Journal of Chromatography, 183 (1980) 65—71
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 582

SIMULTANEOUS ANALYSIS OF DISOPYRAMIDE AND QUINIDINE IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received December 27th, 1979)

SUMMARY

A new reversed-phase high-performance liquid chromatographic method allowing simultaneous measurement of plasma concentrations of disopyramide and quinidine is described. Disopyramide and quinidine were separated on a reversed-phase column using 0.05 *M* phosphate buffer (pH 3.0)—acetonitrile (73:27, v/v), as mobile phase and the peaks were monitored by UV absorbance at the wavelengths of 254 and 325 nm. The drugs were extracted from alkaline plasma with chloroform containing the internal standard. The organic phase was evaporated to dryness and the residue was redissolved in a small volume of the mobile phase before analysis by high-performance liquid chromatography. The method is convenient and reliable in routine monitoring of both drugs.

INTRODUCTION

Both quinidine and disopyramide are used in the treatment and prophylaxis of cardiac arrhythmias. Because of a relatively narrow therapeutic index and intersubject variability in the pharmacokinetics of these drugs, monitoring the serum concentration of these drugs may assist in individualizing the dosage requirements and in assessing patient compliance with the dosage regimen. Methods used previously for measuring disopyramide and quinidine have included fluorometric [1, 2], gas—liquid chromatographic (GLC) [3, 4] and high-performance liquid chromatographic (HPLC) [5—8] assays. The fluorometric assays have proven non-specific and subject to interference by background fluorescence [3]. GLC methods for measuring disopyramide have suffered from frequent lack of linearity at lower concentrations [5]. The most

0378-4347/80/0000-0000/$02.25

promising technique for reliably measuring these drugs in biological fluids appears to be HPLC. Methods capable of measuring groups of drugs simultaneously, e.g. the simultaneous monitoring of drugs such as the anticonvulsant drugs, have been shown to be efficient and desirable, because they provide information on the plasma levels of a number of drugs at the same time with a minimum delay [9]. None of the previously described methods for the measurement of anti-arrhythmic drugs allows the simultaneous measurement of disopyramide and quinidine, both of which may be encountered in the plasma of the same patient. This paper describes an HPLC method for simultaneously measuring plasma concentrations of disopyramide and quinidine.

MATERIALS

Disopyramide [4-diisopropylamino-2-phenyl-2-(2-pyridyl)-butyramide], its mono-N-dealkylated metabolite [4-isopropylamino-2-phenyl-2-(2-pyridyl)-butyramide] and the internal standard [4-diisopropyl-amino-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 & Son (Melbourne, Australia). The chloroform used for extraction was analytical grade obtained from E. Merck (Darmstadt, G.F.R.) and the acetonitrile used for the chromatography was HPLC grade obtained from Waters Assoc. (Eagle Farm, Australia). All other chemicals were analytical grade products available commercially.

METHODS

Sample preparation

One ml of plasma was made alkaline by adding 100 µl of 5 *M* sodium hydroxide. The extraction was performed with 5 ml of chloroform containing the internal standard at the concentration of 2 mg/l. The extraction was carried out in tubes fitted with PTFE-lined screw caps using a rotary mixer at 32 rpm for 20 min. After centrifugation at 600 *g* for 10 min, the upper aqueous layer was aspirated and 3 ml of the remaining chloroform layer were transferred into tapered centrifuge tubes. The chloroform was evaporated under a stream of air. The residue was redissolved in 250 µl of the mobile phase used for the chromatography and 20 µl of this were injected into the high-performance liquid chromatograph.

Absorbance spectra

To determine suitable detection wavelengths the absorbance spectra of disopyramide, its mono-N-dealkylated metabolite and quinidine were recorded using Cary 118-C (Varian Instrument Division, Palo Alto, CA, U.S.A.). The compounds were dissolved in the mobile phase at concentrations of 10 mg/l. The solvent alone was placed in the reference light path.

High-performance liquid chromatography

The HPLC system used consists of a solvent delivery system (Model

M-6000A pump, Waters Assoc., Milford, MA, U.S.A.) and a fixed-volume loop injector, Rheodyne 7120 (Rheodyne, Berkeley, CA, U.S.A.). The eluent was monitored continuously for absorbance changes at 254 nm using a Model 440 detector (Waters Assoc.) and at 325 nm using a Schoeffel SF 770 Spectroflow detector (Schoeffel, Westwood, NJ, U.S.A.). These detectors were connected in series. The output of the detectors was recorded by a dual-pen recorder. LiChrosorb RP-8 column, 10-μm particle size (stainless steel, 250 mm × 4.6 mm I.D.) were made by Brownlee Labs. (Activon Scientific Products, Granville, Australia).

Samples were eluted isocratically with 0.05 *M* sodium phosphate buffer (pH 3.0)—acetonitrile (73:27, v/v) at a constant flow-rate of 1.8 ml/min. The column temperature was maintained at 40°C. The method was calibrated by adding known amounts of disopyramide, 1.0—8.0 mg/l, and quinidine, 1.25—10.0 mg/l into drug-free plasma; these concentrations cover the therapeutic ranges of the drugs. To construct the standard curves the peak height ratios of disopyramide and quinidine to the internal standard were plotted against the amounts of drugs added.

Possible interference by drugs administered to patients having anti-arrhythmic treatment was checked. The following compounds were chromatographed using the same method: lignocaine, procainamide, tocainide and the N-dealkylated metabolite of disopyramide.

RESULTS

Under our chromatographic conditions good separation of disopyramide and quinidine was achieved (Fig. 1). The retention times of quinidine, disopyramide

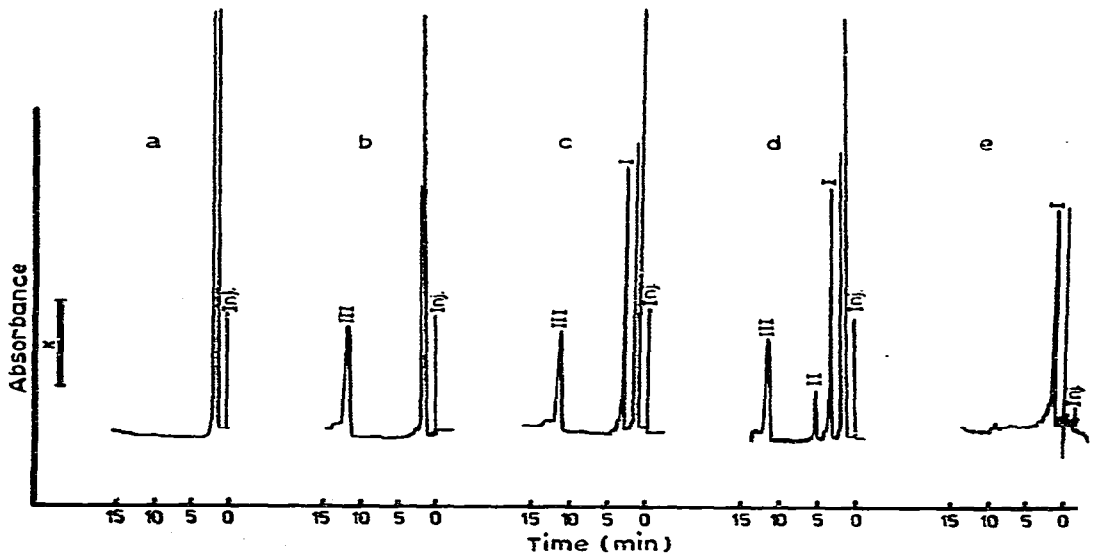

Fig. 1. HPLC tracings obtained for drug-free plasma used for all standards (a); patient plasma prior to quinidine administration (b); plasma from the same patient 2 h after quinidine administration (c); the same plasma after spiking it with disopyramide (d). Peaks: I, quinidine; II, disopyramide; III, internal standard. The tracings a—d were obtained by monitoring the eluent at 254 nm and × corresponds to 0.002 absorbance units. (e) Simultaneous tracing of Fig. 1d monitored at 325 nm and in this case × corresponds to 0.001 absorbance units.

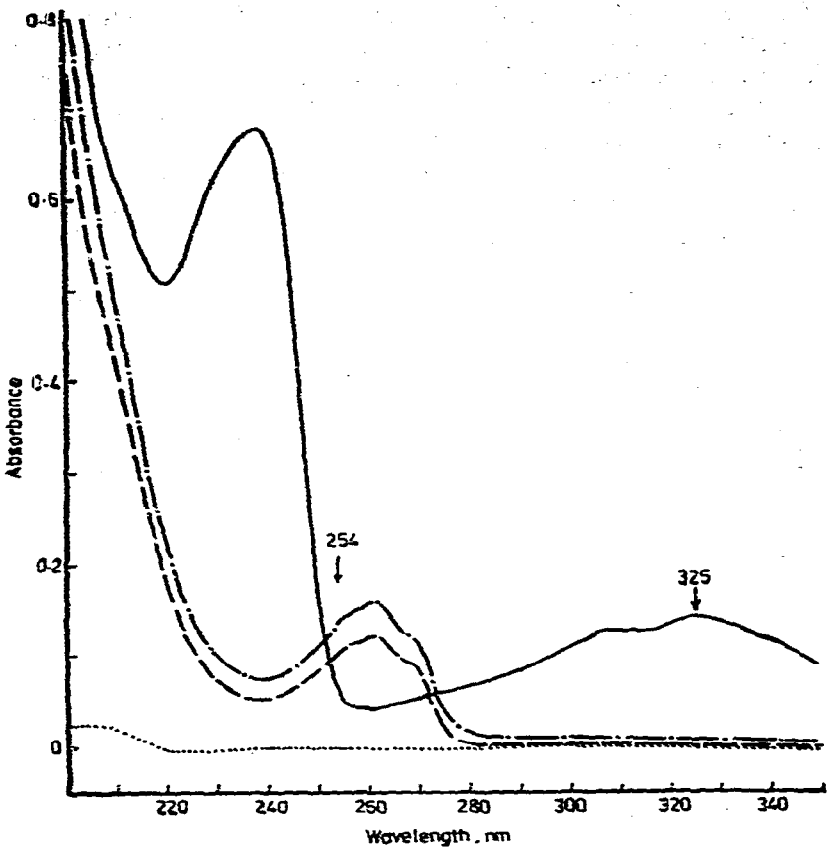

Fig. 2. Absorbance spectra of quinidine (——), disopyramide (— — —) and the mono-N-dealkylated metabolite of disopyramide (—·—·—). In each case the sample cuvette contained the compound in question at a concentration of 10 mg/l dissolved in the mobile phase used for the HPLC analysis. The reference cuvette was filled with the mobile phase. (. . . . . .) denotes the absorbance spectrum when both the sample and the reference cuvettes contained the mobile phase.

TABLE I

RETENTION TIMES OF VARIOUS COMPOUNDS AND THEIR ABSORBANCE RATIOS

	Retention time (min)	Absorbance ratio 325 nm/254 nm
Procainamide	1.9	N.D.*
Tocainide	2.6	N.D.
Mono-N-dealkylated metabolite of disopyramide	3.5	N.D.
Quinidine	3.6	0.248 ± 0.005 (mean ± S.D., $n = 29$)
Lignocaine	3.9	N.D.
Disopyramide	6.0	N.D.
p-Chloro-disopyramide	12.8	N.D.

*N.D.: none of the test compounds was detectable at 325 nm.

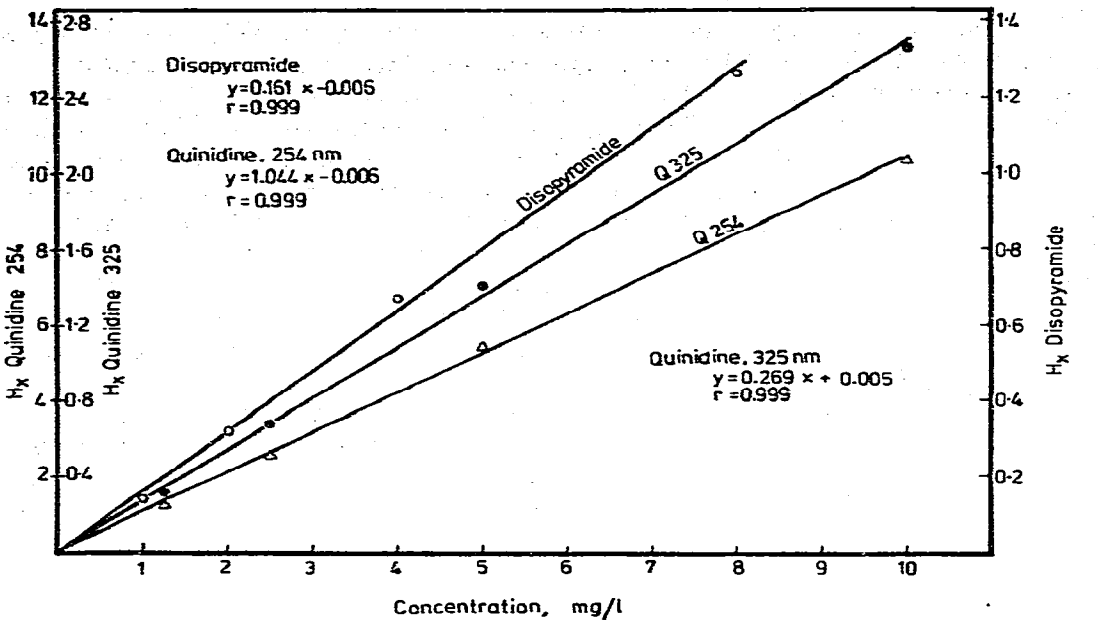

Fig. 3. Standard curves obtained for disopyramide monitored at 254 nm and quinidine monitored at both 254 (Q254) and 325 nm (Q325). H_x is the peak height ratio of the drug in question obtained by dividing the peak height of the drug in question by the peak height of the internal standard. The equations shown were obtained for each set of data by regression analysis.

TABLE II

EXTRACTION EFFICIENCY EXPRESSED AS PER CENT OF PEAK HEIGHT OF AQUEOUS STANDARDS

	n	Mean ± S.D.
Disopyramide	4	76.2 ± 3.4
Quinidine	4	93.0 ± 5.9

TABLE III

ANALYTICAL PRECISION OF THE EXTRACTION AND HPLC ANALYSIS

	X ± S.D. (mg/l)	C.V. (%)	*n*
Disopyramide	1.1 ± 0.1	4.9	10
	2.6 ± 0.2	4.6	9
	4.2 ± 0.2	5.7	10
	8.7 ± 0.4	3.6	10
Quinidine, 254 nm*	1.4 ± 0.1	3.7	10
	3.6 ± 0.1	2.6	9
	5.6 ± 0.2	2.5	10
	11.3 ± 0.6	5.3	10
Quinidine, 325 nm**	1.3 ± 0.04	4.5	10
	3.5 ± 0.1	3.2	9
	5.3 ± 0.1	2.3	10
	10.8 ± 0.5	5.0	10

*Results obtained by monitoring quinidine at 254 nm.
**Results obtained by monitoring quinidine at 325 nm.

and the internal standard were 3.6, 6.0 and 12.8 min respectively. Other plasma constituents did not interfere with the peaks of interest. A compound potentially interfering with quinidine determinations is the mono-N-dealkylated metabolite of disopyramide. This compound eluted immediately before quinidine with a retention time of 3.5 min, however it has essentially no absorbance at 325 nm (Fig. 2) and is not detectable at this wavelength. Dual wavelength monitoring was used routinely and wavelength ratioing results are shown in Table I. The retention times of disopyramide, quinidine, *p*-chloro-disopyramide and some potentially interfering compounds that could be encountered in patient samples were determined (Table I). The standard curves for disopyramide at 254 nm and for quinidine at both 254 and 325 nm were linear as shown in Fig. 3.

The efficiency and the reproducibility of the extraction was acceptable (Table II). The precision of the method was determined at four concentrations of disopyramide and quinidine. In all cases the coefficient of variation is less than 6% (Table III). The total assay time for a single sample is approximately 60 min.

DISCUSSION

Using the HPLC method described in this paper we have achieved good separation of disopyramide and quinidine. The method has allowed simultaneous measurement of these drugs with good reproducibility.

It must be noted that the mono-N-dealkylated metabolite of disopyramide runs very close to quinidine on this HPLC system. With column deterioration it would be possible to have the metabolite underlying the quinidine peak. This may not be a significant limitation, as the metabolite has been detected in only 15% of patients given single therapeutic doses of disopyramide and even then the plasma concentrations were less than 0.4 mg/l [3]. However, to completely eliminate this potential interference and to avoid similar interference going unnoticed, absorbance monitoring at two wavelengths simultaneously was carried out. The mono-N-dealkylated metabolite of disopyramide has no absorbance at 325 nm (Fig. 2) and is not detectable at this wavelength, therefore not interfering with quinidine determinations at 325 nm. It is highly recommended that dual wavelength monitoring and wavelength ratioing is utilized in clinical drug assays [10]. This will greatly reduce the possibility of erroneously high results being released if an unknown peak interferes with the drug being measured. More seriously, an erroneously low value for plasma level of a drug could be obtained if an unknown peak was underlying the internal standard peak. Simultaneous dual wavelength monitoring can be achieved by having two detectors connected in series or most conveniently and inexpensively this can be done using a dual-channel detector.

This assay method provides reliable means of measuring two important antiarrhythmic drugs, both of which could, under some therapeutic change-over situations be found in patients' plasma at combined levels that may represent a threat to the well-being of already dangerously ill patients. Therefore, it is very important to obtain plasma concentrations of these drugs as efficiently as possible. The method described here has the advantage over previous assay

methods for disopyramide and quinidine in that no more effort is required to assay these drugs simultaneously than to assay either drug separately.

ACKNOWLEDGEMENTS

We would like to thank Dr. J. Cameron of Roussel (Castle Hill, Australia) for providing us with samples of disopyramide, its mono-N-dealkylated metabolite and *p*-chloro-disopyramide. We would also like to thank Dr. Tatiana Assaykeen of Astra Chemicals (North Ryde, Australia) for providing us with a sample of tocainide.

Financial support from Merck Sharp & Dohme to one of the authors (J.T.A.) is gratefully acknowledged.

REFERENCES

1 R.E. Ranney, R.R. Dean, A. Karim and F.M. Radzialowski, Arch. Int. Pharmacodyn. Ther., 191 (1971) 162.
2 T. Huynh-Ngoc and G. Sirois, J. Pharm. Sci., 66 (1977) 591.
3 T.C. Hutsell and S.J. Stachelski, J. Chromatogr., 106 (1975) 151.
4 A.M.J.A. Duchateau, F.W.H.M. Merkus and F. Schobben, J. Chromatogr., 109 (1975) 432.
5 K.F. Ilett, L.P. Hackett, L.J. Dusci and R. Tjokrosetio, J. Chromatogr., 154 (1978) 325.
6 P.-O. Lagerström and B.-A. Persson, J. Chromatogr., 149 (1978) 331.
7 R.E. Kates, D.W. McKennon and T.J. Comstock, J. Pharm. Sci., 67 (1978) 269.
8 N. Weidner, J.H. Ladenson, L. Larson, G. Kessler and J.M. McDonald, Clin. Chim. Acta, 91 (1979) 7.
9 S.J. Soldin and J.G. Hill, Clin. Chem., 22 (1976) 856.
10 Waters Associates Application Laboratory, Communication N75, 1977.