J. Pharm. Pharmac., 1973, **25**, 550–553 *Received February 5, 1973*

Spectrophotofluorometric and gas-liquid chromatographic methods for the estimation of mexiletine (Kö 1173) in plasma and urine

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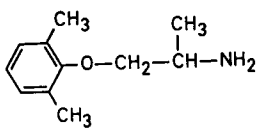
Methods are presented for the spectrophotofluorometric and gas-liquid chromatographic determination in plasma and urine of the anti-arrhythmic compound, mexiletine (Kö 1173). Both methods involve extraction of the drug from alkaline plasma with ether. In the spectrophotofluorometric method the compound is re-extracted into 0·05N HCl and emission intensity determined at 300 nm with activation at 228 nm. In the chromatographic method the drug is acylated during evaporation of the ether. Both butyryl and acetyl derivatives could be used. Use of a nitrogen-sensitive detector increased the sensitivity and selectivity of the method and allowed more rapid analyses. There was good agreement between results obtained by spectrophotofluorometric and chromatographic methods.

Mexiletine* (1-(2,6-dimethylphenoxy)-2 amino-propane, Kö 1173; I) is a primary amine with anticonvulsant activity. It was recently shown by Allen, Ekue & others (1972) to be effective in suppressing experimental ventricular arrhythmias, and these findings have been confirmed in initial clinical studies. It is likely to be of particular value for long-term therapy since it is well absorbed from the gastrointestinal tract and has a long biological half life (Clarke, Julian & others, 1973; Campbell, Kelly & others, 1973). Clinical pharmacological studies have been made in more than 100 patients with ventricular arrhythmias and therapeutic plasma concentrations are in the range 0·5–1·5 μg ml^{-1}. We now describe spectrophotofluorometric and gas-liquid chromatographic methods for the estimation of mexiletine in plasma and urine.



(I)

METHODS

Spectrophotofluorometric method

Fig. 1 shows the activation and emission spectra of mexiletine. Curve A is the activation spectrum of the pure compound (2 μg ml^{-1}) in 0·05 N HCl, obtained with the emission monochromator set at 300 nm and scanning with the activating mono-

* W.H.O. recommended international non-proprietary name.

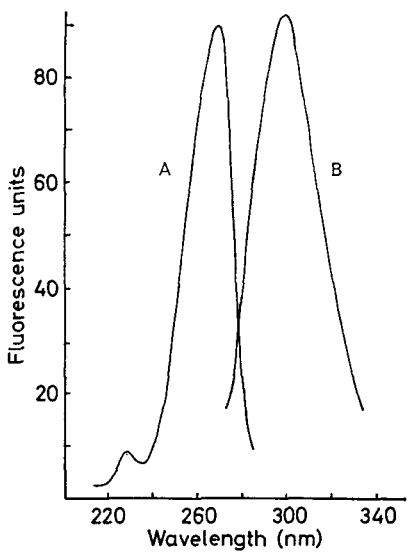


FIG. 1. Activation (A) and fluorescence (B) spectra of mexiletine (2 μg ml^{-1}) in 0·05 N HCl.

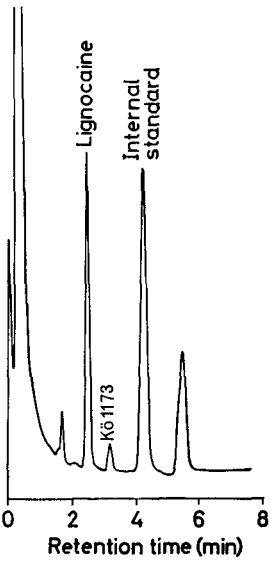


FIG. 2. Chromatogram of plasma extract from a patient receiving mexiletine (Kö 1173) and lignocaine. The plasma concentrations of mexiletine and lignocaine were 0·07 μg ml^{-1} and 0·4 μg ml^{-1} respectively. Mexiletine and the internal standard were chromatographed as the butyryl derivatives. For conditions see text.

chromator. Curve B is the fluorescence spectrum for the same sample with the activation monochromator set at 268 nm while scanning with the emission monochromator. Fluorescence did not vary over the pH range 1–7. Two peaks occur in the activation spectrum. The main peak is at 268 nm and a smaller one occurs at 228 nm. Maximum emission occurs at 300 nm. In the present method, 228 nm is used as the activating wavelength in the determination of mexiletine in plasma since at the wavelength of maximum activation, 268 nm, the advantage of a higher intensity of fluorescence is offset by the marked increase in fluorescence of blank plasma.

Procedure. To plasma (3 ml) in stoppered glass tubes is added 2 N NaOH (0·5 ml) and diethyl ether (5 ml). The tubes are shaken mechanically for 10 min and centrifuged. 3·5 ml of the ether layer is extracted into 3 ml of 0·05 N HCl and the fluorescence of the acid phase is measured in a Perkin Elmer Model MPF-2A fluorescence spectrophotometer at 228 nm activating wavelength and 300 nm fluorescent wavelength. A standard curve is prepared for each set of samples by adding known amounts of the drug samples of blank plasma and carrying these through the above procedure. This calibration curve is linear up to a concentration of at least 20 μg ml^{-1} of mexiletine in plasma.

Mexiletine in urine is determined by a similar procedure except that the urine samples are diluted to the concentration range 0–16 μg ml^{-1}.

Glassware must be scrupulously clean and should be washed in chromic acid and rinsed in distilled water before use. The limit of detection is 0·05 μg ml^{-1}.

Gas-liquid chromatographic method

The initial method employed involved extraction from alkaline plasma into hexane and direct chromatography of the drug on KOH-treated Chromosorb G coated with 2·5% Carbowax 20M (Pollman and Häselbarth, C. H. Boehringer Sohn Ingelheim/Rhein, personal communication). However, the sensitivity and selectivity in the present method using extraction into ether and acylation during the evaporation step are increased by the use of the nitrogen-sensitive flame ionization detector.

Procedure. To plasma or urine (2·0 ml) in round bottomed stoppered glass tubes are added 2N NaOH (0·5 ml) and 0·5 ml of an aqueous solution containing about 2·5 μg ml^{-1} of the 2,4-methyl analogue of mexiletine as the internal standard (Kö 768, Häselbarth & Pollman, personal communication). Redistilled ether (5 ml) is added, the tubes are shaken mechanically for 10 min and then centrifuged. The tubes are frozen, the ether decanted into tapered centrifuge tubes and butyric anhydride (3 μl) added to the ether. The organic solvent is evaporated to approximately 0·5 ml on a rotary vacuum evaporator at room temperature and the tubes are placed on a water bath at 60° for 10 min. The residue is dissolved in 15 μl of ethanol using a vortex mixer and 1–3 μl aliquots taken for injection into the gas chromatograph.

For the assay of unknown plasma samples, a reference standard prepared from pooled blood bank plasma containing 2·5 μg ml^{-1} of mexiletine is carried through the procedure at the same time. If necessary, urine is diluted to contain less than 25 μg ml^{-1} of mexiletine and an appropriate aqueous standard is run with the samples.

Stock standard solutions of the drug and internal standard were made up in 0·1 N HCl and stored at 4°. All glassware was silanized and before use was washed with a mixture of 10% concentrated HCl in methanol followed by a methanol rinse.

Chromatography

The instrument used was a Hewlett-Packard Model 5750 gas chromatograph equipped with a nitrogen-sensitive flame ionization detector using rubidium bromide as the alkali metal salt. The column was glass, 4 ft × ¼ inch o.d. packed with 100/120 mesh Gas-Chrom Q coated with 3% cyclohexane dimethanol succinate* (HI-EFF 8BP), and the column, injection port and detector temperatures were 220, 240 and 420° respectively. The helium carrier gas flow rate was 70 ml min^{-1} and the air flow was 180 ml min.$^{-1}$ The hydrogen flow rate was 26 ± 2 ml min^{-1} with an inlet pressure of 5 atmospheres. The detector was operated with the rubidium bromide crystal at the position of maximum ionization, giving maximum sensitivity to nitrogen and a minimal response to carbon-containing compounds.

RESULTS

Under the above conditions, the butyryl derivatives of mexiletine and the internal standard gave symmetrical peaks with retention times of 3·1 and 4·1 min respectively. Lignocaine can be estimated simultaneously and has a retention time of 2·4 min (Fig. 2). There are no interfering peaks from the plasma extracts. The peak with a retention time of 5·5 min has the same retention time as caffeine and was observed to be much larger when blood samples were taken after consumption of coffee.

The ratio of the peak heights of mexiletine to internal standard plotted against concentration in the range 0·25–2·5 μg ml^{-1} gave a straight line passing through the

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origin. The concentration of drug in an unknown plasma sample (U) is given by: $U = (2{\cdot}5\ X)/Y$, where Y is the peak height ratio of the 2·5 μg ml^{-1} plasma standard and X is the ratio for the unknown sample. Replicate analyses of plasma containing added mexiletine in the concentration range 0·25–1·0 μg ml^{-1} gave a standard deviation of 3·4%. The limit of detection is less than 10 ng ml^{-1}.

Extraction of the drug from alkaline plasma gave peak height ratios which were only 85–90% of those obtained with corresponding aqueous standard solutions. Plasma standards must therefore be used for calibration.

Comparison of spectrophotofluorometric and gas-liquid chromatographic methods. Routine analysis by both methods of duplicate plasma and urine samples containing mexiletine gave good agreement with a correlation coefficient $r = 0{\cdot}99$.

DISCUSSION

The methods described have been found suitable for routine monitoring of plasma concentrations in patients receiving mexiletine. The sensitivity of the fluorometric method can probably be increased by forming a suitable derivative and work is being directed towards this end. No drug likely to be administered concurrently with mexiletine has so far been shown to interfere with the determination. Those tested for interference include lignocaine, quinidine, procainamide, benzodiazepines, propranolol and practolol.

Although we have described the use of the nitrogen sensitive detector, the ordinary flame ionization detector can also be used. We have successfully chromatographed the acetyl derivative of mexiletine both on the HI-EFF 8BP column described above and also on a $\frac{1}{8}$ inch × 2 M stainless steel column packed with 2$\frac{1}{2}$% Carbowax 20 M + 5% KOH on 80/100 mesh Chromosorb G. Oven temperature was 220° and the nitrogen carrier gas flow rate was 50 ml min^{-1}. With ordinary flame ionization detection, methylene chloride or chloroform were used to redissolve the ether residue because of their smaller solvent front.

The nitrogen-sensitive detector has the advantages of a much smaller solvent front, increased sensitivity to nitrogen-containing compounds and decreased interference from compounds which do not contain nitrogen. Analyses can thus be completed in a shorter time than when using a conventional flame ionization detector. Formation of the butyryl derivative of mexiletine was preferable with the nitrogen detector since this gave better separation from lignocaine with the shorter analysis time.

Acknowledgements

We would like to thank Mrs. M. E. Roberts, Mrs. N. Henderson, Mr. W. Leahey and Miss G. McClean for technical assistance. We gratefully acknowledge grants from the Scottish Hospitals Endowment Research Trust, the Northern Ireland Hospitals Authority and Boehringer Ingelheim Ltd. We thank Dr. P. Knowlson for gifts of mexiletine and its 4-methyl analogue.

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