Journal of Chromatography, 145 (1978) 401—411
Biomedical Applications

CHROMBIO. 133

NEW ELECTRON-CAPTURE GAS—LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF MEXILETINE PLASMA LEVELS IN MAN

ARMAND FRYDMAN*

Département de Pharmacologie, Hôpital Broussais, 96 Rue Didot, 75674 Paris Cedex 14 (France)

JEAN-PIERRE LAFARGE

Section Anti-dopage (S.E.J.S.) UER Cordeliers, 15 Rue de l'Ecole de Médecine, 75006 Paris (France)

FRANÇOIS VIAL and ROGER RULLIERE

Service de Médecine Interne-Cardiologie, Hopital Antoine Chantin, Rue Antoine Chantin, 75014 Paris (France)

and

JEAN-MICHEL ALEXANDRE

Département de Pharmacologie, Hôpital Broussais, 96 Rue Didot, 75674 Paris Cedex 14 (France)

(First received May 20th, 1977; revised manuscript received December 12th, 1977)

SUMMARY

A method for the determination of mexiletine in human plasma by gas—liquid chromatography with electron-capture detection is described. Plasma samples are extracted at pH 12 with dichloromethane after addition of the internal standard, the 2,4-methyl analogue of mexiletine. A derivative is obtained using heptafluorobutyric anhydride; according to gas chromatography—mass spectrometry it is a monoheptafluorobutyryl compound. The minimum detectable amount of mexiletine is 5 pg. Accurate determinations of human plasma levels were performed after oral or intravenous treatment.

INTRODUCTION

Mexiletine, [1-(1,6-dimethylphenoxy)-2-aminopropane; Kö 1173] is an anti-arrhythmic drug recently introduced in the management of ventricular arrhythmias [1—6].

As yet, only two methods for the quantification of unchanged mexiletine in biological fluids have been reported. The spectrofluorimetric method [7]

*To whom correspondence should be addressed.

is limited with respect to both specificity and sensitivity. In the gas—liquid chromatographic (GLC) method [7] the detection and quantification of the butyryl derivative of mexiletine is performed by a nitrogen-sensitive flame-ionization detector for which optimal operating conditions can be obtained and rapidly reproduced only with difficulty.

Besides, a GLC technique using heptafluorobutyric anhydride as derivatization reagent was recently described in a short note [8].

The aim of the present experimental study, therefore, was to develop a sensitive and specific GLC method using a linear and stable electron-capture detector (ECD) which should enable us to assess circulating plasma levels in patients after oral and intravenous administration.

EXPERIMENTAL

Reagents

All chemicals used were analytical grade. Heptafluorobutyric anhydride (Merck, Darmstadt, G.F.R.) was used as the derivatization reagent; to protect it from hydrolysis by moist air after opening the ampoule, it was stored in air-tight 3-ml vials (Reactivials, Pierce, Rockford Ill., U.S.A.). For the derivatization reaction the heptafluorobutyric anhydride (HFBA) was diluted (1:25, v/v) in ethyl acetate (J.T. Baker, Phillipsburg, N.J., U.S.A.) just before use. Hydrochloric acid (2 *N*) and sodium hydroxide (2 *N*) were prepared with glass-distilled water and stored in glass bottles. The extraction solvent was dichloromethane (Mallinckrodt, St. Louis, Mo., U.S.A.). Samples of mexiletine [1-(2,6-dimethylphenoxy)-2-aminopropane; Kö 1173] and of internal standard [1-(2,4-dimethylphenoxy)-2-aminopropane; Kö 768] were supplied by Boehringer (Paris, France).

GLC conditions

A Tracor 550 gas chromatograph equipped with a ^{63}Ni ECD was used. A glass column (400 cm × 4 mm I.D.) was packed with a mixed phase of phenylmethyl silicone fluids (2% OV-17 + 1% OV-275) coated on acid-washed, dimethylchlorosilane-treated, high-performance 80—100 mesh Chromosorb G (Johns Manville Products). This column was conditioned at 280° for 72 h (carrier gas nitrogen, 35 ml/min) after temperature programming (2°/min with low nitrogen carrier flow).

The operating conditions were: carrier gas (nitrogen) flow-rate 40 ml/min; scavenger gas (nitrogen) flow-rate 40 ml/min; column temperature 180°; injection port temperature 230° and detector temperature 285°. The ^{63}Ni ECD, which was not used with pulse current, gave a linear response for quantities ranging from 10—1200 pg of mexiletine heptafluorobutyryl (HFB) derivative.

Preparation of the derivative

Mexiletine (as free base) after extraction of its hydrochloride or of plasma was evaporated to a dry residue at 38° under a stream of dry nitrogen. A volume of 500 µl of ethyl acetate solution of HFBA (1:25, v/v) was added to this residue. The tubes were tightly stoppered and shaken and the reagent

solution was left to react for 30 min at 25°. The reaction mixture was then evaporated to a dry residue under a gentle stream of dry nitrogen.

Under the same conditions, the internal standard Kö 768 also reacts with HFBA.

Hexane (Mallinckrodt), 100 μl, was added to the residue; aliquots 90.5—1.5 μl) were injected into the gas chromatograph.

The completeness of the acylation was studied under various conditions: extended reaction times from 20 min—3 h, and the use of increasing amounts of HBFA solution. Increased concentrations of HFBA in these solutions gave identical recoveries, indicating quantitative derivatization conditions for mexiletine and internal standard.

Wentworth and Chen [9] have reviewed the effect of the detector temperature on the electron-capture detection (ECD). Accordingly, ECD temperatures ranging from 250° to 295° were tested for the HFB derivative of mexiletine; the best sensitivity was obtained at a temperature of 285°, which also reduced contamination of the cell detector.

GLC—mass spectrometry (MS) combination

The GLC—MS combination apparatus (Finnigan, Sunnyvale, Calif., U.S.A.) was operated at an accelerating voltage of 3.5 kV, an ionization voltage of 70 eV and a trap current of 65 μA. The column (180 cm × 4 mm I.D.) was packed with 2% OV-1 coated on the same support as already described. The apparatus was equipped with an accelerator voltage alternator for multiple-ion detection; the source was set to monitor in the electronic impact mode.

Mexiletine determination in patients

Protocol. A dose of 250 mg (3.5 mg/kg) of mexiletine hydrochloride was given intravenously (slow 10-min injection) to a 45-year-old patient. A 5-ml volume of heparinized blood was withdrawn 0, 0.5, 1, 2, 3, 4, 6, 9, 12, 18, 24 and 36 h after the dose was injected. After separating the plasma by centrifugation (3500 *g* for 15 min) 2-ml samples were taken for analysis according to the procedure described above.

Another patient was treated orally with 300 mg of mexiletine hydrochloride (5 mg/kg). Blood was withdrawn 0, 1, 2, 3, 4, 6, 9, 12, 18, 24 and 36 h after the dose was administered. Centrifuged plasma was treated following the aforementioned procedure.

Extraction procedure. A 100-μl volume of an aqueous solution (20 μg/ml, as a free base) of the internal standard (hydrochloride Kö 768) and 500 μl of 2 *N* NaOH (pH 12) were added to a 2-ml plasma sample in a glass-stoppered 30-ml centrifuge tube. The sample was shaken with 10 ml of dichloromethane for 15 min and then centrifuged at 3500 *g* for 15 min.

The aqueous phase was removed. The organic phase was mixed with anhydrous Na_2SO_4 (Merck) and then transferred to another tube and dried in a thermostatted bath at 40° under a gentle stream of nitrogen. Derivatization was performed on the dry residue according to the procedure described above.

Standard curve

Mexiletine standards were prepared by dissolving the appropriate amount of

mexiletine hydrochloride in 0.1 *N* HCl to obtain a stock solution of 20 μg/ml. Appropriate dilutions of this stock solution were then made to obtain the following concentrations: 0.20, 0.50, 0.75, 1.00, 1.25 and 1.50 μg/ml.

Control plasma samples (2 ml) were then spiked with these standard solutions and the internal standard (as described above) and were then extracted.

Peak areas of mexiletine and the internal standard were measured with an electronic integrator (Digital Readout CRS 204, Infotronics, Ireland) and their ratios were plotted as a function of mexiletine concentration.

RESULTS AND DISCUSSION

Preparation and structure of the heptafluorobutyryl derivative

Trifluoroacetic anhydride (TFAA) was selected as the acylating agent by Scott et al. [10] who used a GLC method for the isolation and quantification of two urinary metabolites of mexiletine with both the GLC—MS combination and nuclear magnetic resonance (NMR). Trifluoroacetyl derivatives can be obtained using a very large excess of TFAA and heating the reaction medium at 75° for 1 h. But this method, while perfectly convenient for thermal conductivity or flame-ionization detection, as well as for qualitative studies, is not suitable for assessing plasma concentration by GLC—ECD since the detector becomes polluted and there is a risk of its becoming saturated as it is difficult to achieve complete elimination of both the excess TFAA and the fluorinated degradation products of the reaction.

In this study, heptafluorobutylation provided a much higher selectivity and higher sensitivity and detector stability than trifluoroacetylation for determination in complex plasma extracts. HBFA was recently suggested by Willox et al. [8] as a derivatization agent. With their technique, however, the reaction takes place in a heated medium (90° for 30 min) and requires an excess of HFBA, whereas our method of obtaining the heptafluorobutyryl derivative involves a cold medium and less HFBA. Since operating conditions are not the same, it is impossible to know whether the derivatives obtained in each case have similar chemical structures. The conditions under which acylation of mexiletine (and its 2,4-methyl analogue) took place in this study resulted in a derivative containing one heptafluorobutyryl group on the amine moiety (see *m/e* 375 in Fig. 3). The chemical structure has been confirmed by GLC—MS. Fig. 1 shows the mass spectra of mexiletine (top) and its monoheptafluorobutyryl derivative (bottom). The mass spectrum of mexiletine (as free base) exhibits only two prominent peaks: *m/e* 58 (the C_3H_8N ion) which results from fission of the ether—methylene bond and *m/e* 44 (the C_2H_6N ion) which is the result of cleavage of the methylene—methine bond of the side chain. The molecular ion is at *m/e* 179 and the other peaks are at *m/e* 77, 91, 105, 107 and 121, corresponding to the possible fragmentation previously reported by Scott et al. [10] as shown in Fig. 2.

In the mass spectrum of the derivative, the molecular ion (*m/e* 375) is very small; it corresponds to the formation of the monoheptafluorobutyryl compound. In this spectrum the two most abundant ions were observed at *m/e* 254 and *m/e* 122 and correspond to the two ions formed by cleavage of the —O—CH_2— bond. There is retention of the charge on the fragment

m/e 254 and a proton is transferred to the other part of the molecule (*m/e* 121) to form the ion at *m/e* 122.

Fig. 3 shows the possible structure of a few ions (*m/e* 121, 122, 163 and 254). Many of the other peaks correspond to well-known ions.

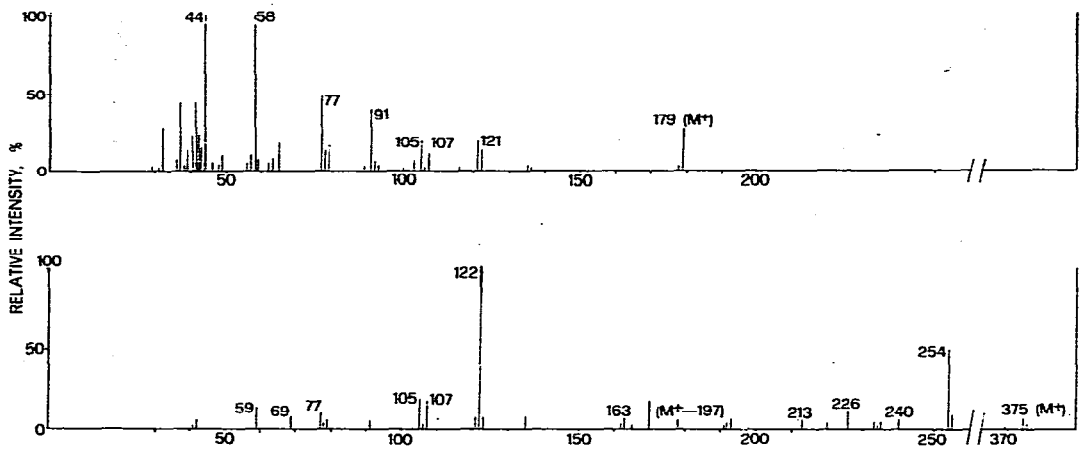


Fig. 1. Normalized mass spectra of mexiletine (top) and of its monoheptafluorobutyryl derivative (bottom).

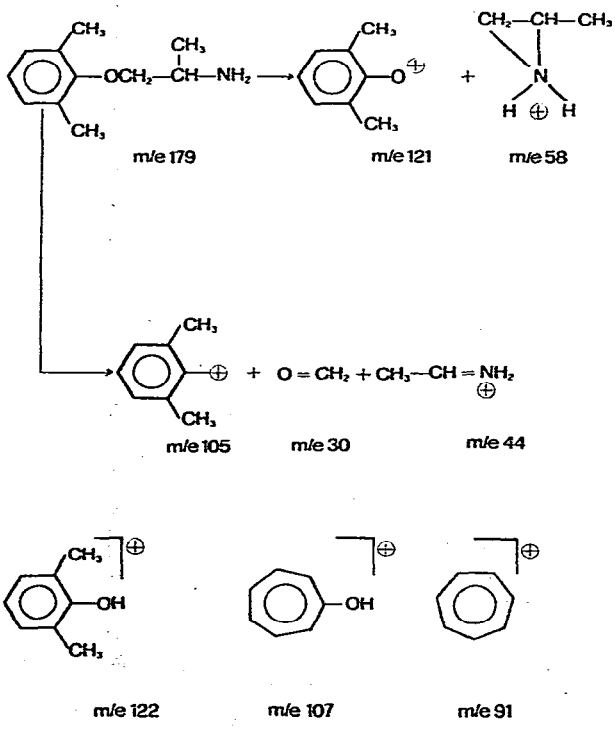


Fig. 2. Fragmentation pattern of mexiletine.

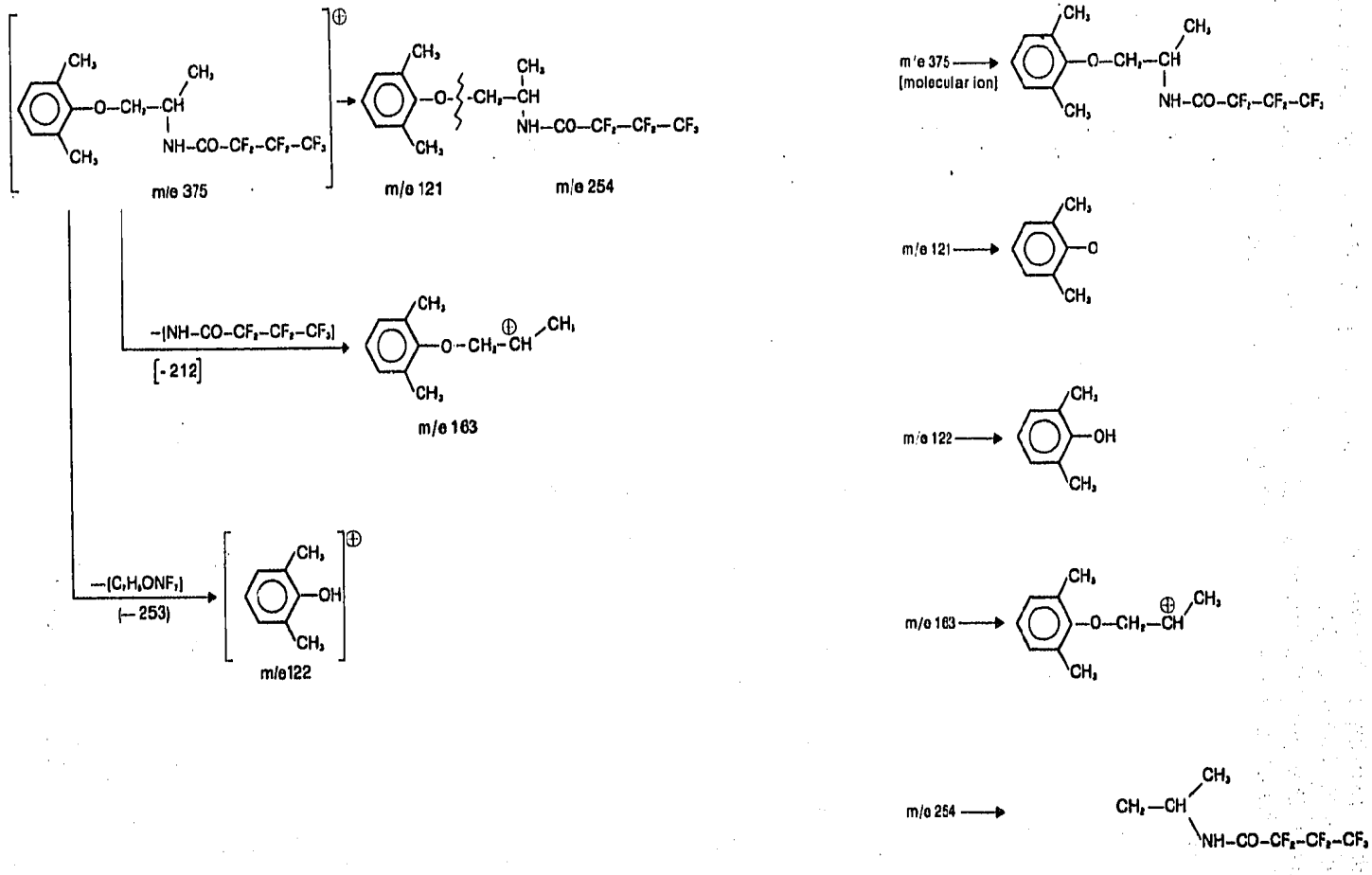


Fig. 3. Possible structure of a few ions resulting from fragmentation of the monoheptafluorobutyryl derivative of mexiletine.

Detector response and stability of the derivative

The monoheptafluorobutyryl derivative has excellent GLC properties and gives a very good response on the ^{63}Ni ECD.

The minimum detectable quantity being defined as the quantity that gives a signal three times that of the background noise level [11, 12], this for mexiletine corresponds to 5 pg under the GLC conditions used here.

This acylation reaction is quantitative and interference in the derivatization reaction by free acid in the anhydride reagent can be avoided by careful handling of HFBA as described in Analytical Procedure. The derivative is stable for at least 72 h at room temperature.

Chromatogram and retention times

With this method, intact mexiletine is measured and the drug is separated from its metabolites usually found in plasma and urine. No interference from normal plasma constituents or from drugs that would possibly be prescribed together with mexiletine (acetylsalicylic acid, α-methyldopa, clonidine, propranolol, pindolol, diazepam, chlordiazepoxide) has been found.

Fig. 4 shows a typical chromatogram obtained with the plasma extracts of

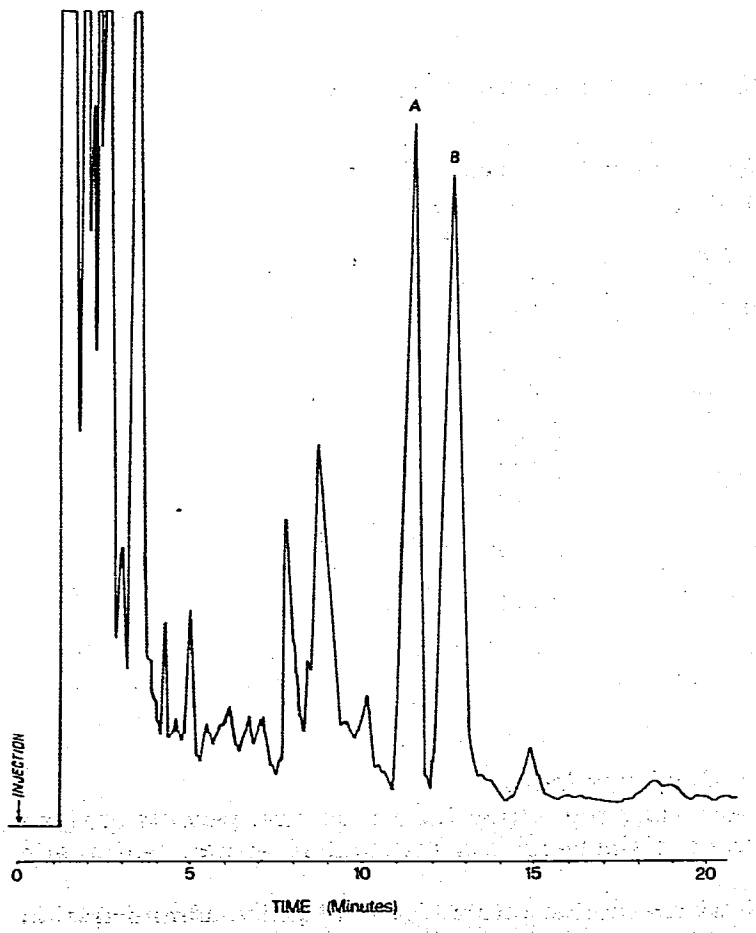


Fig. 4. Gas chromatogram of a calibration curve plasma extract. Retention times are: 11 min 40 sec for mexiletine (peak A) and 12 min 55 sec for internal standard (peak B).

the calibration curve. Retention times are 11 min 40 sec for mexiletine (peak A) and 12 min 55 sec for the internal standard (peak B).

Calibration curve, reproducibility and recovery

The calibration graph is obtained by plotting the ratios of the peak areas of the mono-HFB derivative of mexiletine to that of the internal standard against known amounts of mexiletine added to the plasma. Linearity is observed for concentrations from 0.2—1.0 μg/ml in plasma (r = 0.996; S.D. = 0.008). The precision of the method is adequate, as demonstrated by the recovery and reproducibility studies. The reproducibility of the method was determined in a 10-fold analysis of two plasma samples, one containing 1.0 μg/ml, the other 2.00 μg/ml, of mexiletine. The results are given in Table I. Recovery was assessed by adding known amounts of mexiletine to a pre-analysed plasma sample and calculating the concentration obtained as a percentage of the added concentration. This was carried out for two concentrations of added mexiletine, 0.75 and 1.25 μg/ml. Recoveries were 98.7 and 94.5% respectively.

TABLE I

REPRODUCIBILITY OF MEXILETINE ANALYSIS IN PLASMA

	Mexiletine added to plasma	
	1.00 μg/ml	2.00 μg/ml
	1.030	2.170
	1.020	2.154
	0.885	2.022
	1.011	1.981
	0.860	2.070
	1.030	2.050
	0.990	2.063
	1.075	1.730
	0.995	1.999
	1.085	1.770
mean	0.998	2.0009
S.D.	0.073	0.145
Coefficient of variation (%)	7.3	7.2

Determination of plasma levels of mexiletine

The method described, including the extraction procedure, permits analysis of 15—20 samples per day on a single-column instrument without automatic sampler.

Fig. 5 shows the decline of mexiletine plasma levels after i.v. administration to the 45-year-old patient. Pharmacokinetic data are calculated using a mono-

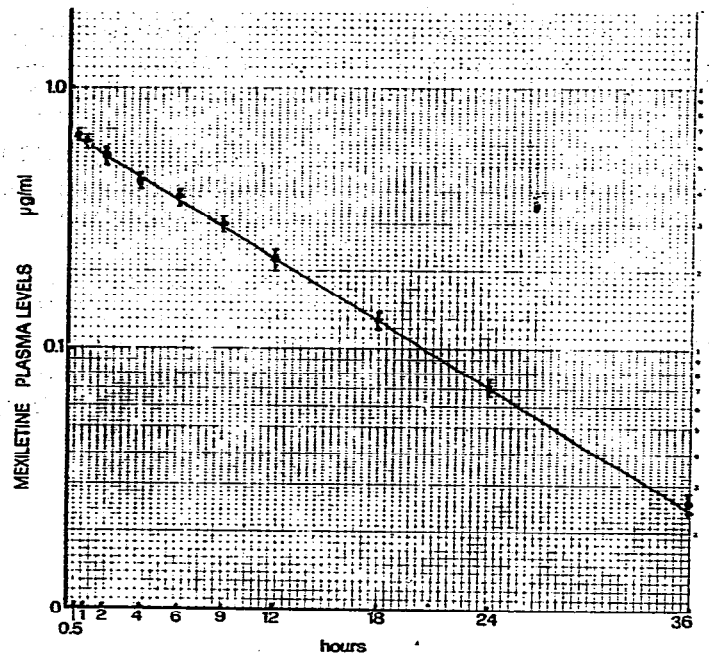


Fig. 5. Mexiletine plasma levels after a 250-mg i.v. dose (3.5 mg/kg) of mexiletine hydrochloride to a 45-year-old patient; each point represents the mean of three determinations (mean ± 2 S.D.).

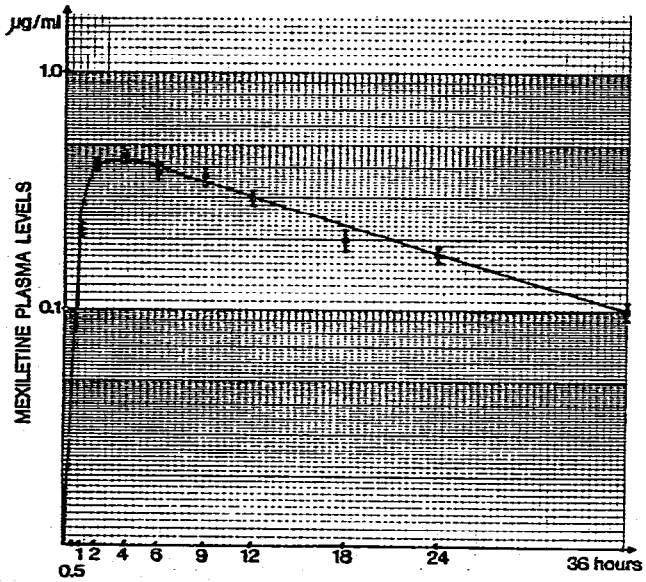


Fig. 6. Plasma elimination curve after a 300 mg oral dose (4.0 mg/kg) of mexiletine hydrochloride. Each point represents the mean of three determinations (mean ± 2 S.D.).

compartmental open model because the loading dose was perfused over a period of 10 min. For this patient, the half-life is 7.9 h, fractional rate constant for elimination is $k_{el} = -0.087\ h^{-1}$ and the central volume of distribution is $V_c = 386$ l.

Fig. 6 shows the plasma elimination curve in an other patient to whom 300 mg of mexiletine hydrochloride were administered per os. In this patient, the calculated pharmacokinetic data are: half-life, 14.9 h; $k_{el} = -0.04649\ h^{-1}$.

Fig. 7 shows a GLC tracing for a plasma sample of this patient.

Urinary elimination of the metabolites (over the 48 h after drug administration) was studied and two metabolites that reacted with both TFAA and HFBA were investigated in particular. After reaction with TFAA their retention times expressed in Kovats indices were similar to those obtained by Scott et al. [10]: i.e. 1770 for compound a and 1837 for compound b. According to the GLC, MS and NMR studies carried out by Scott et al. [10] their structures could correspond to *p*-hydroxymexiletine (a), and to the free base with one of the methyl groups substituted by a $-CH_2OH$ group (b). These structures have also been confirmed by Beckett and Chidomere [13].

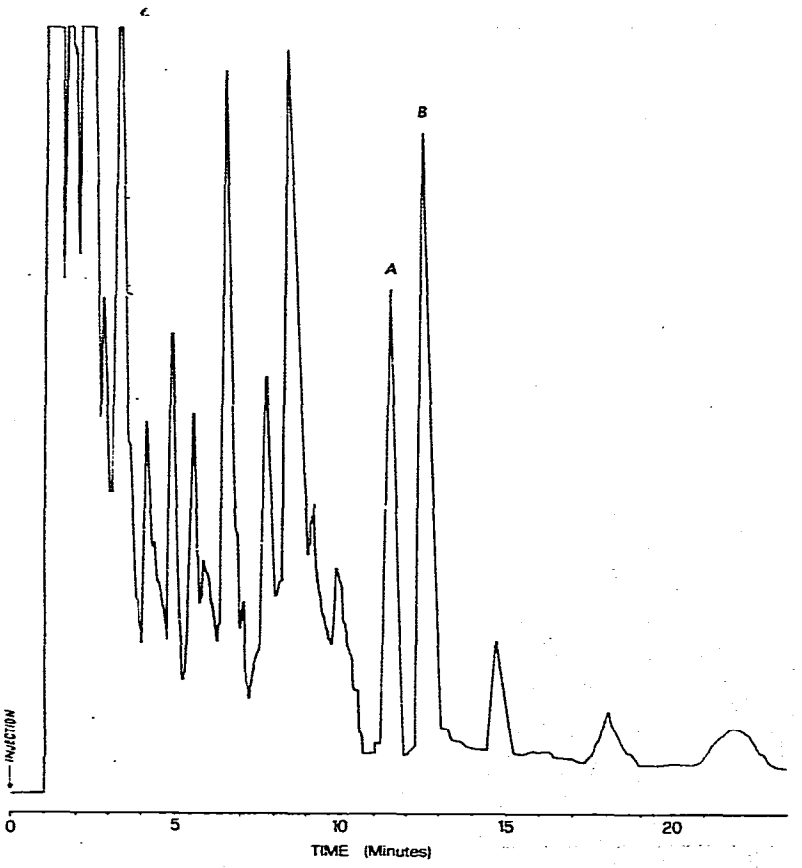
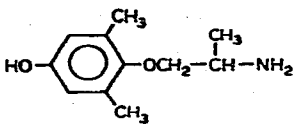
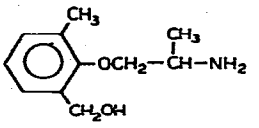


Fig. 7. Gas chromatograph of a plasma extract from a patient receiving a 300 mg oral dose of mexiletine hydrochloride. Peak A and peak B represent mexiletine and internal standard, respectively.



(a)



(b)

CONCLUSION

The present GLC method is simple, highly sensitive and specific because acylated mexiletine (as an unchanged drug) is separated from its metabolites and other drugs commonly used in the treatment of cardiac and vascular diseases. It can be used in single- as well as multiple-administration (particularly with low doses) pharmacokinetic studies.

ACKNOWLEDGEMENT

This study was supported by a grant from Boehringer Ingelheim Laboratories (France).

REFERENCES

1 J.D. Allen, J.M. Kofi Ekve, R.G. Shanks and S.A. Zaidi, Brit. J. Pharmacol., 39 (1970) 183.
2 B.N. Singh and E.M. Vaughan Williams, Brit. J. Pharmacol., 44 (1972) 1.
3 J.D. Allen, J.M. Kofi Ekve, R.G. Shanks and S.A. Zaidi, Brit. J. Pharmacol., 45 (1972) 561.
4 R.A. Clark, D.G. Jullian, J. Nimmo, L.F. Prescott and R.G. Talbot, Brit. J. Pharmacol. 47 (1973) 622.
5 R.G. Talbot, Amer. Heart J., 89 (1975) 537.
6 R.W.F. Campbell, M.A. Dolder, L.F. Prescott, R.G. Talbot, A. Murray and D.J. Jullian, Lancet, i (1975) 1258.
7 J.G. Kelly, J. Nimmo, R. Rae, R.G. Shanks and L.F. Prescott, J. Pharm. Pharmacol., 25 (1973) 550.
8 S. Willox and B.N. Singh, J. Chromatogr., 128 (1976) 196.
9 W.E. Wentworth and E. Chen, J. Chromatogr. Sci., 5 (1967) 170.
10 K.N. Scott, M.W. Couch, B.J. Wilder and C.M. Williams, Drug Metabol. Dispos., 1 (1973) 506.
11 T. Walle, J. Pharm. Sci., 63 (1974) 1885.
12 T. Walle and H. Ehrsson, Acta Pharm. Suecica, 7 (1970) 389.
13 A.H. Beckett and E.C. Chidomere, presented at the Fédération Internationale Pharmaceutique, September 1975, Dublin, Ireland.