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## Note

# Sensitive gas chromatographic method for the estimation of a new antiarrhythmic compound, mexiletine (Ko1173), in biological fluids

S. WILLOX and B. N. SINGH*

*Department of Medicine (Section of Clinical Pharmacology), University of Auckland, and Department of Cardiology, Greenlane Hospital, Auckland (New Zealand)*

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Numerous potent antiarrhythmic compounds are now available for the elective management of clinical cardiac arrhythmias[1]. However, long-term antiarrhythmic prophylaxis remains unsatisfactory, due either to limiting side effects or to relatively short duration of action of available agents[2]. A new antiarrhythmic agent, mexiletine (Ko1173; Boehringer, Ingelheim, G.F.R.), has recently been introduced[3,4]. It has been found to be effective in controlling serious ventricular arrhythmias in patients after acute infarction and has a potency comparable to that of procainamide or lignocaine[3–5]. Unlike lignocaine, it appears to be active by the oral route with a relatively long plasma half-life[3,4].

In order to determine the pharmacokinetic profile of mexiletine, it appeared desirable to develop an assay procedure which was simple but sensitive to low concentrations of the drug in biological fluids. Previously reported methods for the estimation of mexiletine include spectrofluorimetry and gas chromatography (GC)[6,7]. The GC method reported here utilizes a linear electron capture detector (ECD) which has the merit of high sensitivity, high detector stability, and linearity of response over a wide range of mexiletine concentrations. Unlike the older non-linear ECDs, the linear ECD used here can sustain gas flow, temperature, and column changes and re-stabilize rapidly. It is also virtually impossible to "overload" and the detector stabilizes rapidly after injection of large amounts of electron-capturing compounds.

## MATERIALS AND METHODS

Mexiletine was made available by Boehringer and alprenolol hydrochloride (the internal standard) by A. B. Hassle (Goteborg, Sweden). Heptafluorobutyric anhydride (HFBA) was obtained commercially from Pierce (Rockford, Ill., U.S.A.). Diethyl ether used for extraction was analytical grade. It had been shaken with silver nitrate–sodium hydroxide (5 g $AgNO_3$ in 100 ml 1 *M* NaOH per litre of diethyl ether), washed free of base with distilled water, dried over potassium hydroxide pellets and finally distilled from sodium. The instruments used were a Hewlett-Packard

* Present address: Department of Cardiology, Cedars-Sinai Medical Center, Los Angeles, Calif. 90029, U.S.A.

Model 5710A gas chromatograph fitted with a linear ECD, Model 18713A, and an Autolab Minigrator electronic integrator (Spectra-Physics).

*Preparation of samples*

To a 15-ml glass-stoppered centrifuge tube containing 1 ml plasma, 50 $\mu$l of potassium hydroxide solution (1 mole/l) and 2 ml of diethyl ether were added. The internal standard, alprenolol hydrochloride (500 ng), was added to the plasma sample in an aqueous solution (50 $\mu$l of a 10 $\mu$g/ml solution). After mixing for 2 min on a vortex mixer, the tubes were centrifuged and the diethyl ether layer aspirated. The diethyl ether was mixed for 2 min with hydrochloric acid (0.5 ml of 1 mole/l) in a clean tube and the diethyl ether layer discarded. After washing the acid with 1 ml of clean diethyl ether, 0.5 ml of 1.1 mole/l potassium hydroxide was added and the now basic aqueous phase extracted with 1.5 ml of diethyl ether. The diethyl ether was dried over potassium carbonate (20–30 mg) for 60 min, then transferred to a 1-ml "Reactivial" (Pierce). One boiling chip was added and the ether boiled off in a heating block. The dry residue was taken up in 25 $\mu$l of benzene and 25 $\mu$l of HFBA were added. After incubation at 90° for 30 min the solvent and HFBA were removed with a stream of dry nitrogen. The final dry derivatives were dissolved in 30–50 $\mu$l of ethyl acetate and 1 $\mu$l injected into the GC column.

A standard curve was prepared using reconstituted pooled human plasma to which had been added the internal standard (500 ng/ml) and mexiletine (20 ng–4 $\mu$g/ml). Peak areas were obtained with the integrator and the ratios of mexiletine peak area to that of the internal standard plotted against mexiletine concentrations. All glassware was silylated and was washed with 10% hydrochloric acid in methanol followed by clean methanol before use.

*Gas chromatographic conditions*

A 3 m × 4 mm all-glass column packed with 1% cyclohexane dimethanol succinate (HiEFF8BP, Applied Science Labs., State College, Pa., U.S.A.) on 100–120 mesh Gas-Chrom Q was used in conjunction with a heated injection port. The carrier gas was 5% methane in argon, flowing at 45 ml/min. Both injection port and detector were at 250° and the column oven was kept at 180°.

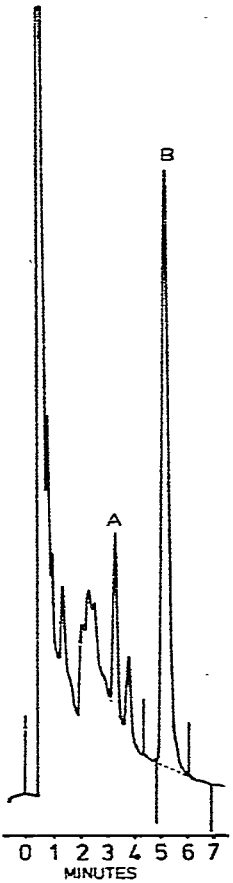
## RESULTS AND DISCUSSION

With the above GC conditions, mexiletine and alprenolol had retention times of 3.3 and 5.2 min, respectively (Fig. 1). The standard (Fig. 2) curve was taken to be the best fit straight line through the points obtained by regression using the method of least squares and had a correlation coefficient $> 0.99$.

The method described here has been used to assay mexiletine in plasma, urine, and bile. In the case of bile and urine, however, samples were diluted to concentrations of 500 ng to 2 $\mu$g/ml.

Studies in metabolism and pharmacokinetic characteristics of mexiletine in rats, dogs, and man have been undertaken and the results will be reported in detail in a separate communication. It may nevertheless be mentioned that our results confirm the previous observations[3,4] that mexiletine has a longer plasma half-life after oral ingestion than currently available antiarrhythmic drugs. With an oral dose of 200 mg, peak plasma levels of 1–2 $\mu$g/ml occur 30–60 min after ingestion and decline to about 100 ng/ml after 24 h. Further clinical and experimental evaluation of

this compound may therefore be of therapeutic interest, especially in relation to the recent report[5] that mexiletine may have the advantage of easier administration and lower toxicity when compared to procainamide during prophylactic treatment in patients after acute myocardial infarction.



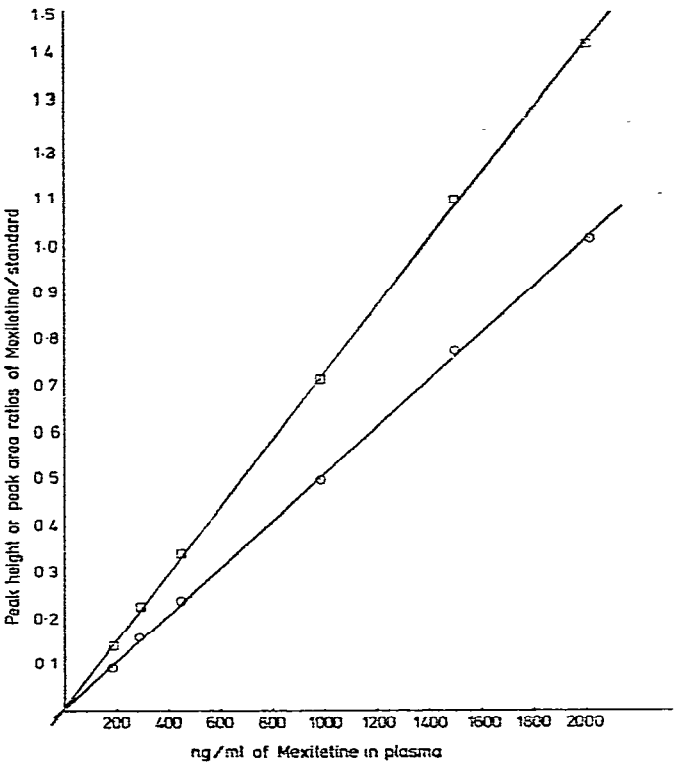




Fig. 1. Chromatogram of mexiletine (A) in human plasma. The internal standard is alprenolol (B).
Fig. 2. Calibration curves for mexiletine in human plasma. ○, Peak area ratios; ⊡, peak height ratios.

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