Pergamon

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 3, pp. 425–427, 1994

0731-7085/94 $6.00 + 0.00

Short Communication

Determination of disopyramide in plasma by high-performance liquid chromatography

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Keywords: *Disopyramide; HPLC determination; plasma.*

Introduction

Disopyramide [4-di-isopropylamino-2-phenyl-2-(2-pyridyl)-butyramide] is frequently used in the suppression and prevention of cardiac arrhythmias [1]. Because of the relatively narrow therapeutic index and the intersubject variability in the pharmacokinetics of this drug, monitoring of its concentration in plasma may assist in individualizing the dosage requirements. Chromatographic methods have been developed for the quantitation of disopyramide in biological material; the main method was HPLC [2–15] although gas chromatography (GLC) has also been used. However, GLC requires relatively complex and time-consuming sample preparation, including a complicated derivatization. Most HPLC methods with UV monitoring of the eluent at 254 nm [2–4, 9, 10, 13] had poor sensitivity and lacked specificity owing to interference by other drugs. Two of the published HPLC methods required expensive, sophisticated columns and were used for separation of the *R* and *S* enantiomers [6, 11] and not for routine measurements in the clinical laboratory. The major disadvantages of some other procedures are the lack of an internal standard, a relatively moderate recovery of about 73% disopyramide or a time-consuming extraction procedure.

The extraction of disopyramide from human plasma has been examined and a rapid, selective, accurate and simple HPLC assay has been developed for determining this drug in plasma. The internal standard employed, procaine hydrochloride, is readily available; this substance is structurally dissimilar from disopyramide but has similar solubility and chromatographic and absorptive characteristics.

Experimental

Substances, reagents and apparatus

Disopyramide phosphate and procaine hydrochloride (internal standard) were obtained from Pharmaceutical Works 'Polfa' (Poland). All reagents and solvents were analytical grade; ethyl ether was distilled before use. A liquid chromatograph (model 730, Laboratorni Přistroje Praha, CSFR) with an injection valve fitted with a 20-μl sample loop was used in conjunction with a variable wavelength spectrophotometric detector.

HPLC assay for quantitation of disopyramide

Chromatographic conditions. A 250 × 4 mm i.d. column packed with 10-μm LiChrosorb RP-18 was used at ambient temperature. The mobile phase was acetonitrile–methanol–phosphate buffer (pH 3.2); (6:1:3, v/v/v). The flow rate was 1.0 ml min^{-1} and the column effluent was monitored at 269 nm at a sensitivity of 0.02 aufs.

Calibration standards. Stock methanolic solution containing 0.1 mg ml^{-1} of disopyramide phosphate and 0.1 mg ml^{-1} of procaine hydrochloride (internal standard) was prepared. Working solutions were prepared by pipetting volumes of disopyramide stock sol-

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ution (corresponding to 40–240 μg of drug) and a fixed volume of stock internal standard into a 10-ml volumetric flask and diluting to volume with methanol.

Twenty-microlitre volumes of each solution were injected on to the column. Calibration graphs were constructed by plotting the peak-height ratio of disopyramide to procaine (h/h') versus the mass of the drug in 20 μl of each standard solution and they were calculated by the least-squares regression method. The correlation coefficient was 0.9998 indicating a high degree of linearity in the calibration curve over the concentration range studied. The regression equation for standard solutions was:

$$y = 2.41x + 0.012,$$

where $y = h/h'$ and x = amount of dysopyramide (μg) in 20 μl.

Plasma sample preparation and analysis

Each plasma sample (2 ml) was mixed in a centrifuge tube with 1.0 ml of methanolic solutions of disopyramide phosphate containing 2–12 μg of drug, 1.0 ml of methanolic solution of procaine hydrochloride (= 1.45 μg of substance) and 2.0 ml of methanol. After centrifugation for 10 min at 1100*g*, 3.0 ml of supernatant from each tube was made alkaline with 2 ml of 0.01 M NaOH and extracted with two-5-ml portions of ethyl ether. The separation of the phases was carried out by freezing of an aqueous solution in a cooling bath (−80°C). The dried ether extracts were evaporated under nitrogen and the residues were dissolved in 200 μl of mobile phase.

Twenty-microlitre volumes of each solution were submitted to HPLC. For the calculation of disopyramide the peak-height ratio of disopyramide to procaine (h/h') was plotted versus the mass of the drug in 20 μl. The regression equation for plasma samples spiked with disopyramide was:

$$y = 4.72x + 0.17,$$

where $y = h/h'$, and x = amount of disopyramide (μg) in 20 ml. The correlation coefficient was 0.9882.

Results and Discussion

An extraction of disopyramide from plasma was carried out from basic medium (pH about 9.5) with ethyl ether, which was the best solvent of those examined (chloroform, dichloromethane, hexane and heptane with 1–2% (v/v) isopropanol).

For quantitative determination of disopyramide in plasma, HPLC method was used with UV spectrophotometric detection at 269 nm, the λ_{max} of the drug. The recovery of the extraction of the drug was estimated by comparing peak heights obtained by injections of standard solutions to those obtained by extracts of plasma spiked with disopyramide and internal standard (procaine). The recovery was 89.4–100% (mean 96.2%) for disopyramide. The recovery of the internal standard at a concentration of 0.72 μg ml^{-1} was 98.7 ± 3.2% (n = 9). The retention times were 6.08 min for disopyramide and 4.48 min for procaine; thus the separation of the peaks was good and the time of the analysis was not too long.

In certain instances, where it is known for certain that other drugs in the unknown sample are absent, the elution process can be speeded by increasing the acetonitrile content of the mobile phase; however, if the absence of other drugs is not certain, the elution parameters should not be changed.

Some drugs which may be given concomitantly with disopyramide, or in place of disopyramide, in cardiac arrhythmias were tested for interference in the assay. The drugs tested included amiodarone, the cardiac glycosides digitoxin and digoxin, dipyridamole, flecainide acetate, lignocaine (lidocaine) HCl, lorcainide HCl, procainamide HCl, propranolol HCl, quinidine sulphate and verapamil. These drugs were tested by direct injection into the liquid chromatograph and their retention times (if an additional peak appeared) were compared with those of disopyramide and the internal standard. The drugs did not show any interference with the disopyramide assay, because of either a different retention time or very little UV absorption at 269 nm.

The proposed method is simple, selective and accurate because of the reproducible chromatographic elution system, the judicious choice of internal standard and the simple extraction procedure. The range 1–6 μg of the drug per ml of plasma was chosen since the blood concentration of disopyramide after the usual dose of the drug is 2–5 μg ml^{-1} [16]; linearity in this range makes the method useful for routine determination of the drug in blood,

e.g. for therapeutic monitoring in individual patients.

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[*Received for review 27 July 1993*]