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Note

Gas chromatographic method for the routine serum monitoring of mexiletine

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Mexiletine [1-(2,6-dimethylphenoxy)-2-aminopropane] is a class 1 antiarrhythmic drug effective in the management of ventricular arrhythmias [1, 2]. Its electrophysiological properties are similar to those of lidocaine, but it differs from the latter in that it is active orally and that it has a long elimination half-life. Effective plasma concentrations fall in the range 0.75—2.0 mg/l [3, 4]; an unacceptable frequency of serious side effects occurs at plasma concentrations higher than 2.0 mg/l [5].

Various gas chromatographic (GC) methods for the determination of mexiletine in serum or plasma have been published. In addition to flame-ionization detection [6, 7], both nitrogen-sensitive [8—12] and electron-capture [13—17] detection have been used. Some procedures [11—13] require a back-extraction of the drug in order to obtain a clean sample and all except one method [7] require 1.0 ml or more of serum or plasma. So far, only one of these methods has been adapted to monitor serum mexiletine levels during therapy [7]. A disadvantage of this method is that 2,7-dimethylquinoline, the external standard used, besides being very different in structure from mexiletine, is kept in solution in chloroform, a solvent that is known to react with amino compounds [18, 19]. Also due to the volatility of this solvent, the concentration of the reference standard may vary on storage. A modification of this method has recently been reported [20]. In this case, 2,7-dimethylquinoline is kept in acidic aqueous solution and used as internal standard and chloroform is replaced by dichloromethane as extracting solvent.

A simple GC method using flame-ionization detection for the routine monitoring of mexiletine serum levels is described herein. Only 500 μl of serum or plasma are required and the internal standard used, besides being a primary aliphatic amine like mexiletine, is stable for several months when kept in aqueous solution.

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EXPERIMENTAL

Reagents and materials

All chemicals used were analytical reagent grade: isopropanol, *n*-pentane and diethyl ether were distilled before use. Mexiletine hydrochloride and its two metabolites, 1-(2,6-dimethyl-4-hydroxyphenoxy)-2-aminopropane hydrochloride and 1-(2-hydroxymethyl-6-methylphenoxy)-2-aminopropane oxalate were gifts from Boehringer Ingelheim Canada (Burlington, Canada), whilst rimantadine hydrochloride (1-methyl-1-adamantylmethylamine) was given by Du Pont de Nemours (Wilmington, DE, U.S.A.).

Synthesis of N-acetylmexiletine

To 500 mg of mexiletine base dissolved in sodium-dried diethyl ether, 1 ml of acetic anhydride was added and the resulting solution was stirred for 3 h at room temperature. The solvent in the reaction mixture was then evaporated under vacuum and the residue was redissolved in 100 ml of diethyl ether. This ethereal solution was washed successively with 15 ml of 2 mol/l hydrochloric acid, 15 ml of 2 mol/l sodium hydroxide and water, dried over anhydrous sodium sulphate, filtered and evaporated to dryness to give an oily residue which was crystallized from methanol as a white solid; m.p. 70.5°C (uncorrected); IR (KBr disc) 3250 (NH), 1615 (CO), 1530 (NH) cm^{-1}; electron-impact mass spectrum (solid inlet, 70 eV) *m/z* 100 (100%), 58 (80%), 44 (37%), 43 (35%), 77 (14%), 91 (11%), 122 (7%).

Instrumentation

A Hewlett-Packard gas chromatograph Model 5711A equipped with a flame-ionization detector and an integrator was used. The coiled glass column (1.2 m × 4 mm I.D.) which was packed with 100—120 mesh Gas Chrom Q, coated with OV-17 3% was preconditioned at 250°C for 16 h and treated with hexamethyldisilazane before use. The injection and detector temperatures were set at 250°C and 300°C, respectively, while the column temperature was kept at 180°C. The nitrogen (carrier gas) flow-rate was 60 ml/min and the hydrogen and air flow-rates were 60 and 240 ml/min, respectively.

Extraction procedure

A 0.5-ml aliquot of serum sample was rendered alkaline by the addition of 1.0 ml of sodium hydroxide (2 mol/l); 0.5 ml of rimantadine hydrochloride solution containing 2.5 mg/l of the base was added as internal standard and the mixture was extracted twice with *n*-pentane containing 3% isopropanol. The organic extracts were combined and evaporated to dryness in a water-bath at 44°C. The residue was dissolved in 100 μl of diethyl ether, 2 μl of acetic anhydride was added and the resulting solution was returned to the water-bath for 10 min and afterwards analyzed by gas—liquid chromatography (GLC).

Calibration curves

Aqueous solutions of mexiletine hydrochloride and of rimantadine hydrochloride were added to serum or plasma. The concentration range of mexiletine used was 0.1—5.0 mg/l and the concentration or rimantadine hydrochloride

was 2.5 mg/l. All the samples were extracted and analyzed using the procedure described above. Calibration curves based on the area ratios of mexiletine to the internal standard were constructed using six different concentrations of mexiletine analyzed in duplicate for each sample. The data were subjected to linear-regression analysis to give the appropriate calibration factor.

RESULTS AND DISCUSSION

The structure of synthesized acetylmexiletine was confirmed by solid-inlet mass spectrometry. A sample of this compound gave a single peak having the same retention time as that of the derivatized mexiletine in the serum samples. Analysis of mexiletine as its acetyl derivative was necessary in order to eliminate interference from the serum or plasma constituents. Rimantadine is also a primary amine and it reacts with acetic anhydride under the same conditions as mexiletine. Derivatization of both compounds was optimal and reproducible using the conditions described herein.

Acetic anhydride was used by Kelly [6] to form the acetyl derivative of mexiletine prior to GLC analysis. The reaction was carried out using a large volume of the reagent (20 μl) and excess reagent was evaporated at 60° C under nitrogen. The present method uses only 2 μl of acetic anhydride and therefore no evaporation of excess reagent is necessary. The reaction is complete within 10 min.

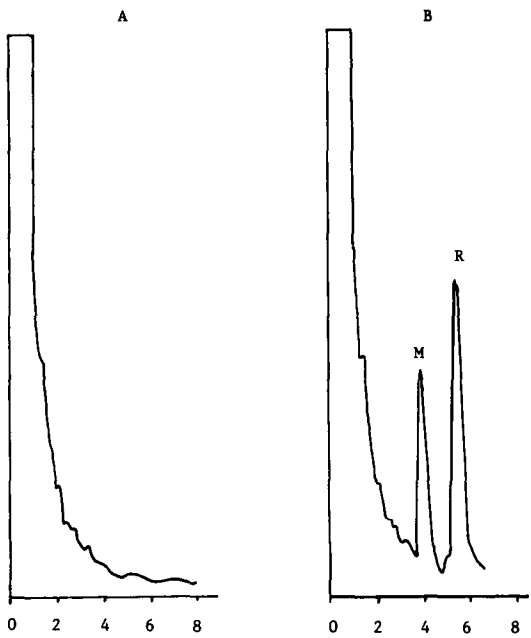

Fig. 1. Representative gas—liquid chromatograms of (A) blank human serum and (B) serum sample containing 0.8 mg/l mexiletine obtained from a patient stabilized on 200 mg of mexiletine four times a day. Peaks: M = mexiletine; R = rimantadine, internal standard.

n-Pentane containing 3% isopropanol was preferred to other low-boiling-point extracting solvents such as diethyl ether or dichloromethane since it gave a cleaner extract. When serum samples were spiked with mexiletine to give final concentrations of 0.5, 2.0 and 5.0 mg/l and extracted as described except that rimantadine was used as an external standard, the recoveries were 94%, 96% and 99%, respectively. The same procedure was repeated for rimantadine, with similar results. In this case, mexiletine was used as the external standard.

Fig. 1A is a chromatogram of an extract of blank serum treated with acetic anhydride while Fig. 1B is a chromatogram of a similarly treated sample containing 0.8 mg/l mexiletine; the internal standard was added prior to extraction. The retention times of the acetyl derivatives of mexiletine and rimantadine were 4.0 and 5.7 min, respectively.

The calibration curve was linear between 0.1 and 0.5 mg/l. The mean concentration, standard deviation and coefficient of variation for intra- and inter-day analysis of spiked serum samples containing 0.2, 1.0 and 5.0 mg/l mexiletine are given in Table I. The selectivity of the assay was checked against the parahydroxy and the ring monohydroxymethyl derivatives of mexiletine which have been shown to be the two major metabolites of mexiletine [21, 22] as well as against the following cardiovascular drugs, hypnotics and tranquillizers: disopyramide, lidocaine, procainamide, diltiazem, nifedipine, verapamil, isosorbide nitrate, prazosin, hydralazine, amiloride, flurazepam, diazepam, lorazepam, triazolam, chlorpromazine and theophylline. Interference from the above mentioned drugs was checked by analyzing the serum of several cardiac patients taking one or more of these compounds thus indicating selectivity of the mexiletine assay against the metabolites of such drugs as well.

TABLE I

INTRA- AND INTER-ASSAY VARIATIONS IN GC MEASUREMENT OF SERUM MEXILETINE ($n = 4$)

Concentration (mg/l)	Intra-assay		Inter-assay	
	Mean ± S.D. (mg/l)	C.V. (%)	Mean ± S.D. (mg/l)	C.V. (%)
0.2	0.21 ± 0.01	4.76	0.21 ± 0.01	4.76
1.0	0.95 ± 0.03	3.16	0.99 ± 0.04	4.04
5.0	5.01 ± 0.08	1.60	4.93 ± 0.19	3.85

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