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Short Communication

Mexiletine determination in serum by capillary gas chromatography with nitrogen-selective detection*

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Introduction

Mexiletine (Fig. 1), an antiarrhythmic drug closely resembling lidocaine in chemical structure and pharmacological properties, is used for the suppression of various ventricular arrhythmias. Monitoring of mexiletine levels in serum is beneficial in the clinical management of patients receiving this drug. The therapeutic range for mexiletine is 0.5–2.0 mg l^{-1} [1], with the majority of patients exhibiting neurologic side effects (tremor, dizziness, diplopia, seizures) at serum concentrations greater than 2.0 mg l^{-1}. Gastrointestinal and cardiovascular toxicities may also occur.

Analytical methods previously described for mexiletine involve either high performance liquid chromatography (HPLC) [2–12] or gas chromatography (GC) [13–24], and require derivatization [3, 4, 6–8, 14, 16, 19, 20, 23], back-extraction [4, 20, 22–24], a relatively large sample size (≥1.0 ml) [4, 5, 7, 9, 10, 13, 14, 19–21, 23, 24], freezing during extraction [3, 4, 12, 23], duplicate extraction [2], or a long chromatographic analysis time (≥10 min) [4–6, 10, 15, 20]. Furthermore, some of these methods do not evaluate mexiletine metabolites for potential assay interference [2, 4, 5, 8, 9, 12–14, 17, 18, 21–24] and most do not involve automated integrator calculation of results [4–16, 18–24]. A simple, rapid, specific quantitative method has been developed for routine therapeutic drug monitoring of mexiletine in serum. The assay does not permit the quantitation of mexiletine enantiomers, a practice which has not yet proven clinically necessary.

Experimental

Materials

Mexiletine HCl reference standard (Lot F) was purchased from the United States Pharmacopeial Convention, Inc. (Rockville, MD, USA). 4-Methylmexiletine HCl (Lot KOE-1307), hydroxymethylmexiletine oxalate (Lot C) and 4-hydroxymexiletine HCl (Lot ST16) were supplied by Boehringer Ingelheim

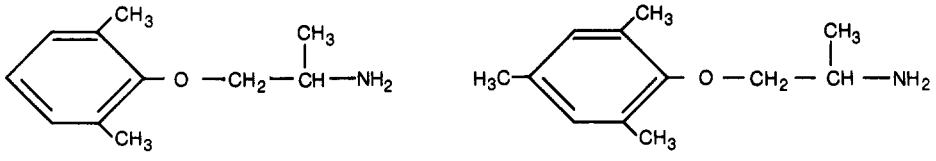

Figure 1
The structures of mexiletine and 4-methylmexiletine.

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(Canada) Ltd (Burlington, ON, Canada). Reacti-Vials® (0.3 and 3.0 ml) were purchased from Pierce (Rockford, IL, USA). Mexiletine Serum Toxicology Control (Lot 5515; Utak Laboratories, Inc., Canyon Country, CA, USA) was kindly provided by NCS Diagnostics Inc. (Mississauga, ON, Canada). Albumin (human) 5% Solution USP, was purchased from Miles Canada Inc. (Etobicoke, ON, Canada). Distilled in glass grade 1-chlorobutane and toluene were purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada) and BDH Inc. (Toronto, ON, Canada) respectively. All other chemicals used were reagent grade.

Apparatus

A Hewlett–Packard (HP) 5890A gas chromatograph (Palo Alto, CA, USA), consisting of a N/P detector and a HP 3392A integrator was used. A HP-5 (cross-linked 5% phenylmethylsilicone) capillary column (11.2 m × 0.2 mm i.d. × 0.5 μm film thickness) was used.

Chromatographic conditions

Helium was utilized as carrier and detector make up gas with the following flow/pressure settings: column + auxillary — 28 ml min^{-1} (column head pressure: 14.5 psi); split vent — 52 ml min^{-1}; septum purge — 4 ml min^{-1}. Air and hydrogen flows were 75 ml min^{-1} and 6 ml min^{-1}, respectively.

The injection port and detector temperatures were 280 and 325°C, respectively. The following temperature program was used: initial column temperature (140°C) held for 0.33 min followed by a 30°C min^{-1} increase to 200°C (held for 0.27 min), followed by a second 30°C min^{-1} increase to 260°C (held for 0.30 min), for a total analysis time of 5.0 min.

Extraction

To 100 μl of serum in a 3.0 ml Reacti-Vial®, internal standard was added (5 μl of a 50 mg l^{-1} aqueous 4-methylmexiletine solution). Saturated sodium tetraborate buffer, pH 9.0 (50 μl) was added and mixed. The solution was vortexed with 0.5 ml of 1-chlorobutane for 20 s and centrifuged at 2500 rpm for 5 min. The upper organic layer was transferred to a 0.3 ml Reacti-Vial® and evaporated just to dryness under nitrogen. The residue was redissolved in 40 μl toluene and a 1–3 μl aliquot was injected into the gas chromatograph.

Quantitation

For initial assessment of assay linearity, standard solutions containing mexiletine at final concentrations ranging from 0.1 to 4.0 mg l^{-1} were prepared to final 100 μl volumes using 5% albumin and appropriate aliquots of aqueous mexiletine solutions (2 mg l^{-1}, 10 mg l^{-1}). Following extraction, calibration (peak area ratios of mexiletine vs internal standard over the specified concentration range) characteristics were determined [correlation coefficient (r); standard error of slope; y intercept].

Having established linearity, for mexiletine quantitation, a single 4.0 mg l^{-1} calibrator was prepared, extracted and injected three times into the gas chromatograph, using the internal standard calibration protocol [25]. Concentrations in subsequent specimens were obtained by direct readout from the integrator.

Results and Discussion

Chromatography

A chromatogram of a calibrator spiked with mexiletine (4.0 mg l^{-1}) and internal standard, 4-methylmexiletine, is presented in Fig. 2. The two compounds were resolved in 3 min with retention times of 2.08 and 2.48 min, respectively.

Linearity and quantitation

Linearity (peak area ratios of mexiletine vs internal standard) over the concentration range of 0.1–4.0 mg l^{-1} was established (r = 0.9976–0.9999; SE of slope = 0.0606) and the line passed through the origin (y intercept = −0.023–0.010). Therefore, automated calculation of mexiletine was made possible and was performed using the internal standard mode on the HP 3392A integrator. Examples of automated integrator printouts of mexiletine concentrations are shown in Figs 2 and 3.

Detection limit

The assay detection limit, assessed at a signal to noise ratio of 3, was 0.1 mg l^{-1} for extraction of a 100 μl serum specimen.

Precision and accuracy

For evaluation of intra-assay precision, aliquots (n = 10) of the Utak commercial mexiletine control specified in the Materials section were analysed on the same day, resulting in an RSD of 3.0%. For inter-assay precision, the

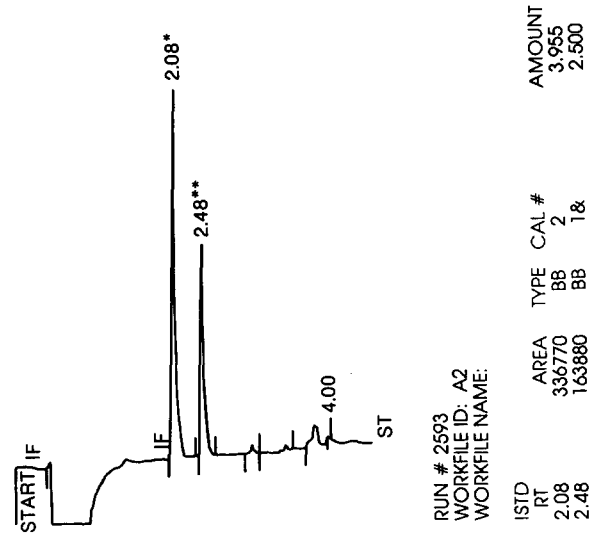

Figure 2
Chromatogram from the analysis of a 4.0 mg l^{-1} mexiletine calibrator containing mexiletine (*) and the internal standard, 4-methylmexiletine (**).

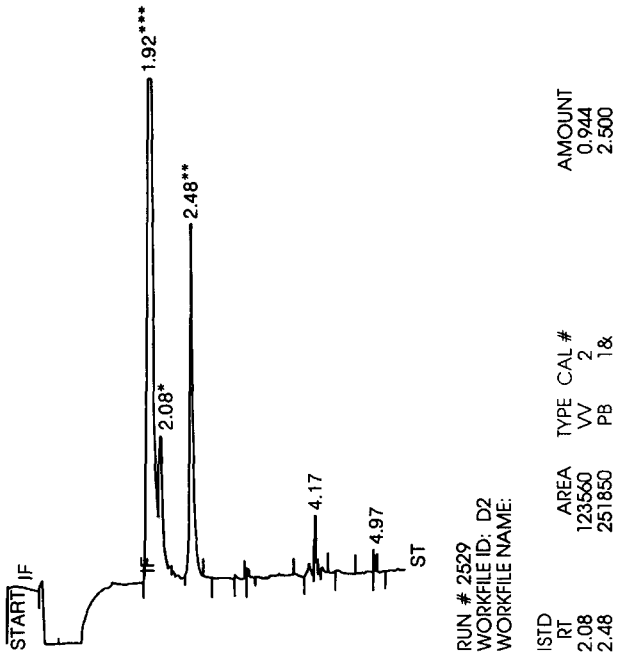

Figure 3
Chromatogram from the analysis of a 5% albumin sample containing mexiletine (*) (1.0 mg l^{-1}), 4-methylmexiletine (**) and nicotine (***) (5.0 mg l^{-1}).

RSD in aliquots (n = 7) of the control analysed on different days was 6.7%.

Assay accuracy was assessed by quantitating mexiletine in aliquots (n = 16) of the Utak control (Target concentration = 1.0 mg l^{-1}). The mean value was 1.01 mg l^{-1} ± 0.07 mg l^{-1}, representing 101.0% of target concentration.

Analytical recovery

Analytical recovery of mexiletine from 5% human albumin at a concentration of 1.0 mg l^{-1} was determined as follows: four 100 µl aliquots spiked with mexiletine and internal standard were extracted. In addition, a sample spiked only with internal standard was extracted, with mexiletine added to the extract after extraction.

Mexiletine concentrations in the aliquots to which the drug was added prior to extraction were compared with the concentration in the aliquot to which mexiletine was added after

extraction. Recovery was 73.8 ± 4.7% (RSD = 6.4%).

Selectivity

No chromatographic interference from endogenous serum constituents and other drugs was found. Nicotine elutes just prior to mexiletine (1.92 min), but did not interfere with the quantitation of the latter, even if present at a concentration of 5.0 mg l^{-1} (Fig. 3), which is far in excess of peak nicotine serum concentrations found in smokers (0.04–0.05 mg l^{-1}) [26].

The two major mexiletine metabolites (hydroxymethylmexiletine, 4-hydroxymexiletine) were evaluated for interference. Under the previously described gas chromatographic conditions, the two metabolites did not co-elute with either mexiletine or 4-methylmexiletine. In fact, neither metabolite (5 mg l^{-1} concentration) was detected when the total analysis time was extended to 20 min. Verification of a lack of interference was also accomplished by electron-impact gas chromatography–mass spectrometry (GC–MS) (HP 5985A GC–MS; column and chromatographic operating conditions similar to those for GC in Method section). Mass spectra of mexiletine in extracts from a spiked 5% albumin sample and a serum sample of a patient prescribed mexiletine ([] = 2.0 mg l^{-1}) were similar, with no additional ions noted in the latter. The *m/z* 58 ion was common to the mass spectra of mexiletine and the two metabolites ($C_3H_8N^+$), whereas the *m/z* 179 ion is unique to mexiletine [27, R.T. Coutts, Faculty of Pharmacy, University of Alberta, personal communication, 1991]. If mexiletine metabolites were contaminating the mexiletine mass spectrum in the patient extract, the ratio of ions 179/58 would be decreased compared with the mass spectrum for mexiletine in the standard extract. However, the ion ratios in spectra from patient and spiked extracts were 0.026 and 0.024, respectively, suggesting no interference from metabolites.

Conclusions

A simple, accurate, precise, specific, sensitive and rapid assay requiring minimal sample volume (100 µl) has been developed for the determination of mexiletine in serum. This method could easily be adapted to any clinical laboratory interested in initiating routine therapeutic drug monitoring of this antiarrhythmic drug.

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