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Note

Mexiletine analysis in blood and plasma using gas chromatography and nitrogen-selective detection

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A number of gas chromatographic methods have been described for the analysis of mexiletine in biological fluids [1—8]. These methods require the derivatization of mexiletine prior to chromatography [1—4, 6, 7], the use of comparatively large sample volumes, typically 2 ml of plasma [1, 2, 4, 6], or electron-capture detection [3, 6, 7]. We describe a method for the analysis of mexiletine without derivatization, which requires small sample volumes, is more sensitive and has comparable reproducibility to previously reported methods.

EXPERIMENTAL

Materials

Mexiletine [1-(2,6-dimethylphenoxy)-2-aminopropane] and the internal standard 1-(2,4-dimethylphenoxy)-2-aminopropane, were supplied by Boehringer Ingelheim, Sydney, Australia. Diethyl ether and dichloromethane were of analytical grade (Merck, Darmstadt, G.F.R.). All other reagents were of reagent grade.

Sample preparation

Plasma or blood, 0.05—1.0 ml, is placed in a 15-ml capacity PTFE-lined screw cap culture tube, with 1250 ng or 250 ng of internal standard contained in 250 μl of water. Diethyl ether (5 ml) and 0.25 ml of 1 *N* sodium hydroxide are added and the sample extracted using a vortex mixer for 1 min. The organic and aqueous phases are separated by centrifugation for 5 min and the lower aqueous phase frozen in a dry-ice—acetone bath. The upper organic phase is

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poured into a second tube which has an elongated cone at its base of approximately 50 μl capacity. Dilute sulphuric acid (0.5 ml of 0.2 *M*) is added to the tube which is mixed with a vortex mixer (30 sec), the phases are again separated, frozen and the diethyl ether discarded. The tubes are placed in a vortex evaporator (Buchler Instruments, Fort Lee, N.J., U.S.A.) and evaporated at reduced pressure and room temperature for 5 min to remove traces of diethyl ether. Sodium hydroxide solution (0.25 ml of 1 *N*) and 50 μl of dichloromethane are added to the tubes which are mixed for 30 sec and centrifuged. Aliquots (1 μl) of the dichloromethane phase, sampled through the aqueous phase, are injected into the gas chromatograph.

Chromatographic conditions

A Hewlett-Packard Model 5730A gas chromatograph fitted with a nitrogen/phosphorus-selective flame ionisation detector was used for the analysis. A 1.8 m × 2 mm I.D. glass column packed with 1.5% Carbowax 20M and 5% KOH on Supelcoport 80—100 mesh was used for the separation. The injector port, oven and detector were maintained at 200°, 130° and 250° respectively. The flow-rates of the nitrogen carrier gas, hydrogen and air, were 30 ml/min, 3 ml/min and 50 ml/min respectively. Prior to use, the column was conditioned with the above carrier gas flow-rate at 220° for 16 h. Chromatograms were recorded on a Hewlett-Packard Model 7123A recorder.

Calibration and accuracy

The method was calibrated by adding known amounts of mexiletine and internal standard, each contained in 100 μl of aqueous solution, to plasma or blood which was then analysed. Calibration curves were established covering two ranges. The higher range was calibrated with samples containing 50, 100, 250, 500, 1000 and 2000 ng of mexiletine and used 1250 ng of internal standard. The lower range was calibrated with samples containing 5, 10, 20, 50, 75 and 100 ng of mexiletine and used 250 ng of internal standard.

A normalised peak height ratio was determined by dividing the peak height ratio of mexiletine to internal standard by the amount of mexiletine in each standard. The mean normalised peak height ratio was used to calculate the amount of mexiletine in unknown samples and the coefficient of variation was used to establish the reproducibility of the method over the entire range of each calibration curve.

RESULTS AND DISCUSSION

Under the chromatographic conditions described, the retention time of mexiletine was 3.5 min and that of the internal standard 4.75 min (Fig. 1A). Control plasma or blood samples did not contain peaks that interfered with that of mexiletine or internal standard (Fig. 1B). No peaks were observed which interfered with the peaks due to mexiletine or the internal standard when samples of lignocaine, procainamide, N-acetylprocainamide, tocainide, disopyramide or propranolol were injected into the gas chromatograph.

A total of 18 calibration curves in the range of 50—2000 ng were prepared from plasma (1 ml) over a period of approximately three months. The average

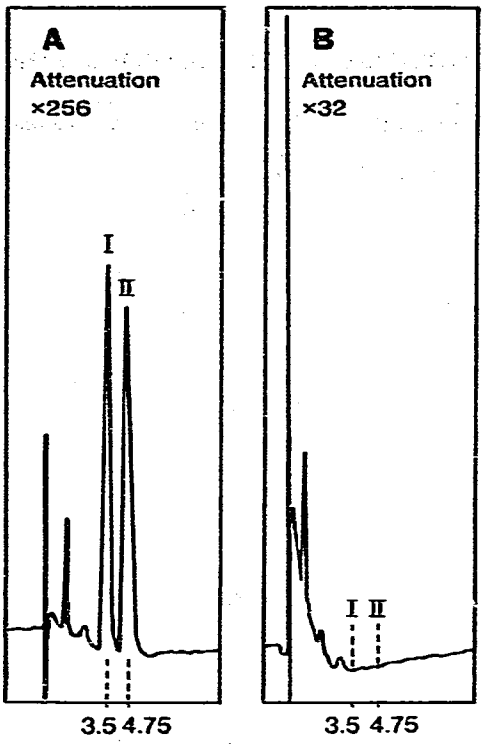

Fig. 1. Chromatograms of (A) a plasma sample (1 ml) containing 850 ng of mexiletine (I) and 1250 ng of internal standard (II); (B) a control plasma sample, I and II indicating the times that would correspond to mexiletine (3.5 min) and internal standard (4.75 min) peaks. Note that the sensitivity in B is 8 times that shown in A.

coefficient of variation of the normalised peak height ratio was 5.86%, the lowest value being 3.4% and the highest 9%. The calibration curves were linear, the mean correlation coefficient of the 18 curves being 0.997. The method was also calibrated in the range of 5—100 ng of mexiletine using 1-ml samples of plasma. The coefficient of variation of the normalised peak height ratio in this range was 4.9%. Calibration curves from whole blood samples had similar slopes and reproducibility to those from plasma. Given the ability of the method to accurately measure 5 ng of mexiletine, this would enable the bottom of the therapeutic range (500 ng/ml) to be measured with 25 μl of plasma.

Some analytical characteristics of previously reported methods for the analysis of mexiletine are compared with the present method in Table I. Willox and Singh [3] and Perchalski et al. [7] have described methods using polyfluoroalkylamide derivatives of mexiletine which give sensitivities comparable to that reported in the present method. All other methods report calibration curves with the lowest concentration an order of magnitude above that used with the present method. The reproducibility of the present method is comparable to or better than that of other methods in Table I. The choice of a suitable stationary phase results in symmetrical peaks (Fig. 1A) without derivatization. Similar-

TABLE I

COMPARISON OF THE ANALYTICAL CHARACTERISTICS OF METHODS FOR MEXILETINE IN BIOLOGICAL FLUIDS

Biological fluid	Sample size (ml)	Derivative	Detector*	Reproducibility (C.V. %)**	Calibration range (ng/ml)	Reference
Plasma Urine	2	butyramide	NPD	3.4	250—2500	1
Plasma	2	acetamide	FID	–	–	2
Blood Plasma Urine	1	hepta-fluoro-butyramide	EC	***	20—2000	3
Plasma	2	acetamide	FID	–	100—?	4
Plasma	1	underivat-ised	NPD	8.7	100—?	5
Plasma	2	hepta-fluoro-butyramide	EC	7.25	200—1000	6
Plasma	2	pentafluoro-propion-amide/tri-fluoroacet-amide	EC/ FID	6	7—2000	7
Blood Plasma	0.2	underivat-ised	FID	4.3	500—16,000	8
Blood Plasma	0.05—1.0	underivat-ised	NPD	5.8	5—2000	This paper

*NPD = nitrogen/phosphorus-selective detector; FID = flame ionisation detector; EC = electron-capture detector.
**C.V. = coefficient of variation.
***$r = 0.99$.

ly the use of nitrogen-selective detection, in addition to producing good sensitivity, enhances the selectivity of the method, compared to those analyses using flame ionization or electron-capture detection.

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