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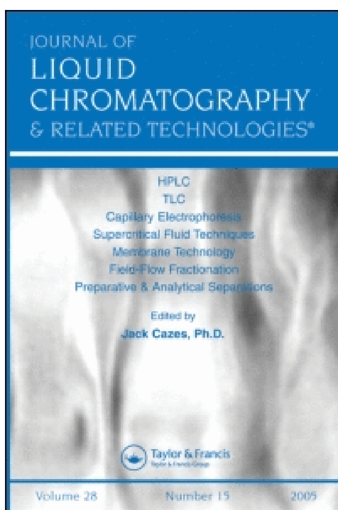

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MICROBORE LIQUID CHROMATOGRAPHY FOR ANALYSIS OF PLASMA PINDOLOL CONCENTRATIONS

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ABSTRACT

Isocratic high-performance liquid chromatographic assay of pindolol with use of a microbore column was developed. The sample preparation involves extraction of alkalized plasma into ether and back extraction into 0.05 N H_2SO_4. Metoprolol was used as the internal standard. Chromatographic separation is performed on a microbore C18 (5 µm) column using acetonitrile-disodium hydrogenphosphate buffer (37:63) containing 20 mM sodium dodecyl sulfate as the mobile phase. The detection is achieved by using a fluorescence detector operated at the excitation and emission wavelengths of 260 and 310 nm, respectively. Acceptable reproducibility and accuracy data are presented over the concentration range normally encountered in human plasma samples. The lower detection limit is 2.5 ng/ml. This sensitivity has been found to be adequate for routine analysis of pindolol in human plasma samples, making the method applicable to pharmacokinetic studies and clinical trials.

INTRODUCTION

Pindolol, *d,l*-4-(2-hydroxy-3-isopropylaminopropoxy) indole is a potent β-adrenoceptor antagonist used clinically in the treatment of hypertension and angina pectoris (1,2). It possesses a high bioavailability and low first-pass effect as compared to other β–adrenoceptor blocking agents, so low effective daily doses

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ranging from 10-45 mg are possible (3,4). As the doses administered are very low and the elimination half-life is comparably short (1-3 hours), sensitive assays are necessary for the determination of pindolol in plasma when performing pharmacokinetic and pharmacodynamic studies.

Several high-performance liquid chromatographic (HPLC) methods have been published for the assay of pindolol in human plasma (5-8). Most of these methods involve tedious sample clean-up procedures, require large samples and some use specialised instruments. This paper reports a simple, rapid and economical HPLC procedure with fluorescence detection. The assay provides sufficient sensitivity for the monitoring of plasma pindolol concentrations following single or chronic therapeutic doses of pindolol. The present method has been used routinely for the analysis of pindolol in human plasma samples in bioavailability studies demonstrating its reliability and reproducibility.

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MATERIALS AND METHODS

Reagents and Chemicals

HPLC-grade acetonitrile, sodium dodecyl sulfate (SDS), orthophosphoric acid and diethyl ether were purchased from BDH Chemicals Limited (Poole, UK). Pindolol and metoprolol succinate were kindly provided by Pacific Pharmaceutics Limited (Auckland, New Zealand). All other chemicals were of analytical grade. Water was double glass distilled and filtered (MilliQ). Glassware was silanized with Aquasil®, (Pierce Chemical Co., Rockford, IL, USA) before use.

Reference Solutions and Internal Standard

Metoprolol was used as the internal standard for the pindolol assay. Pindolol stock solution, 1 mg/ml was prepared by dissolving the drug in methanol. Internal standard stock solution (1 mg/ml metoprolol) was also prepared in methanol. These solutions were stored at -20°C until required and were found to be stable for at least one week.

Sample Preparation

Blood samples were taken by venipuncture into lithium heparin tubes and plasma was separated by centrifugation. Plasma samples were stored at -70°C until analysis. Aliquots (1 ml) of plasma were placed in 10-ml polytetrafluoroethylene

lined screw-capped glass tubes. The samples were alkalized by addition of 150 µl of 1 N NaOH. The internal standard solution (100 µl of 1 µg/ml metoprolol in water) was added. The contents were shaken gently with 6 ml of diethyl ether for 10 min to avoid emulsion formation. The samples were centrifuged at 3000g for 5 min at 4°C. The organic layer was transferred into a 10-ml conical glass tube and 250 µl of 0.05 N H_2SO_4 was added. The tube was shaken for 10 min and centrifuged for 5 min. The upper organic layer was carefully discarded by aspiration using a Pasteur pipette. Approximately 120 µl of the acidic phase was introduced into a 0.25 ml polyethylene tube of the autosampler and 50 µl was injected onto the HPLC column.

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Chromatographic Conditions

The HPLC system used in this study included a model 250 Perkin Elmer pump, (Perkin Elmer Corporation, Norwalk, CT, USA), equipped with a Waters® WISP 712 autosampler, (Waters Assoc., Milford, MA, USA), a variable wavelength fluorescence detector, a F-1050 Hitachi fluorescence spectrophotometer (Hitachi, Tokyo, Japan) and a Hitachi D-2500 integrator. A C18 reversed-phase microbore column (2 mm I.D. x 100 mm) slurry packed with 5 µm ODS Hypersil (Shandon, London, UK) was used. The mobile phase consisted of acetonitrile-water (37:63, % v/v) containing 10 mM Na_2HPO_4 and 20 mM SDS. The pH of the mobile phase was adjusted to 2 with orthophosphoric acid. The flow rate was 0.5 ml/min with a column back pressure of 1300 psi at room temperature. The excitation and emission wavelengths of the fluorescence detector were set at 260 and 310 nm, respectively.

Calibration

Calibration standards were prepared by adding 100-500 µl of appropriately diluted solution of pindolol in water to 10 ml of drug-free plasma. These produced plasma standards with concentrations of pindolol ranging from 5 to 200 ng/ml. The standard curve was constructed by the linear least-squares regression analysis of peak height ratios (pindolol/internal standard) vs pindolol concentrations.

RESULTS AND DISCUSSION

Pindolol is a basic drug with a pKa of 8.8 and is ionized in the acidic and neutral solvents commonly used in reversed-phase HPLC. Chromatographic retention and

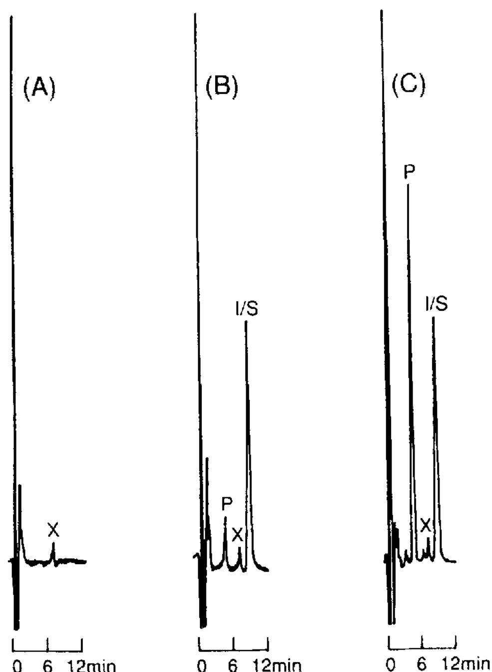

FIGURE 1
High-performance liquid chromatograms of extracts from human plasma. (A) Control drug-free plasma (i.e. blank plasma); (B) plasma standard spiked with 5 ng/ml pindolol; (C) subject's plasma 2 h after a single 30 mg oral dose of pindolol (concentration = 103 ng/ml). Peaks: P = pindolol; x = plasma endogenous peak; I/S = internal standard, metoprolol.

separation of pindolol and the internal standard metoprolol (pKa 9.7) was achieved by the addition of the anionic hydrophobic pairing ion sodium dodecyl sulfate (SDS), to the mobile phase at low pH. A mobile phase of an acetonitrile-aqueous phosphate buffer (10 mM, pH2) mixture (37:63, % v/v) containing 20 mM SDS was chosen as it provides a good resolution between pindolol and the internal standard. With these chromatographic conditions, retention times of pindolol and the internal standard (metoprolol) were 5.1 and 9.4 min, respectively.

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Pindolol and the internal standard are isolated from plasma and are are well separated from any analytical artefacts (Figure 1). No interfering peaks have been detected in the plasma used for blank standards or from subjects prior to administration of the drug. Some plasma samples showed a small peak with a retention time of 7.5 min which does not interfere with either pindolol or the internal standard peak.

Absolute recovery was assessed by comparing the peaks of pindolol and the internal standard with those obtained by direct injection of equivalent quantities of the pure drug in water. The mean recovery for pindolol (n = 4) from plasma was 79 ± 12% (S.D.) at 5 ng/ml and 73 ± 3% at 100 ng/ml. The absolute recovery for the internal standard metoprolol was 82 ± 9% (n = 4).

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Daily calibration curves (n = 5) for pindolol in plasma resulted in a linear concentration-response relationship over the concentration range tested, i.e. 5 to 200 ng/ml. The correlation coefficients of the lines were >0.99. A typical least-squares linear regression of the peak height ratio (y) versus concentration (x) gave y = 0.0141 x, with the y-intercept (a) being insignificant. The analytical system using a different column (with the same stationary phase) showed remarkable stability. The day-to-day coefficient of variation (C.V.) of the slope of several calibration curves (n = 14) was 7.5% over one month.

The precision and accuracy of the assay was evaluated for three concentrations of pindolol (5, 100 and 200 ng/ml) by analysing each one four times on the same day. The intra-assay C.V. for pindolol were 6.9% at 5 ng/ml, 3.6% at 100 ng/ml and 2.6% at 200 ng/ml. Between day variation of the assay was assessed by re-analysing at random selected subject samples (n = 24). The results indicate that the accuracy of the assay for pindolol was greater than 90% (mean ± S.D. = 98.9 ± 8.5) with C.V. of 8.6%.

The predicted value of five 2.5 ng/ml plasma pindolol standards gave values of 2.1, 2.6, 2.4, 2.0 and 2.7 ng/ml. This data gives a mean and S.D. of 2.4 ± 0.3 ng/ml with a C.V. of 12.9%. This C.V. is much lower than the generally accepted C.V. of 20% for minimum quantifiable concentration (MQC). Thus the MQC or detection limit of sensitivity for this assay was assigned at 2.5 ng/ml.

TABLE 1

Mean Plasma Pindolol Concentrations After a Single Oral Dose of 30 mg Visken® in Twelve Subjects

Time after dose (h)	Concentration (mean ± S.D.) (ng/ml)
0.0	0
0.25	54.0 ± 28.5
0.5	108.7 ± 68.7
1.0	115.5 ± 50.6
1.5	106.3 ± 52.6
2.0	94.5 ± 50.9
2.5	88.5 ± 44.8
3.0	83.9 ± 42.2
3.5	77.4 ± 41.6
4.0	70.9 ± 38.3
6.0	49.7 ± 31.8
9.0	30.3 ± 21.4
12.0	15.5 ± 10.8
16.0	7.2. ± 6.7
20.0	3.8 ± 2.8
24.0	2.4 ± 3.0

Area under the curve, AUC from 0 to ∞ (mean ± S.D.) = 830 $ng.h.ml^{-1}$
Elimination half-life (mean ± S.D.) = 3.7 ± 0.9 h

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Plasma samples frozen at -70°C for up to three months showed no signs of decomposition, and practically the similar concentration values were obtained (n = 6). This suggests that pindolol is stable in human plasma under these storage conditions for at least three months.

As pindolol and the internal standard (metoprolol) are basic drugs, the nature of the extraction is such that acidic and neutral drugs are not expected to interfere with this assay. Several basic drugs with native fluorescence were tested to determine possible interference. None of the drugs tested had a retention time which overlapped with those of pindolol and metoprolol. The retention time was either less than 4.5 min (morphine, atenolol, practolol, sotolol) or greater than 10 min (propranolol, alprenolol, desipramine, labetalol, quinidine, quinine and verapamil).

The applicability of the method was tested by analysing human plasma samples from volunteers taking part in pindolol bioavailability studies. Approximately 900 plasma samples have been analysed using this procedure. In one study, twelve male volunteers (age 20-30 years) were given 30 mg of pindolol (Visken®, The Sandoz Pharma Ltd, New Zealand) and serial blood samples were drawn to determine post-dosing plasma pindolol concentrations. The majority of the subjects had plasma pindolol concentrations above the detection limit of this assay (2.5 ng/ml) and could be followed for 4-5 half-lives. Table 1 shows the mean plasma pindolol concentrations in the twelve subjects after the oral dose of 30 mg pindolol (Visken®) together with the pharmacokinetic parameters obtained.

In summary, the method described here employs a relatively simple sample preparation, maintenance-free and selective fluorescence detection and provides sufficient sensitivity for the determination of pindolol in plasma. The assay has been shown to be suitable for use in pharmacokinetic studies and clinical trials. With the use of a 2 mm I.D. microbore column, the cost of acetonitrile used in the mobile phase can be substantially reduced. It also provides greater sensitivity with no significant effect of band broadening or diffusion on the quality of the observed chromatography.

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