renal clearance was calculated by determining the ratio of the 24-h excretion rate of pindolol to the area under the plasma concentration—time curve [9]. Absorption and elimination half-lives and the renal clearance of pindolol were compared to those published previously.

RESULTS AND DISCUSSION

Fig. 1 shows three typical chromatograms for serum and urine. Extracts of drug-free serum showed no interfering peaks (Fig. 1A). The extract of patient serum obtained 2 h following a 10-mg oral dose of pindolol is shown in Fig. 1B. Fig. 1C depicts the extract from a patient urine sample (diluted 1:10 with water) which was collected for 24 h following a 5-mg oral dose of pindolol. The detector response for the patient serum and urine chromatograms was to 116 and 31 ng/ml pindolol, respectively. Retention times of pindolol and alprenolol were 5.5 and 9.5 min, respectively. The limit of sensitivity for pindolol was 1.2 ng, using the criterion of a peak height/noise ratio of 4:1.

Table I lists the retention times of the drugs tested on the system. Quinidine, *n*-acetylprocainamide and lidocaine interfered with the pindolol peak. Disopyramide, oxprenolol and levobunolol interfered with the internal standard peak. Therefore, caution must be exercised when this method is used to quanti-

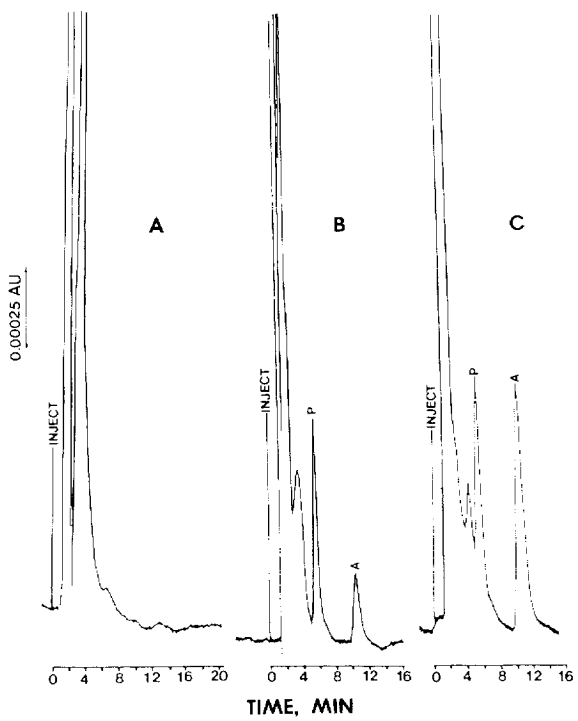

Fig. 1. Typical chromatograms of drug-free serum (A), patient serum 2 h following a single 10-mg oral dose of pindolol (B) and patient urine (diluted 1:10 with water) collected for 24 h following a single 5-mg oral dose of pindolol (C). Peaks: P = pindolol; A = alprenolol (internal standard).

TABLE I

RETENTION TIMES FOR SELECTED DRUGS

Drug	Retention time (min)	Drug	Retention time (min)
Alprenolol	9.5	Pindolol	5.5
Atenolol	2.5	Practolol	3.0
Caffeine	3.5	Procainamide	3.5
Disopyramide	10.5	Pronethalol	12.5
Levobunolol	8.5	Propranolol	25.0
Lidocaine	4.5	Quinidine	5.5
n-Acetylprocainamide	5.0	Sotalol	2.0
Nadolol	3.5	Timolol	1.8
Oxprenolol	10.5		

tate pindolol in plasma and urine of patients taking other drugs. In samples from eleven patients analyzed using this method, no interferences were observed with the internal standard peak.

Urine samples assayed according to the present method were diluted 1:10 with distilled water. Although pindolol peak-height measurements were possible, the pindolol peak was not sufficiently resolved from the solvent front, as depicted in Fig. 1C. Injections of extracts of urine samples diluted 1:100 with distilled water resulted in a pindolol peak which was sufficiently resolved from other peaks. This is only possible if the concentration of pindolol in the urine samples is high enough to allow such a dilution.

Of the radioactivity added to glass and polypropylene tubes, 98% (S.D. = 4.76, range: 90—107%) was recovered and recovery was concentration-independent between 15 and 215 ng/ml. The average (± S.D.) recoveries of radiolabeled pindolol from buffer and plasma (non-extracted) were 98 ± 4.98 and 99.5 ± 4.44%, respectively. These results indicate that pindolol does not bind to either glass or polypropylene tubes.

Peak heights of pindolol measured from replicate injections of pindolol in dilute sulfuric acid (pH 2.2) over a 2-h period remained constant. Peak heights ranged between 39 and 41 mm and no trend with time was observed, indicating that pindolol is stable in dilute sulfuric acid (the final phase of the extraction procedure).

Pindolol and alprenolol extraction efficiencies from plasma were 92 and 97%, respectively, at the low concentrations (Table II). Respective extraction efficiencies of pindolol and alprenolol at high concentrations were 92 and 101% from plasma and 96 and 102% from urine.

Table III shows that the coefficient of variation for within-day precision of pindolol ranged between 6.8 and 7.8% and for day-to-day precision of pindolol over a two-month period ranged between 6.0 and 9.9%. Day-to-day variation in the standard curve was low; the coefficients of variation of the slope were 10.7 and 7.6% for the low and high curves, respectively.

The present method is reproducible as indicated by the results of the within-day and day-to-day precision studies and the day-to-day variability in the slope of the standard curve. New batches of standards were prepared in both stripped donor plasma and human serum albumin [4 g per 100 ml phosphate

TABLE II

ANALYTICAL RECOVERY OF PINDOLOL AND ALPRENOLOL FROM PLASMA AND URINE (n = 4)

Drug	Concentration (ng/ml)	Medium	Extraction efficiency (mean ± S.D.) (%)	Coefficient of variation (%)
Pindolol	50	Plasma	91.92 ± 2.163	2.353
	200	Plasma	91.52 ± 3.623	3.959
	200	Urine	96.14 ± 2.3419	2.436
Alprenolol	218	Plasma	96.47 ± 3.033	3.144
	873	Plasma	100.70 ± 4.752	4.719
	873	Urine	101.60 ± 2.958	2.912

TABLE III

REPRODUCIBILITY OF THE ANALYSIS OF PINDOLOL

Nominal concentration (ng/ml)	Measured concentration (mean ± S.D.) (ng/ml)	Coefficient of variation (%)
Within-day reproducibility (n = 10)		
12.5	10.90 ± 0.7409	6.800
100.0	97.28 ± 7.6280	7.841
Day-to-day reproducibility (n = 10)		
12.5	13.30 ± 1.313	9.875
100.0	102.40 ± 6.126	5.983

buffer (as was used for stripping plasma)] and the slopes of the standard curves from these new batches were not significantly different from the slopes of the curves from the old batch.

Pindolol may be light-sensitive [10] and therefore, all pindolol solutions were kept wrapped in foil. No trend was evident in the concentrations of the quality controls over the two-month period, indicating that no degradation of pindolol occurred. Pindolol, therefore, is stable when stored in the refrigerator or freezer, wrapped in foil, for at least two months.

Fig. 2 shows that use of a one-compartment model to fit the plasma concentration—time data following the administration of a 10-mg oral dose to a healthy volunteer was reasonable. The least-squares estimates (mean ± S.D.) of the absorption and elimination rate constants were 2.39 ± 0.671 and 0.236 ± 0.032 h^{-1}, respectively. These correspond to absorption and elimination half-lives of 0.290 and 2.94 h, respectively, and are in excellent agreement with published values in healthy volunteers [11—13]. The renal clearance of pindolol in this subject was 185 ml/min, and agreed with published values [11]. These data suggest that this method is reliable for performing pharmacokinetic studies of pindolol.

Spectrofluorometric methods for pindolol determination were developed

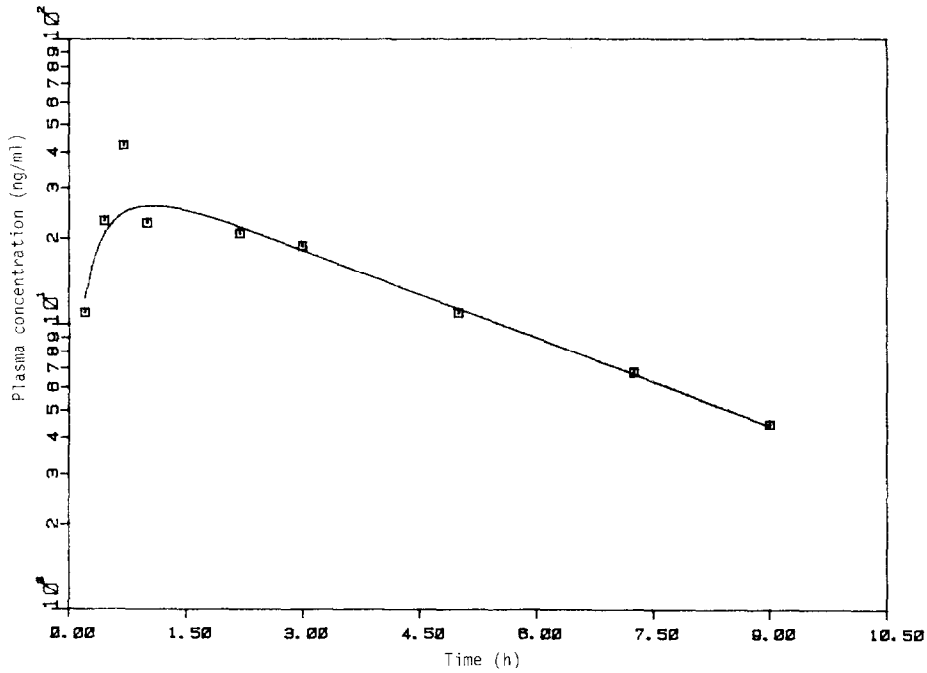

Fig. 2. Plasma concentrations of pindolol (□) at various times following the oral administration of a 10-mg tablet in a healthy volunteer. The symbols were measured, the line was computer fitted.

by Mohamed et al. [6] and Pacha [7]. The method of Mohamed et al. [6] was not used for plasma or urine samples. The method of Pacha [7] involves reaction with *o*-phthaldehyde and a tedious sample preparation. This method [7] allows detection of 20 ng of pindolol when 1—4 ml of plasma or 5 ml of urine are extracted and is not sensitive enough for use in single-dose pharmacokinetic studies.

The electron-capture detection—GC methods [2, 3] were sensitive enough to be used in pharmacokinetic studies, but involved special glassware clean-up procedures, tedious extraction procedures and required derivatization.

Lefebvre et al. [14] developed a reversed-phase HPLC method for pindolol with ultraviolet detection, and concluded that the method is not sensitive enough for plasma level determinations. The present method achieves a sensitivity of 3.125 ng/ml when 0.5 ml of plasma is extracted, whereas Lefebvre et al. [14] were only able to achieve a sensitivity of 20 ng/ml when 1 ml of plasma was extracted. The greater sensitivity with the present method is most likely attributed to the use of a variable-wavelength detector, optimized at 220 nm, rather than a fixed-wavelength detector with a 280-nm filter [14]. Differences in column efficiencies (C_{18} used by Lefebvre et al. [14], CN in the present paper) is less likely to explain the different sensitivities as the particle size of the two columns is the same.

Bangah et al. [4] developed an HPLC method with fluorescence detection for pindolol which has adequate sensitivity, but requires four times the amount of sample as the present method. It also involves a tedious extraction procedure

and does not have an internal standard, thus necessitating the use of measured transfers.

The HPLC method of Diquet et al. [5] using amperometric detection involves an extraction procedure similar to that of the present method; however, no internal standard was used. This necessitates the use of measured transfers. They reported no interferences with pindolol, but they did not test the drugs that were found to interfere with pindolol in the present method. The sensitivity of both methods is similar.

The present method has the following advantages over the other methods: it uses ultraviolet detection, requires a smaller sample volume for extraction and is more economical because less extracting solvent is required and no organic solvent is used in the mobile phase. If the analyst so desires, analysis time may be shortened to 6 min by eliminating the internal standard. Use of an internal standard, however, makes the extraction procedure easier and faster and overcomes the difficulty of measured transfers caused by the volatility of diethyl ether.

ACKNOWLEDGEMENTS

Supported by the National Institutes of Health (Grant No. GCRC-34), the S.J. Roessler Research Foundation and the Central Ohio Heart Chapter of the American Heart Association.

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