Journal of Chromatography, 230 (1982) 433—437
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1266

Note

Simultaneous high-performance liquid chromatographic assay for quinidine, disopyramide and the mono-N-dealkylated metabolite of disopyramide

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(First received November 30th, 1981; revised manuscript received February 17th, 1982)

It is of considerable use in the management of patients with disturbances of cardiac rhythm to have the means to monitor the serum concentration of the drug [1]. Although quinidine has been available for many years it has been shown that its pharmacokinetics vary considerably between individual patients and there is an advantage in individualising the dose of the drug based on serum concentrations measured at steady-state. This also applies to disopyramide which has become available more recently. The therapeutic drug monitoring of disopyramide is made more complex because one of its metabolites has pharmacological actions similar to the parent drug [2, 3]. The method described in this paper allows the simultaneous measurement of quinidine, disopyramide and its mono-N-dealkylated metabolite (MND) by a rapid, accurate and reproducible method suitable for use in a routine therapeutic drug assay laboratory.

EXPERIMENTAL

Materials

Disopyramide [4-diisopropylamino-2-phenyl-2-(2-pyridyl)-butyramide], mono-N-dealkylated disopyramide [4-isopropylamino-2-phenyl-2-(2-pyridyl)-butyramide] and the internal standard *p*-chlorodisopyramide [4-diisopropylamine-2-*p*-chlorophenyl-2-(2-pyridyl)-butyramide] were supplied by Roussel (Castle Hill, Australia). Quinidine was obtained from ICN K+K Labs. (Cleveland, OH, U.S.A.). Lignocaine and tocainide were from Astra Chemicals (North Ryde, Australia) and procainamide was from E.R. Squibb and Son (Melbourne,

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0378-4347/82/0000—0000/$02.75

Australia). Mexilitine was obtained from Boehringer Ingelheim, Artarmon, Australia). Dichloromethane used for extraction and acetonitrile used in the mobile phase were both high-performance liquid chromatographic (HPLC) grade solvents obtained from Waters Assoc. (Eagle Farm, Australia). All other chemicals were analytical grade products available commercially.

Sample extraction

To each 12-ml glass culture tube, 500 μl of sample or standard were added. This was made alkaline by the addition of 50 μl of 5 *M* sodium hydroxide. Before sealing the tubes with PTFE-lined screw caps, 5 ml of dichloromethane, containing 2 mg/l *p*-chlorodisopyramide as internal standard, were added. The tubes were then mixed on a rotary mixer at 32 rpm for 15 min. After centrifugation at 600 *g* for 3 min, the upper, aqueous layer was aspirated and 3 ml of the remaining organic layer were transferred into tapered centrifuge tubes. The dichloromethane was evaporated using a stream of air and the residue re-dissolved in 100 μl of the mobile phase, from which 20 μl were injected onto the column.

Chromatography

The HPLC system consisted of a Waters Model 6000A solvent delivery system (Waters Assoc., Milford, MA, U.S.A.) with a 20-μl fixed-volume loop injector (Rheodyne, Berkeley, CA, U.S.A.). Eluent was monitored continuously for absorbance changes at 254 and 313 nm using a dual-channel Model 440 UV detector (Waters Assoc.,), the output of which was recorded by a dual-pen Omniscribe recorder (Houston Instrument, Austin, TX, U.S.A.). A Brownlee Labs. Spheri-5 RP-8 column (Activon Scientific Products, Granville, Australia) was used (particle size of the sorbent on these columns is 5 μm).

Samples were eluted isocratically at 2.5 ml/min using 40% acetonitrile (v/v) in 0.05 *M* sodium dihydrogen orthophosphate buffer (pH 3.00). The column was maintained at room temperature (ca. 21°C). Standard curves were prepared using blank plasma spiked with all three components to be assayed. Concentration ranges for disopyramide (0.8—8.1 mg/l) and quinidine (0.9—9.3 mg/l) cover their therapeutic ranges and the concentration range of MND covers the maximum expected in patients having therapeutic concentrations of the parent drug (0.4—3.5 mg/l). The ratios of the peak heights of the drug to internal standard were plotted against concentration to obtain the standard curves. Peak heights for quinidine were determined at 313 nm while those of disopyramide, MND and the internal standard were measured at 254 nm.

Possible interference by other antiarrhythmic drugs was evaluated by chromatographing lignocaine, procainamide, tocainide and mexilitine also.

RESULTS AND DISCUSSION

Using the conditions described, adequate separation of MND, quinidine, disopyramide and *p*-chlorodisopyramide was obtained (Fig. 1) with retention times of 2.2, 3.1, 4.2 and 7.2 min, respectively (Table I). Table I also shows the retention time of procainamide, tocainide, lignocaine and mexilitine to be 1.8,

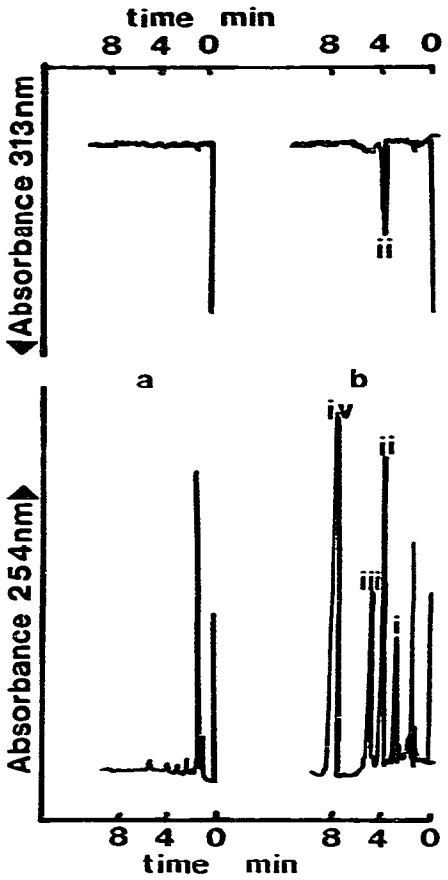

Fig. 1. HPLC tracing obtained using drug-free plasma (a) before spiking (extraction without internal standard), (b) after spiking with (i) mono-N-dealkylated disopyramide (ii) quinidine, (iii) disopyramide (extraction with internal standard iv).

TABLE I

RETENTION TIMES OF VARIOUS COMPOUNDS AND THEIR ABSORBANCE RATIOS

	Retention time (min)	Absorbance ratio (313 nm/254 nm)
Procainamide	1.8	0.69
Tocainide	2.0	N.D.*
MND	2.2	N.D.
Quinidine	3.1	0.28
Lignocaine	3.3	N.D.
Mexilitine	3.6	N.D.
Disopyramide	4.2	N.D.
p-Chlorodisopyramide	7.2	N.D.

*N.D., compound not detected at 313 nm.

TABLE II

EXTRACTION EFFICIENCY (n = 9)

Extraction efficiency expressed as percent of peak height of standard solutions prepared using mobile phase as solvent.

	$\bar{x}$ ± S.D.	μg/ml
MND	97.5 ± 6.1	3.45
Quinidine	94.8 ± 6.2	9.33
Disopyramide	77.5 ± 6.1	8.08

2.0, 3.3 and 3.6 min, respectively. These retention times indicate the lack of interference from these drugs.

Both efficiency and reproducibility of the extraction were acceptable (Table II). Standard curves using single assays of spiked plasma standards were prepared. These curves had the following parameters: MND, $y = 0.212x - 0.016$, $r = 0.999$; quinidine, $y = 0.128x - 0.010$, $r = 0.999$; and disopyramide $y = 0.106x - 0.0068$, $r = 0.999$. To assess the precision of the method a further nine samples from each spiked plasma standard were assayed as unknowns (Table III). The largest coefficients of variation were 5.5, 5.0 and 3.3% for disopyramide, MND and quinidine, respectively.

The reproducibility, precision and accuracy of this method are suitable for use both in pharmacokinetic studies and routine therapeutic drug monitoring. It is an example of how HPLC methods can be adapted to simultaneously measure multiple drugs and their metabolites often providing considerable

TABLE III

PRECISION OF ANALYSIS (n = 9)

	$\bar{x}$ ± S.D. (μg/ml)	C.V. (%)
MND*	0.35 ± 0.02	5.0
	0.86 ± 0.02	2.5
	1.75 ± 0.02	1.1
	3.46 ± 0.06	1.8
Quinidine**	0.97 ± 0.03	3.3
	2.3 ± 0.04	1.6
	4.7 ± 0.07	1.4
	9.3 ± 0.18	2.0
Disopyramide*	0.87 ± 0.05	5.5
	2.0 ± 0.06	2.8
	4.0 ± 0.15	3.6
	7.9 ± 0.35	4.4

*Analyte measured at 254 nm.
**Analyte measured at 313 nm.

advantages over alternative assays such as enzymeimmunoassay and radioimmunoassay. Simultaneous determination of a drug and its metabolite may allow better correlation of serum concentrations with drug effect and toxicity as has been suggested for the tricyclic antidepressants and procainamide [4—6].

This method is an improvement on a previously published method [1] because smaller samples of only 500 μl are acceptable, and the mono-N-dealkylated metabolite is measured simultaneously.

Since this method has been available in our therapeutic drug assay laboratory no interference from other drugs has been encountered, nor have any of the blank plasma samples contained any interfering compounds.

ACKNOWLEDGEMENTS

Financial support from the Lions Kidney and Medical Research Foundation is gratefully acknowledged. J.T.A. is a recipient of Grant No. 247 from the N.H. & M.R.C. of Australia.

We would also like to thank Dr. J. Cameron of Hoechst Roussel (Castle Hill, Australia) for assistance with equipment and provision of disopyramide, mono-N-dealkylated disopyramide and *p*-chlorodisopyramide.

This work has been undertaken in partial fulfillment of the requirements of a Master of Applied Science Degree at the Queensland Institute of Technology by R.L.G. Norris.

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