Journal of Chromatography, 307 (1984) 439—444
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2037

Note

High-performance liquid chromatographic method for the measurement of mexiletine and flecainide in blood plasma or serum

R.K. BHAMRA, R.J. FLANAGAN and D.W. HOLT*

Poisons Unit, Guy's Hospital, London SE1 9RT (U.K.)

(First received July 18th, 1983; revised manuscript received December 21st, 1983)

Mexiletine and flecainide are orally effective Class 1 antiarrhythmic agents. Plasma mexiletine measurement is an accepted aid in the administration of the drug, which has a narrow therapeutic range [1, 2]. Flecainide has been used as an investigational compound in the U.S.A. and U.K. [3] and a simple, selective assay is needed for use in the investigation of its clinical pharmacology.

Two high-performance liquid chromatographic (HPLC) methods for the measurement of mexiletine in biological fluids have been published. Both use a relatively large sample size (0.2—0.4 ml [4], 1 ml [5]), a lengthy extraction procedure, and UV absorption detection either following derivatisation with 2,4-dinitrofluorobenzene [4] or at 254 nm [5]. Published methods for the measurement of flecainide in biological fluids use HPLC with either fluorescence [6] or ultraviolet absorption detection at 308 nm [7]. Both use a relatively large sample size (1 ml) to achieve limits of accurate measurement of 50 μg/l and 22 μg/l, respectively.

The method described here for the measurement of mexiletine and flecainide is both sensitive and selective, and is based on the extraction of a small (50 μl) sample volume with an organic solvent at alkaline pH, followed by the direct analysis of the resulting extract. The chromatographic system used employs a microparticulate (5 μm) silica column together with a non-aqueous ionic eluent [8] and fluorescence detection.

EXPERIMENTAL

Mexiletine hydrochloride [1-methyl-2-(2,6-xylyloxy)ethylamine hydrochloride] and flecainide acetate [2,5-bis-(2,2,2-trifluoroethoxy)-N-(2-piperidylmethyl)benzamide acetate] were obtained from Boehringer Ingelheim U.K.

0378-4347/84/$03.00

(Bracknell, U.K.) and Riker (Loughborough, U.K.), respectively. The internal standard, benzimidazole, was obtained from BDH (Poole, U.K.) and was used as a 0.3 mg/l solution in glass-distilled water, this latter solution being prepared by dilution from a 1.0 g/l methanolic solution. Methanol, 2,2,4-trimethylpentane and methyl-*tert.*-butyl ether were all HPLC grade (Rathburn, Walkerburn, U.K.). *d*-10-Camphorsulphonic acid monohydrate was obtained from Aldrich (Gillingham, U.K.). Tris-(hydroxymethyl)-methylamine (Tris) was analytical reagent grade (BDH) and was used as a 2 *M* solution in glass-distilled water.

High-performance liquid chromatography

The solvent delivery system was a constant-flow reciprocating pump (Applied Chromatography Systems, Model 750/4) and sample injection was performed using a Rheodyne Model 7125 syringe-loading valve fitted with a

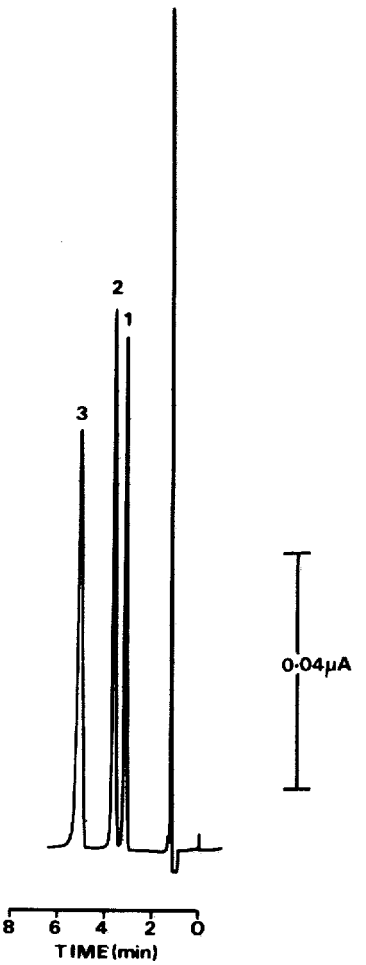

Fig. 1. Chromatogram obtained on analysis of an extract of a standard solution prepared in heparinised human plasma containing mexiletine (1) and flecainide (2) at concentrations of 1.5 and 0.5 mg/l, respectively; 100 μl injection. The initial benzimidazole (3) concentration was 0.3 mg/l.

100-μl sample loop. Stainless-steel tubing (0.25 mm I.D.) was used to connect the outlet port of the valve to the analytical column, a stainless-steel tube 125 × 5 mm I.D. packed with Spherisorb S5W Silica (Hichrom, Woodley, U.K.) which was used at ambient temperature (normally 22°C). The mobile phase was methanol—2,2,4-trimethylpentane (80:20, v/v) containing 1 mmol/l (0.25 g/l) *d*-10-camphorsulphonic acid and was used at a flow-rate of 2.0 ml/min. The column effluent was monitored using a Schoeffel Model FS 970 fluorescence detector at an excitation wavelength of 200 nm, without emission filter, and at a time constant of 0.5 sec. Integration of peak areas was performed using a Hewlett-Packard 3390A recording integrator.

The chromatography on this system of an extract of a plasma standard containing both mexiletine and flecainide is illustrated in Fig. 1. The retention times, measured relative to the internal standard, of mexiletine, flecainide and some additional compounds of interest are given in Table I.

TABLE I

RETENTION TIMES OF MEXILETINE, FLECAINIDE AND SOME OTHER COMPOUNDS RELATIVE TO BENZIMIDAZOLE

Compound	Relative retention time	Compound	Relative retention time
Desalkylflurazepam	0.55	Dipyridamole	0.82
Nitrazepam*	0.61	Trimipramine	0.88
Mexiletine	0.61	Desipramine	0.90
4-Hydroxypropranolol	0.65	Norverapamil	0.90
Ajmaline	0.68	Prazosin	0.93
Nadolol	0.68	Terazosin	0.94
Pyrimethamine*	0.68	Protriptyline	0.97
Pindolol	0.69	Benzimidazole	1.00
Triamterene	0.69	Dextropropoxyphene*	1.10
Penbutolol	0.71	Trazodone	1.16
Propranolol	0.71	Orphenadrine	1.47
Nordextropropoxyphene*	0.71	Butriptyline	1.48
Metoprolol	0.71	Mianserin	1.65
Oxprenolol*	0.75	Verapamil	1.68
Flecainide	0.75	Imipramine	1.85
Prajmalium	0.79		

*Poor fluorescence under the conditions of the assay.

Sample preparation

Plasma or serum (50 μl) was pipetted into a small glass (Dreyer) test tube (Poulton, Selfe and Lee, Wickford, U.K.). Internal standard solution (20 μl), Tris solution (50 μl) and methyl-*tert.*-butyl ether (200 μl) were added using Hamilton gas-tight glass syringes fitted with Hamilton repeating mechanisms. The contents of the tube were vortex-mixed for 30 sec and centrifuged at 9950 *g* for 2 min in an Eppendorf 5412 centrifuge (Anderman, East Molesey, U.K.). Subsequently, a portion (approximately 110 μl) of the extract was taken and used to fill the sample loop of the injection valve. Duplicate sample analyses were performed and the mean result taken.

Instrument calibration

Standard solutions containing mexiletine at concentrations equivalent to 0.5, 1.0, 2.0 and 3.0 mg/l free-base were prepared in heparinised human plasma by serial dilution of an aqueous solution of mexiletine hydrochloride equivalent to 1.0 g/l free-base. Standard solutions containing flecainide at concentrations equivalent to 0.1, 0.25, 0.5, 0.75 and 1.0 mg/l free-base were prepared in heparinised human plasma by serial dilution of an aqueous solution

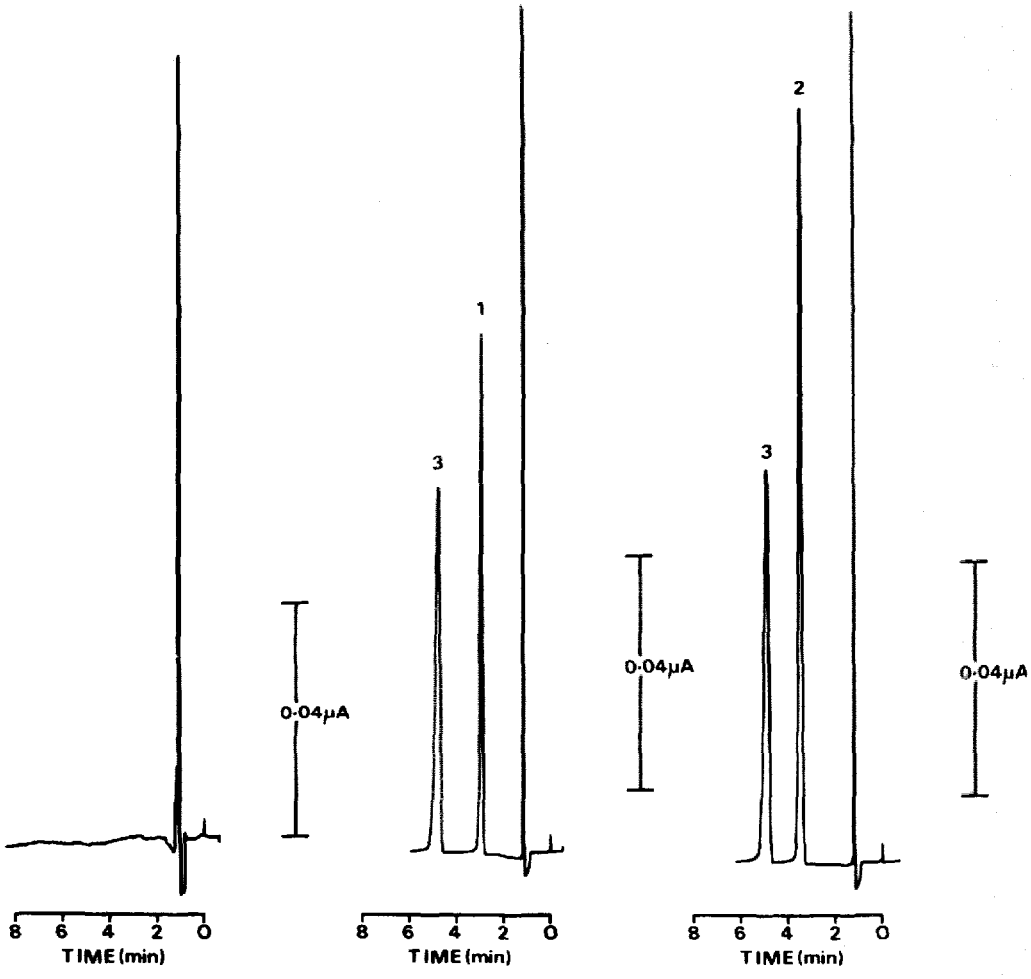

Fig. 2. Chromatogram obtained on analysis of an extract of mexiletine- and flecainide-free human plasma without addition of an internal standard; 100 μl injection.

Fig. 3. Chromatogram obtained on analysis of an extract of a plasma sample obtained from a patient receiving mexiletine (600 mg per day); 100 μl injection. The initial benzimidazole (3) concentration was 0.3 mg/l, and the plasma mexiletine (1) concentration was found to be 1.7 mg/l.

Fig. 4. Chromatogram obtained on analysis of an extract of a plasma sample obtained from a patient receiving flecainide (200 mg per day); 100 μl injection. The initial benzimidazole (3) concentration was 0.3 mg/l, and the plasma flecainide (2) concentration was found to be 0.75 mg/l.

of flecainide acetate equivalent to 1.0 g/l free-base. In addition, an internal quality assurance sample containing mexiletine and flecainide at concentrations of 1.5 and 0.5 mg/l, respectively, was prepared in heparinised human plasma by dilution from independent stock solutions of the drugs.

These standards were stable for at least three months if stored in 250-μl aliquots at −20°C in tightly closed containers in the absence of light. On analysis of these solutions the ratio of the peak area of the analyte to the peak area of the internal standard, when plotted against analyte concentration, was linear and passed through the origin of the graph in each case.

RESULTS AND DISCUSSION

No endogenous sources of interference have been observed. A chromatogram obtained on analysis of an extract of mexiletine- and flecainide-free human plasma is illustrated in Fig. 2, whilst those obtained on analysis of specimens from patients receiving either mexiletine or flecainide are shown in Figs. 3 and 4, respectively.

Compounds which were extracted under the conditions of the assay were studied further as potential sources of interference (Table I). Desalkylflurazepam, ajmaline, prajmalium, nadolol, pindolol, triamterene, penbutolol, propranolol and metoprolol elute close to either mexiletine or flecainide but were, at least, partially resolved. Although nitrazepam elutes with mexiletine and oxprenolol elutes with flecainide (Table I), no interference from these compounds was seen at the concentrations attained during normal therapy owing to their relatively poor fluorescence under the conditions of the assay. In addition, 4-hydroxypropranolol and nordextropropoxyphene were not detected owing to the instability of the former and the poor fluorescence of the latter. Finally, although protriptyline and dextropropoxyphene elute close to benzimidazole, both were partially resolved and dextropropoxyphene has a poor fluorescence relative to benzimidazole.

Some other cardioactive drugs and metabolites studied (amiodarone, desethylamiodarone, disopyramide, lignocaine, procainamide, tocainide, quinidine, sotalol, atenolol, labetalol, nifedipine, lorcainide and methyldopa) were not detected on this system.

TABLE II

THE INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION FOR MEXILETINE AND FLECAINIDE

	Mexiletine		Flecainide	
	Concentration (mg/l)	C.V. (%) (n = 10)	Concentration (mg/l)	C.V. (%) (n = 10)
Intra-assay	1.5	1.2	0.88	1.6
	0.5	2.8	0.40	2.3
	0.2	3.7	0.10	3.1
Inter-assay	2.0	2.9	0.50	2.9

The intra- and inter-assay coefficients of variation (C.V.) for replicate analyses of standard solutions of mexiletine or flecainide, prepared in heparinised human plasma, are shown in Table II. Using a sample size of 50 μl, the limits of accurate measurement for mexiletine and flecainide were 50 μg/l and 20 μg/l, respectively (intra-assay C.V. at these concentrations 8.4% and 8.9%, respectively; n = 10 in both cases).

CONCLUSIONS

The method described here has been used for the measurement of the plasma concentrations of both mexiletine and flecainide attained during therapy. Only 100 μl of specimen are required for a duplicate analysis, which can be completed together with the analysis of a quality control specimen, within 30 min and few potential sources of interference have been identified. An increase in sample size would facilitate the measurement of both compounds at the concentrations attained following single oral dosage.

REFERENCES

1 S.P. Joseph and D.W. Holt, Eur. J. Cardiol., 11 (1980) 115.
2 D.W. Holt, D.E. Chadwick and R.W.F. Campbell, Clin. Ther., 5 (1983) 268.
3 M. Hodges, J.M. Haugland, G. Granrud, G.J. Conrad, R.W. Asinger, F.L. Mikell and J. Krejci, Circulation, 65 (suppl. V) (1982) 879.
4 H. Breithaupt and M. Wilfling, J. Chromatogr., 230 (1982) 97.
5 R. Kelly, D. Christmore, R. Smith, L. Doshier and S.L. Jacobs, Ther. Drug Monit., 3 (1981) 270.
6 J.W. de Jong, J.A.J. Hegge, E. Harmsen and P.Ph. de Tombe, J. Chromatogr., 229 (1982) 498.
7 S.F. Chang, T.M. Welscher, A.M. Miller and R.E. Ober, J. Chromatogr., 272 (1983) 341.
8 R.J. Flanagan, G.C.A. Storey, R.K. Bhamra and I. Jane, J. Chromatogr., 247 (1982) 15.