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Short Communication

Automated determination of disopyramide and N-monodealkyldisopyramide in plasma by reversed-phase liquid chromatography with a column switching system

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ABSTRACT

A rapid and simple column liquid chromatographic method involving a column switching system for the determination of disopyramide and its N-monodealkyl metabolite (NMD) in plasma is described. The deproteinized plasma is applied to an automated system. Purification and concentration were performed using a precolumn connected to a six-position valve; analytical separation was done on-line using a cyano reversed-phase column with a mobile phase consisting of 10 mmol/l trimethylamine (pH 2.5, adjusted with phosphoric acid)–acetonitrile–tetrahydrofuran (78:20:2, v/v/v). Absorbance was measured at 265 nm, with a minimum detectable amount of disopyramide and NMD of 0.1 μg/ml. The method can be applied to drug monitoring and pharmacokinetic studies.

INTRODUCTION

Disopyramide [4-diisopropylamino-2-phenyl-2-(2-pyridyl)butyramide] is a class Ia antiarrhythmic agent with a cardiac depressent action. It is used for the prevention and treatment of ventricular and supraventricular arrythmias. Its principal metabolite is the N-monodealkyldisopyramide (NMD). A knowledge of its pharmacokinetics and disposition is of great interest for appropiate interpretation of plasma concentration data.

Gas chromatography (GC) was first used for the determination of these compounds. Hutsell and Stachelski's method [1] involved multiple phases of extraction, evaporation and derivatization of the metabolite, before chromatographic analysis using an OV-17 stationary phase and flame ionization detection. A single extraction–evaporation sample preparation step was required with a nitrogen detector [2–4]. An EMIT immunoenzyme assay procedure has been used to measure total and unbound disopyramide plasma concentrations [5,6].

Recently, several methods used high-performance liquid chromatography (HPLC) have been reported [7–11]. These assays involve an extraction and evaporation step for the preparation of the sample before the chromatographic separation. Absorbance is measured at 258 nm. The HPLC method overcomes problems such as the interferences encountered with the GC procedure.

This paper describes a simple HPLC method

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using a column switching system for the purification, concentration and analytical separation of disopyramide and NMD in plasma.

EXPERIMENTAL

Chemicals

Disopyramide (**I**), its N-monodealkylated metabolite (**II**) and the internal standard, *p*-chlorodisopyramide (**III**), were kindly supplied by Roussel Uclaf (Paris, France). Trimethylamine (TMA) (10 mmol/l), sodium carbonate (1.2 mol/l) and perchloric acid (0.5 mol/l) were obtained from Merck (Darmstadt, Germany) and chromatographic grade acetonitrile and tetrahydrofuran from SdS (Peypin, France).

Stock and working standard solutions

Stock standard solutions of **I, II** and **III** were prepared in methanol at concentrations of 1 mg/ml and stored at 4°C. Working standard solutions were prepared by adding 0 and 0.5–25.0 μg/ml **I** and **II** to drug-free human plasma and stored at −25°C.

The intra-assay reproducibility was tested by adding known amounts of **I** and **II** to plasma, which was then measured as described below (twelve samples at each concentration). The inter-assay reproducibility was determined by adding the same amounts of **I** and **II** as previously to plasma which was frozen at −25°C and measured in each daily run (twelve samples at each concentration). For intra- and inter-assay reproducibilities we used three quality control samples of disopyramide (low, medium and high levels), the theoretical concentrations of which are reported in Table I.

Mobile phase

Solvent A was distilled water. Solvent B consisted of 10 mmol/l TMA (pH 2.5, adjusted with phosphoric acid)–acetonitrile–tetrahydrofuran (78:20:2, v/v/v).

Sample preparation

Plasma sample (100 μl) was added to **III** (1.25 μg) and perchloric acid (150 μl) and vigorously mixed for 45 s. After centrifugation at 2000 *g* for 10 min, the supernatant (200 μl) was made alkaline (pH 7.5–8) by the addition of sodium carbonate (1.2 mol/l) and transferred into vials in order to inject 200 μl into the liquid chromatograph.

Chromatographic analysis

The liquid chromatographic apparatus used was a system from Waters (Millipore–Waters, Milford, MA, USA) composed of an automatic injector (712 WISP), two high-pressure pumps (M 501 and M 510) and a six-position valve (P/N 600 57). The precolumn was a 7 cm × 4.6 mm I.D. cyano reversed-phase column, particle size 5 μm (Beckman Instruments, Altex Division, San Ramon, CA, USA) and the analytical column was a 7 cm × 4.6 mm I.D. XL cyano reversed-phase column, particle size 3 μm (Beckman).

Absorbance was measured with a Model 490 E detector (Millipore) at 265 nm. Acquisition, integration and processing of the chromatograms were performed by a Baseline 810 workstation (Millipore) using an NEC APC IV microcomputer (NEC Information Systems, Boxborough, MA, USA).

Column switching procedure

The described method employs column switching to concentrate, clean up and separate components **I, II** and **III** present in plasma samples. The flow-rates of the pumps were adjusted to 2 ml/min for pump A and 1 ml/min for pump B. The two phases of concentration/purification and elution were determined by the position of the valve (Fig. 1). At the beginning of the assay (time zero), the valve takes position 1 and the sample is injected from the autoinjector. The injected sample, dissolved in solvent A, is fist passed through the precolumn, where **I, II** and **III** are firmly retained on a polar bonded phase while the other components in the samples are flushed away to the drain with water. Compounds **I, II** and **III** were retained near the head of the precolumn. During the first period, the mobile phase is continuously flowing through pump B to the column. At time 2 min after sample injection, the valve is switched

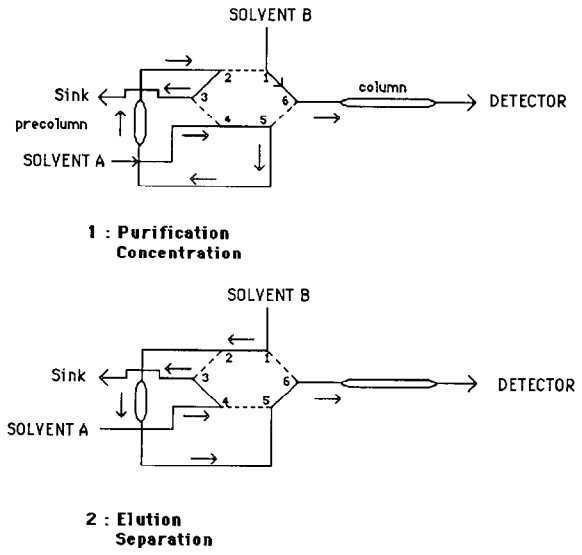

Fig. 1. Schematic diagram of the switching system.

to position 2. Solvent B passes from the precolumn into the column in the backflush mode, where resolution of the components is effected. During time 2–10 min solvent A is continuously flowing from position 4 to 3 and is flushed to the drain. At 10 min after sample injection, the valve is turned back to the original position (1).

TABLE I

PRECISION OF RESULTS (n = 12)

Control (μg/ml)	Disopyramide		Metabolite	
	Mean ± S.D. (μg/ml)	C.V. (%)	Mean ± S.D. (μg/ml)	C.V. (%)
Intra-assay				
1.7	1.7 ± 0.03	1.78	1.7 ± 0.03	1.89
7	6.6 ± 0.06	1.01	7.2 ± 0.06	0.82
13.5	13.2 ± 0.18	1.40	14.5 ± 0.21	1.43
Inter-assay				
2	1.9 ± 0.03	1.54	2.1 ± 0.06	2.89
6.5	6.5 ± 0.14	2.13	6.2 ± 0.16	2.60
13.5	12.9 ± 0.18	1.70	13.7 ± 0.35	2.55

RESULTS AND DISCUSSION

Chromatograms of a human plasma sample spiked with 10 and 15 μg/ml **I** and **II** are shown in Fig. 2. The retention times of **I, II** and **III** are 4.10, 4.69 and 6.29 min, respectively. The baseline is stable.

The calibration graphs exhibited a correlation coefficient (r) of 1.00 for peak response (ratio of peak heights of **I** and **II** to **III**). The response was linear over the concentration range 0.5–25 μg/ml.

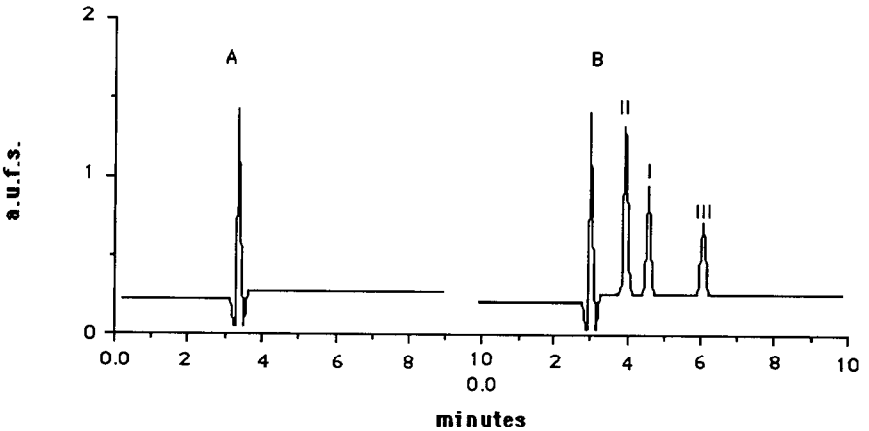

Fig. 2. Elution profiles for (A) a blank plasma and (B) a human plasma sample spiked with (I) disopyramide (10 μg/ml), (II) N-monodealkyldisopyramide (15 μg/ml) and (III) *p*-chlorodisopyramide.

The intra- and inter-assay precision, expressed as the coefficient of variation (C.V.), was less than 3% (Table I); the C.V. values were determined using therapeutic concentrations.

The assay permits the determination of disopyramide and its metabolite with a lower limit of detection than earlier methods [7–10]. The minimum detectable concentration of disopyramide was 0.1 μg/ml in biological samples. Moreover, only 100 μl of plasma were necessary for the dosage of **I** and **II**, whereas other HPLC methods often require 1 ml of plasma.

This method is accurate and fast. The analysis time for one sample, including sample preparation, is less than 30 min. The assay has been used extensively with an automated injection and peak integration system, allowing overnight running of a large number of samples and application in routine analysis. This method is valuable for pharmacokinetic studies and therapeutic drug monitoring.

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