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Determination of the metabolites of mexiletine in human plasma

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Abstract: A liquid chromatographic method for the determination of mexiletine and unconjugated metabolites in plasma has been developed. A reversed-phase C_{18} column is used with isocratic elution and either UV or amperometric detection. Sample pretreatment involves double extraction of the metabolites. The method enables the measurement of four mexiletine metabolites at levels as low as 10 ng ml^{-1}, with both precision and accuracy of about 6%. Forty-nine samples from patients receiving mexiletine were analysed. Metabolite **VII** was found to be the major metabolite (mean concentration 225 ng ml^{-1}), metabolites **IX** and **VI** were also found at mean concentrations of 95 and 10 ng ml^{-1}, respectively, whilst metabolite **VIII** was not detected.

Keywords: *Mexiletine; metabolites; human plasma; reversed-phase high-performance liquid chromatography.*

Introduction

Mexiletene is a new antiarrhythmic drug similar in structure to lidocaine, but is effective when administered orally and has a longer plasma elimination half-life. Whereas there have been numerous reports on the clinical pharmacology and analysis of mexiletine [1–3] there appear to have been few studies of its metabolism. Mexiletine is eliminated primarily by metabolism which takes place largely in the liver [4].

The main sources of information on mexiletine metabolism result from the investigations of Beckett and Chidomere [5,6]. These workers developed a sensitive gas–liquid chromatographic method for the analysis of mexiletine and its metabolites and identified eight metabolites in human urine. Four of them are considered major metabolites (Fig. 1). Conjugation of three of these (**VI**, **VIII**, and **IX**), as well as mexiletine itself was also found to occur. Prescott *et al.* [4] found that the urinary concentration of mexiletine increased several-fold after incubation of the urine with bovine liver β-glucuronidase, suggesting that mexiletine *N*-glucuronide is also a metabolite. However, it is unknown whether mexiletine metabolites possess the pharmacological activity of the parent drug.

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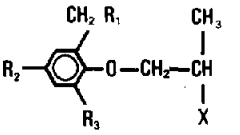

Substance	R_1	R_2	R_3	X
I. mexiletine (Kö 1173)	H	H	CH_3	NH_2
VI (Kö 2127)	H	OH	CH_3	NH_2
VII (Kö 2259)	OH	H	CH_3	NH_2
VIII (Kö 2619)	H	OH	CH_3	OH
IX (Kö 2618)	OH	H	CH_3	OH
internal standard (Kö 768)	H	CH_3	H	NH_2

Figure 1
Structure of mexiletine, its metabolites, and the internal standard. Symbols are as used by Beckett [5, 6]. The code designations used by the manufacturer are shown in brackets.

Recently, Farid and White [7] reported a selective and sensitive HPLC method for the simultaneous determination of mexiletine and two unconjugated metabolites in human plasma. The present study is devoted to obtaining more information about unconjugated metabolites using a procedure that employs an improved analyte extraction and an HPLC method with enhanced specificity of detection. This has enabled substances of interest (**VII** and **IX**) to be detected by absorbance measurements at 214 nm whilst another two (**VI** and **VIII**) are determined using an amperometric detector, which is sensitive to the presence of phenolic groups. Additionally, mexiletine is analysed by a modification of the method of Kelly *et al.* [8].

Experimental

Reagents

Mexiletine, its metabolites **VI**, **VII**, **VIII** and **IX** and 1.(2,4-dimethylphenoxy)-2-aminopropane [15] were gifts from Boehringer Ingelheim Ltd. All other chemicals were analytical grade and the organic solvents for liquid chromatography were of HPLC grade.

Extraction procedures

Extraction A — for the basic metabolites **VI** and **VII**.

(i) Add 0.1 ml of 1.0 M HCl to 1 ml of standard or patient's plasma;
(ii) Add 5 ml of methylene chloride–ethyl ether (50:50, v/v) and shake at 250 vibrations min^{-1} for 10 min;
(iii) Centrifuge at 1600 ***g*** for 5 min and discard the upper organic phase;
(iv) To the lower aqueous phase 0.1 ml of 60 M NH_4OH–6.0 M NH_4Cl (pH 10.0) (60:10, v/v) buffer and agitate for 10 min with methylene chloride (8 ml);
(v) Centrifuge at 1600 ***g*** for 5 min, remove the upper organic phase, add 50 μl of 0.3% HCl in methanol remove the solvent by evaporation at 40°C in a stream of dry air.
(vi) Dissolve the residue in 100 μl of mobile phase "a" (described later) and inject 85 μl of the solution into the liquid chromatograph.

Extraction B — for hydroxylic metabolites **VIII** and **IX**.

(i) Add 0.2 ml of 0.5 Na_2HPO_4 phosphate buffer adjusted to pH 10.0 with 0.5 M NaOH to 1.0 ml of standard or patient's plasma;

(ii) Add methylene chloride (6 ml) and shake at 250 vibrations min^{-1} for 10 min;
(iii) Centrifuge at 1600 ***g*** for 5 min and discard the upper aqueous phase.
(iv) Shake the lower organic phase with 2 ml of 0.1 M HCl for 10 min;
(v) Centrifuge at 1600 ***g*** for 5 min, remove the upper aqueous phase and evaporate the organic phase at 40°C in a stream of dry air;
(vi) Dissolve the residue in 100 μl of mobile phase "a" and inject 85 μl into the liquid chromatograph.

Extraction C — for mexiletine.
(i) Add 0.5 ml of 2.0 M NaOH and 200 μl of internal standard solution (containing 2 μg of standard) to 1.0 ml of standard or patient's plasma;
(ii) Add 5 ml of hexane–isopropyl ether (90:10, v/v) and shake at 250 vibrations min^{-1} for 5 min;
(iii) Separate the mixture by centrifuging at 1600 ***g*** for 5 min;
(iv) Transfer the upper organic phase into a conical tube, add 50 μl of 0.3% v/v HCl in methanol and evaporate as in the previous extraction procedures;
(v) Dissolve the residue in 100 μl of mobile phase "b" (described later) and inject 85 μl of this solution on to the chromatographic column.

Liquid chromatography

Equipment. A Waters Chromatographic system, consisting of model 6000 A pump model U6K injector, a μBondapak C_{18} column (10 μm) (300 × 3.9 mm i.d.) a model 441 UV detector 441 (set at wavelength of 214 nm) and data module M730, was used. The data module records and integrates two detector signals simultaneously. The results were checked by peak height measurement.

A model LC-4B amperometric detector (manufactured by Bioanalytical Systems, West Lafayette, Indiana) and connected in series after the UV detector was also used.

Mobile phase "a". A 150 ml portion of acetonitrile and 20 ml of 1.0 M Na_2HPO_4 buffer (pH 6.5) were added to water and made up to 1000 ml. After mixing all the components together, the solution was degassed using a flow of helium and filtered through a nylon ultipor filter (0.45 μm); the pH was measured and adjusted to 6.5 by addition of H_3PO_4. This mobile phase was used for the elution of metabolites **VI**, **VII**, **VIII**, and **IX**. The retention times at a flow rate of 2.0 ml min^{-1} were as follows: **VI**, 5.1 min; **VII**, 7.2 min; **VIII**, 8.3 min; **IX**, 10.4 min. For comparison, mexiletine under the same conditions has a retention time of 22.6 min.

Mobile phase "b". A 250 ml portion of acetonitrile and 50 ml of 1.0 M Na_2HPO_4 buffer (pH 5) were added to water and made up to 1000 ml. After mixing all the components together, degassing, and filtering as described for solvent "a", the pH was measured and adjusted to 5.0 by addition of H_3PO_4. Retention times at a flow rate of 2.0 ml min^{-1} were 6.4 and 9.2 min for mexiletine and the internal standard, respectively.

Amperometric detection

Two of the substances of interest (**VI** and **VIII**) are electroactive by virtue of a phenolic group. In order to obtain hydrodynamic voltammograms, 30 μl of a standard solution containing 81 ng of **VIII** and 97 ng of **VI** were injected repeatedly into the system, whilst the applied potential of the amperometric detector was increased by increments of 0.1 V

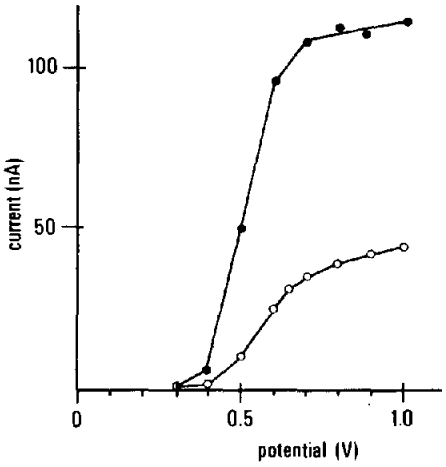

Figure 2
Hydrodynamic voltammograms of ● metabolite **VI** and ○ metabolite **VIII**. Injected amounts are 97 ng and 81 ng, respectively.

after each run, starting from +0.3 V. The results are shown in Fig. 2. Based upon these results a potential of +0.6 V was chosen for metabolite **VI** and +0.7 V for **VIII**. Both these potentials are about 0.1 V short of their respective plateaus, and hence the sensitivities achieved are about 85% of the maximum values. However, in the authors' view the advantage of having a more stable baseline (which is related to the lower voltage) overcomes an immediate loss of about 15% in terms of sensitivity. For routine purposes a voltage of +0.7 V was chosen in order to obtain optimum sensitivity for both metabolites.

Results and Discussion

The precision and accuracy of the method was determined over several days by analysing 1.0 ml aliquots of a plasma sample spiked with a standard solution of mexiletine metabolites. The results are summarized in Table 1.

Subsequently 49 plasma samples from 29 patients undergoing long-term mexiletine therapy were analysed. The mean daily oral dose of mexiletine was 800 mg (range 400–1200 mg) in three or four equal administrations. The mean time of blood sampling after administration was 4.5 h (range 0.5–9 h). The mean plasma concentration of mexiletine was found to be 1610 ng ml^{-1} (as free base, range 310–4320 ng ml^{-1}, S.D. = 820).

In four cases metabolite **VII** was detected at levels which were within the expected therapeutic range for mexiletine, namely 500–2500 ng ml^{-1}. In five more cases, concentration of the metabolite exceeded 400 ng ml^{-1}. On the average, the concentration was 225 ng ml^{-1} (S.D. = 170). Almost every patient also had metabolite **IX** present in their plasma; the mean concentration was 95 ng ml^{-1} (S.D. = 55). Metabolite **VI** was present in the plasma of some patients at a concentration of only 10 ng ml^{-1}, however, more than half of the patients had undetectable levels of metabolite **VI**. In every case metabolite **VIII** was undetectable. Typical chromatograms are shown in Fig. 3.

The relationship between the concentration of mexiletine and metabolite **IX** in plasma is shown in Fig. 4.

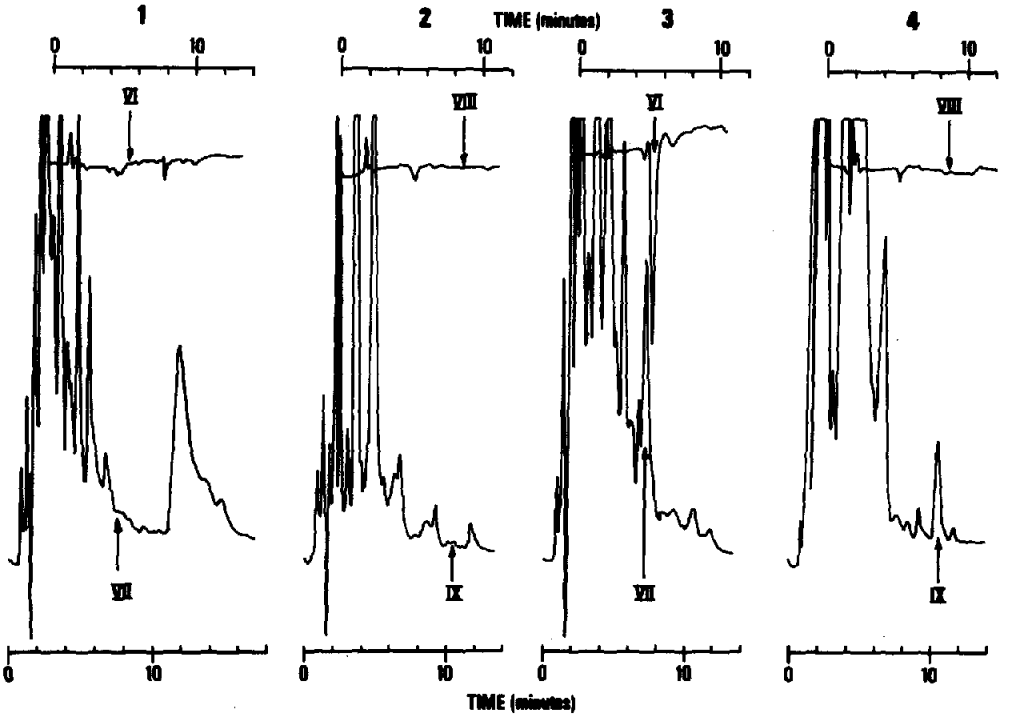

Figure 3
Chromatograms of plasma samples. Section 1 and 2: patient not receiving mexiletine; Section 3 and 4: patient receiving mexiletine. Plasma samples shown in sections 1 and 3 were extracted by procedure A, in sections 2 and 4 by procedure B. Arrows indicate where the respective peaks are expected.

Lower trace — UV detector set at 214 nm, 0.01 a.u.f.s.

Upper trace — amperometric detector set at +0.6 V (sections 1, 3), and +0.7 V (sections 2, 4), 20 nA full scale.

The peaks recorded by the amperometric detector are inverted. Retention times for that detector are approximately 0.3 min longer than those given the UV detector.

Operating conditions: Column μ Bondapak C_{18} (10 μm) 300 × 3.9 mm i.d. Mobile phase: acetonitrile ≃ 0.02 M Na_2HPO_4 (pH 6.5) (15:85, v/v). Flow rate 2.0 ml min^{-1}.

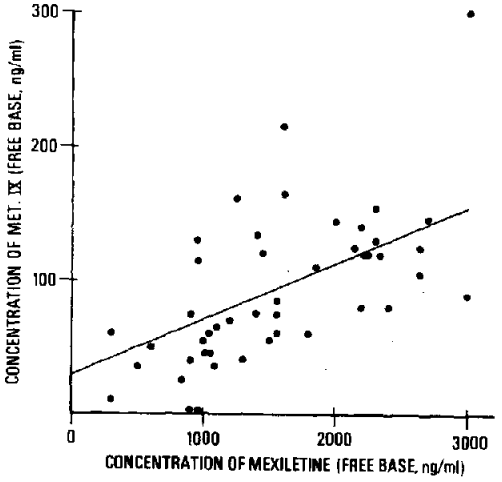

Figure 4
Correlation between the concentrations of mexiletine and metabolite **IX** in plasma.

Table 1
Evaluation of the precision of the analytical method

	Metabolites VI	VII	VIII	IX
Extraction procedure	A	A	B	B
Mobile phase	a	a	a	a
Detection	Amperometric +0.6 V	UV 214 nm	Amperometric +0.7 V	UV 214 nm
Expected (ng ml^{-1}) concentration	79 Free base	101 Free base	79	239
Day to day				
n	6	6	5	5
Observed mean concentration (ng ml^{-1})	78	104	80	242
$\frac{\text{observed}}{\text{expected}} 100\%$ (%)	99	103	101	101
SD	2.7	6.9	4.2	8.4
Relative SD (%)	3.5	6.6	5.3	3.5
Between — run				
n	6	6	6	6
Observed	76	105	76	241
$\frac{\text{observed}}{\text{expected}} 100\%$ (%)	96	104	96	101
SD	2.6	6.3	3.3	14.3
Relative SD (%)	3.4	6.0	4.4	5.9

Plasma concentration of metabolite **IX** was about 4% of that of mexiletine, and the correlation coefficient between the two was 0.614 ($n = 44$, $t = 5.04$, $p < 0.001$). Correlation between mexiletine and metabolite **VII** was even less ($r = 0.419$, $t = 2.96$, $p < 0.005$, $n = 43$). On average, the mexiletine/metabolite **VII** ratio was 9.6, but the range was great (S.D. = 8.5, relative S.D. = 88%). The relative concentration of metabolite **VII** was inconsistent, occasionally reaching 90% that of mexiletine.

Thus far it is unknown whether mexiletine metabolites possess the pharmacological activity of the parent drug. It is to be expected that some might have pharmacological activity, for, as Kates pointed out in a review [9], the great majority of the newer antiarrhythmic drugs have at least one active metabolite. Metabolite **VII** occurs in the greatest concentration and a search for pharmacological activity might profitably be directed towards that metabolite. On the basis of the present analytical results metabolites **VI**, **VIII**, and probably **IX** would seem to be less significant from the clinical point of view.

The method described in this paper also might be suitable for conjugated metabolites with the addition of hydrolysis with beta glucuronidase/aryl sulphatase before extraction [5].

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