Short Communication

Simplified, rapid and inexpensive extraction procedure for a high-performance liquid chromatographic method for determination of disopyramide and its main metabolite mono-N-dealkylated disopyramide in serum

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ABSTRACT

A simplified, rapid and inexpensive extraction procedure for the determination of the antiarrhythmic drug disopyramide and its main metabolite mono-N-desalkylated disopyramide in serum by high-performance liquid chromatography has been developed. The analysis uses ultraviolet detection at 254 nm, and a 5 μm reversed-phase column with a mobile phase of water–triethylamine–acetonitrile–PIC-B8 reagent. Serum extraction is performed with dichloromethane and 1 M sodium hydroxide. p-Chlorodisopyramide is used as internal standard. Recovery rates were 94.5% (S.D. 5.7%) for disopyramide, 96.8% (S.D. 2.2%) for mono-N-desalkylated disopyramide and 97.9% (S.D. 2.8%) for the internal standard.

INTRODUCTION

Disopyramide is a widely used antiarrhythmic drug with class Ia activity, according to the Vaughan and Williams classification [1,2]. In clinical trials it has been shown to be effective for treatment of atrial fibrillation [3–5] and ventricular arrhythmias [4,6–11]. Although successful treatment of arrhythmias may not be directly correlated with serum levels, determination of serum levels is helpful to check compliance, especially in out-patient treatment, when side-effects occur, and to exclude sub-optimal dosing, especially in oral therapy.

Serum concentrations of mono-N-desalkylated disopyramide (MND) reach *ca.* 30% of the disopyramide serum concentration during long-term oral therapy [12] and are increased by enzyme induction or renal failure [12,13]. As MND is reported to have a lower antiarrhythmic activity but marked anticholinergic activity [13], determination of MND seems to be useful, when anticholinergic side-

effects, such as reduced salivation, accommodation disturbances or prostation occur.

The aim of the present study was to develop a rapid, inexpensive and precise method for determination of serum concentrations of disopyramide and MND.

EXPERIMENTAL

Chemicals and reagents

Disopyramide, MND and *p*-chlorodisopyramide were provided by Albert Roussel Pharma (Wiesbaden, Germany). Acetonitrile, hydrochloric acid, triethylamine, dichloromethane and sodium hydroxide were obtained from Merck (Darmstadt, Germany). 1-Octanesulphonic acid (5 M, PIC-B8 reagent) was purchased from Waters Assoc. (Eschborn, Germany).

Instruments

The high-performance liquid chromatographic (HPLC) system consisted of a Model 721 system controller, a data module Model 730, an HPLC pump, a WISP 710B injector block and a Lambda-Max Model 481 variable-wavelength detector (all from Waters Assoc.).

Extraction procedure

A 2.0-ml volume of dichloromethane, 1.0 ml of serum, 100 μl of 1 M sodium hydroxide and 100 μl of the stock solution of the internal standard were introduced into a glass centrifuge tube, obtained from Kästner (Tübingen, Germany). The tube was stoppered with a plastic stopper, obtained from Sarstedt (Nümbrecht, Germany), vortex-mixed for 60 s and centrifuged at 3600 g for 10 min. Subsequently the aqueous phase was discarded, and 1.0 ml of the dichloromethane phase was transferred to a clean glass tube and evaporated to dryness at room temperature under nitrogen. The residue was dissolved in 500 μl of 0.01 M hydrochloric acid, and 200 μl were injected into the column.

Standards

The stock standard solutions were prepared by dissolving 3 mg of disopyramide, 3 mg of MND and 3 mg of *p*-chlorodisopyramide each in 100 ml of 0.01 M hydrochloric acid. In this way stock standard solutions, containing 30 μg/ml of each substance, were obtained, which proved to be stable for at least two months at 4°C. A working standard solution was prepared by combining 100 μl of each stock standard solution with 700 μl of 0.01 M hydrochloric acid. This working standard solution contained disopyramide, MND and the internal standard *p*-chlorodisopyramide in concentrations of 3 μg/ml each.

Detection wavelength

To find the optimal detection wavelength, UV absorption spectral analysis was performed over a wavelength range of 200–500 nm with the stock standard solutions of disopyramide and MND. Both substances showed a maximum of absorption at 254 nm.

Chromatographic conditions

The analysis was performed at room temperature using a Shandon ODS reversed-phase column (125 mm × 4.6 mm I.D., particle size 5 μm). The mobile phase consisted of 70% A (974 ml of water, 25 ml of PIC-B8 reagent and 1 ml of triethylamine) and 30% B (acetonitrile). The concentration of 1-octanesulphonic acid in part A of the mobile phase was 0.0125 M. The flow-rate was 1.0 ml/min. The column effluent was monitored at 254 nm, using a detector or range of 0.02 a.u.f.s. and a chart-speed of 0.5 cm/min. The injection volume was 200 μl.

Lower limit of detection

The lower limit of detection was determined by diluting the working standard solution to concentrations of 1.0, 0.5, 0.25, 0.1 and 0.05 μg/ml for disopyramide, MND and the internal standard. A signal-to-noise ratio of 3:1 was set as the limit for detection of a peak.

Recovery rates

Recovery rates were determined by comparing the peak areas of disopyramide, MND and the internal standard, obtained by analysing ten spiked serum samples (100 μl of each stock solution, added to 1.0 ml of normal pool serum), with peak areas obtained by direct injection of the standard working solution.

Technical error

The technical error was determined by injecting the same sample ten times. Ten peak areas were compared and given as percentage values.

Linearity test

The linear relationship of peak areas to substance concentrations was demonstrated by analysing five standard solutions, containing concentrations of 6.00, 3.00, 1.50, 0.75 and 0.375 μg/ml disopyramide, MND and the internal standard. Peak areas were plotted *versus* substance concentrations in orthogonal scales and resulted in a linear curve.

The same procedure was performed for spiked serum samples, containing similar concentrations of disopyramide, MND and the internal standard.

Quantification

Because a linear relationship between substance concentrations and peak areas was obtained, no daily calibration graph was necessary. Calculation was per-

formed by comparing peak areas of the standard working solution (analysed before every serum sample) to peak areas of serum samples, obtained by the described extraction procedure.

Sample collection

Blood was taken by venous puncture of a cubital vein, and serum was obtained by centrifugation at 900 g, avoiding haemolysis. Twenty-two serum samples from normal volunteers (twelve male, ten female) were pooled (normal pool). This drug-free normal pool serum was used for blank chromatograms and for preparing spiked serum samples for the linearity test and for the recovery rate.

Eleven undiluted serum samples from patients treated with cardiovascular drugs, such as acetylsalicylic acid, heparin, phenprocoumone, nifedipine, diltiazem, verapamil, nitrendipine, betaxolol, metoprolol, isosorbide mononitrate, furosemide, xipamide, spironolactone, hydrochlorothiazide, triamterene, allopurinol, bezafibrate, enalapril, captopril, digitoxin, metildigoxin, digoxin and diazepam, were analysed with the above described assay.

Fifteen serum samples from fifteen patients (three female, twelve male) undergoing chronic oral therapy (at least five days) with two 250-mg sustained-release tablets per day were analysed individually. Samples were taken as trough levels at 8:00 a.m. before the morning dose was taken. None of the patients had signs of congestive heart failure or kidney or liver dysfunction.

RESULTS

A linear relationship between the concentration of the substances and the peak areas for the standards, as well as for spiked serum samples, could be demonstrated. The mean recovery rates were 94.5 ± 5.7% for disopyramide (coefficient of variation, C.V. 6.03%), 96.8 ± 2.2% for MND (C.V. 2.27%) and 97.9 ± 2.8% for the internal standard (C.V. 2.86%).

The inter-assay C.V. (n = 10) were 6.0% for disopyramide and 2.3% for MND. The intra-assay C.V. (n = 10) were 2.7% for disopyramide and 1.4% for MND. The technical error of the apparatus was less than 1% for disopyramide, MND and the internal standard. Disopyramide serum levels were 2.00 ± 0.85 μg/ml and MND levels were 0.60 ± 0.33 μg/ml, in fifteen patients taking 270 ± 14 mg of disopyramide per square metre of body surface orally (data given as mean ± S.D.).

None of the above-mentioned drugs showed interference with the determination of disopyramide. There was no significant correlation either between dosage per body surface area or serum concentrations of disopyramide or MND in fifteen patients during chronic oral drug treatment. Serum concentrations of MND were 23.7% (S.D. 10.4%) of disopyramide serum concentrations.

Typical chromatograms obtained from the analysis of a blank from the normal pool, a standard working solution and a serum of a patient taking disopyramide
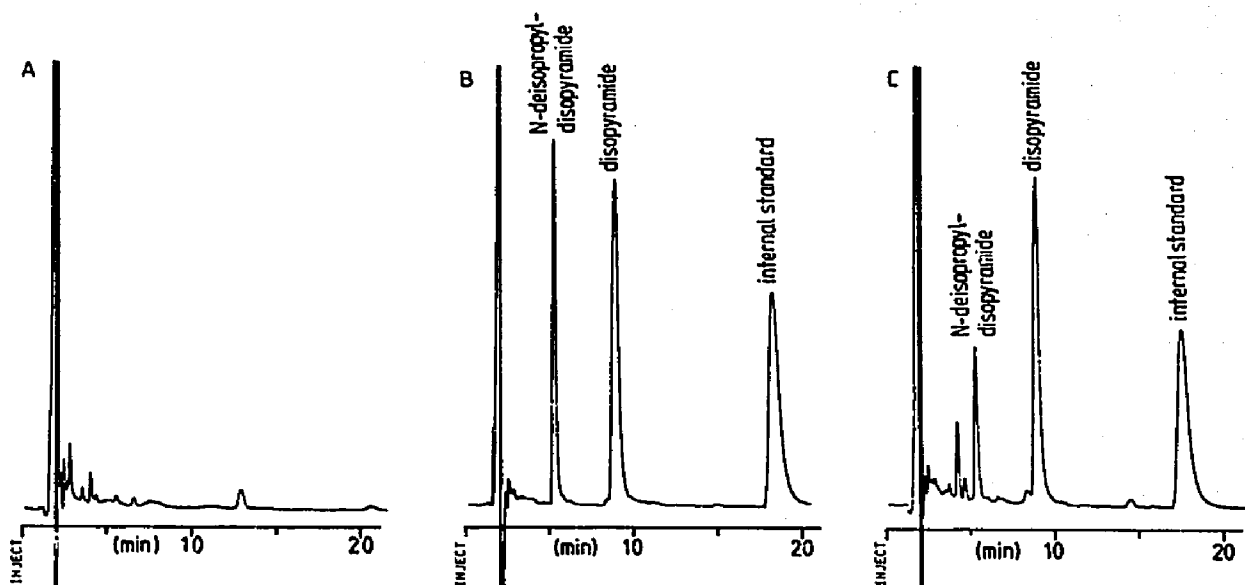
Fig. 1. Chromatograms of a blank of the normal pool (A), of a standard working solution (B) and of the serum of a patient receiving disopyramide (C).
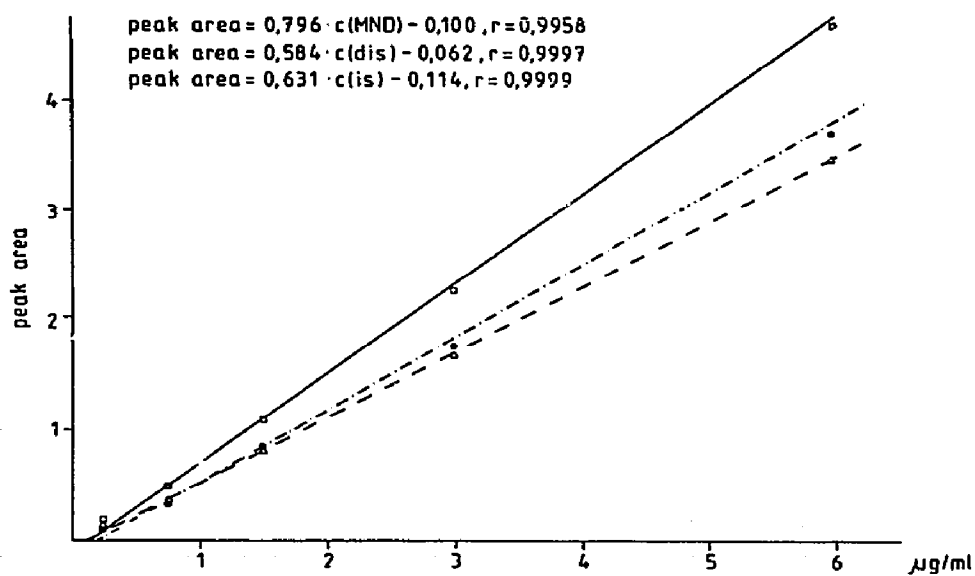

peak area = 0,796·c(MND) - 0,100, r = 0,9958
peak area = 0,584·c(dis) - 0,062, r = 0,9997
peak area = 0,631·c(is) - 0,114, r = 0,9999

Fig. 2. Linearity test for ($\triangle$) disopyramide, ($\square$) MND and ($\bullet$) the internal standard in the standard solution. c(MND) = concentration of MND; c(dis) = concentration of disopyramide; c(is) = concentration of internal standard.

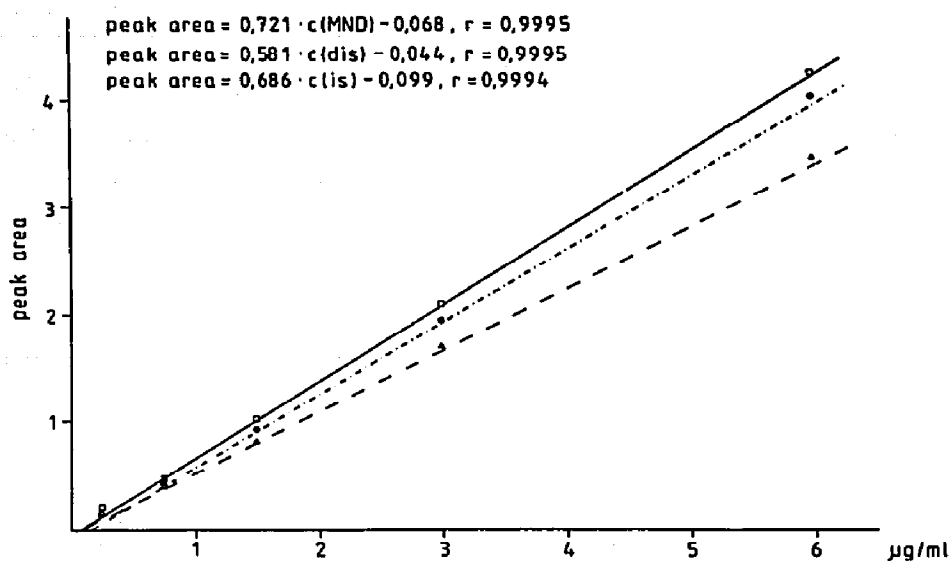peak area = 0,721 · c(MND) - 0,068 , r = 0,9995
peak area = 0,581 · c(dis) - 0,044 , r = 0,9995
peak area = 0,686 · c(is) - 0,099 , r = 0,9994

Fig. 3. Linearity test for (△) disopyramide, (▢) MND and (●) the internal standard in a spiked serum sample. c(MND) = concentration of MND; c(dis) = concentration of disopyramide; c(is) = concentration of internal standard.

are shown in Fig. 1. Linearity tests on the standard solution and on a spiked serum sample are demonstrated in Figs. 2 and 3.

DISCUSSION

Gas chromatographic methods have proved to be highly specific and sensitive for measuring serum levels of disapyramide [14–19], but have the disadvantage that the samples have to be derivatized and therefore the analyses are more time-consuming.

Immunological methods for measuring disopyramide levels with monoclonal antibodies have been developed [20–23] but quantification of MND is not possible.

A variety of HPLC methods with UV detection for determination of disopyramide have been published [16,24–33]. Some give essentially lower analytical recovery rates for disopyramide or its main metabolite MND [26,29,30]. Without an internal standardization some methods need a time-consuming calibration graph to be prepared before every analysis [27,28]. Ilett et al. [16] used organic liquid extraction with dichloromethane, but quantitative determination of MND and analytical recovery rates were not described in this publication. The method published by Angelo et al. [24] gives excellent recovery rates for disopyramide and MND, but their use of dimethyl ether for extraction has several disadvantages (working with an inflammable, explosive and narcotic substance in closed rooms). Methods published by Radwan et al. [31], Sweezy and Ponzo [32] and

Broussard and Frings [25] do not allow determination of MND. Wang *et al.* [33] used a silica gel column and an aqueous mobile phase, consisting of dibasic ammonium phosphate. This method gives high recovery rates for disopyramide and MND, but is rather expensive, because H_2SO_4, the solvent for probe injection, quickly destroys the analytical column, despite the presence of a guard column.

The method described in this paper for determination of disopyramide and its main metabolite MND in serum is easy to handle, rapid to perform and inexpensive. Nearly complete analytical recovery of disopyramide and MND is obtained. The range of detection (0.1–6.0 µg/ml) is sufficient for monitoring subtherapeutic levels (*e.g.* single-dose studies) as well as toxic levels.

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