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**Note**

# Determination of lidocaine and its desethylated metabolites in plasma by capillary column gas–liquid chromatography

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The desethylated metabolites of lidocaine, monoethylglycinexylidide (MEGX) and glycinexylidide (GX), have local anaesthetic[1] and anti-arrhythmic[2,3] activity. It could therefore be of interest to measure the levels of these metabolites together with the levels of the parent product when monitoring patients on lidocaine therapy.

Although several workers have reported the determination of lidocaine together with MEGX in plasma[4–6], there have been only a few reports on the simultaneous determination of lidocaine and both desethylated metabolites. Difazio and Brown[7] used gas–liquid chromatography (GLC) with a flame-ionization detector but gave only an incomplete description of their method. Adjepon-Yamoah and Prescott[8] described a time-consuming method involving nitrogen-detection GLC with column temperature programming, after acetylation of the metabolites. Strong and co-workers[1,2] used mass fragmentography, but concentrations of GX below 0.5 μg/ml could not be measured.

We have developed an isothermal GLC method using a capillary column and a nitrogen-specific detector for the rapid simultaneous determination of lidocaine, MEGX and GX in human plasma samples, with derivatization of the metabolites.

## EXPERIMENTAL

### *Materials*

Lidocaine hydrochloride (Xylocaine; Astra, Läkemedel AB, Södertälje, Sweden) was obtained as a 2% solution. Monoethylglycinexylidide hydrochloride and glycinexylidide hydrochloride were provided by Rune Sandberg (Astra). Trimecaine (W-45184) was donated by Astra Pharmaceutical Products (Worcester, Mass., U.S.A.). The derivatizing reagent was trifluoroacetic anhydride (TFAA; Pierce, Rockford, Ill., U.S.A.).

### *Instrumental conditions*

The analysis was performed on a Hewlett-Packard Series 5730A gas chromatograph, equipped with a dual nitrogen–phosphorus flame-ionization detector (Model 18789A).

A 20 m × 0.5 mm I.D. glass capillary column, wall coated with OV-17 (RSL,

St. Martens-Latem, Belgium) was used. Hydrogen was used as the carrier gas at a flow-rate of 4 ml/min, 30 ml/min of helium as make-up gas and 100 ml/min of air were also added to the detector. The temperatures used were injector 300°, column 190° and detector 300°. Direct on-column injection without stream splitting was used.

The peak areas were recorded on a recording integrator (Hewlett-Packard 3380A).

*Procedure*

All glassware was silanized as described previously[9]. A 4-$\mu$g sample of the internal standard trimecaine and 40 $\mu$l (0.5 *M*) of triethylamine (to prevent adsorption on the glassware[10]) in methylene chloride were added to 2 ml of acidified plasma in a silanized glass-stoppered 10-ml centrifuge tube. The solution was shaken for 5 min with 5 ml of redistilled methylene chloride. After centrifugation, the organic phase was discarded and to the aqueous phase 0.5 ml of 5 *M* sodium hydroxide solution was added. The sample was extracted twice for 5 min with 5 ml of methylene chloride. The organic extract was transferred into a 5-ml glass-stoppered conical tube and evaporated to dryness under nitrogen. Immediately after evaporation, 200 $\mu$l of *n*-hexane and 40 $\mu$l of trifluoroacetic anhydride were added and the tube was tightly capped. The mixture was allowed to react for 5 min at room temperature. The sample was taken to dryness under a stream of nitrogen and the residue was stored at −18° and analysed within 6 h. A 20-$\mu$l volume of ethyl acetate was added, and 0.2 $\mu$l of the resulting solution was injected immediately into the gas chromatograph.

*Quantitation and reproducibility*

Human plasma samples spiked with increasing concentrations of lidocaine (0.5–16 $\mu$g per 2 ml), MEGX and GX (0.1–6.0 $\mu$g per 2 ml) were carried through the whole procedure. These concentrations were chosen because they have been reported in patients treated intravenously with lidocaine[1,4,6,11,12]. The ratio of the peak area of the test product to the peak area of the internal standard was plotted against the concentration of the test product and a least-squares linear regression analysis was performed. Unknown drug concentrations were determined from this calibration graph.

*Human samples*

Blood samples were taken from patients with acute myocardial infarction who had been treated for ventricular premature beats with intravenous infusions of lidocaine in the usual doses[13] for at least 42 h. Plasma was separated by centrifugation as soon as possible after sampling and to 2 ml of plasma 1 ml of 1 *N* hydrochloric acid was added to prevent loss of MEGX and GX during storage[8]. The acidified plasma was stored at −20° until taken for assay.

## RESULTS AND DISCUSSION

The chromatographic peaks obtained for lidocaine and trimecaine are symmetrical, but the peaks for MEGX and GX tail when these substances are analysed as such. This problem was overcome by making volatile TFA derivatives of MEGX and GX. A typical chromatogram is shown in Fig. 1.

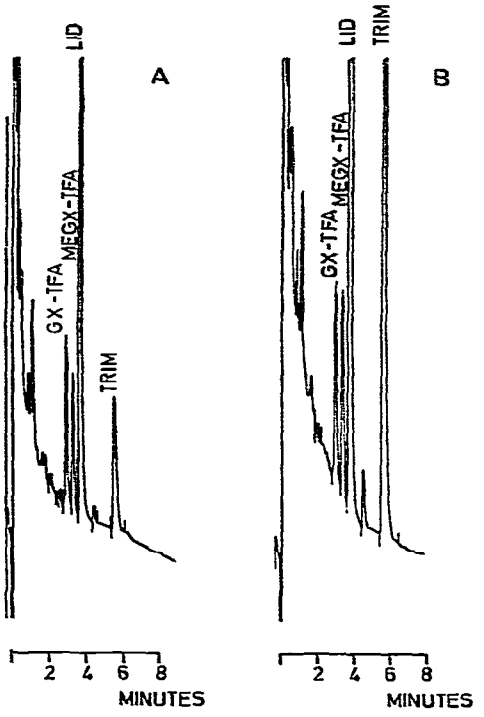


Fig. 1. Gas chromatograms of 2 ml of extracted human plasma. A, Spiked plasma sample: N-trifluoroacetylglycinexylidide (2 $\mu$g), N-trifluoroacetylmonoethylglycinexylidide (2 $\mu$g), lidocaine (12 $\mu$g) and trimecaine (4 $\mu$g). B, Plasma of patient F.D. during intravenous infusion of lidocaine, 4 $\mu$g of trimecaine was added before the extraction procedure. Note the presence of N-trifluoroacetylglycinexylidide and N-trifluoroacetylmonoethylglycinexylidide.

When the ratio of the peak area of lidocaine to that of the internal standard was calculated and plotted against the concentration of the product in plasma, a linear relationship was found for the concentration ranges studied. The same was true for MEGX-TFA and GX-TFA. The relative standard deviations for replicate analyses of plasma containing 1 $\mu$g per 2 ml of plasma of the different substances were 4.16 ($n = 10$) for lidocaine, 6.78 ($n = 10$) for MEGX and 7.32 ($n = 10$) for GX. The lower limit of detection was approximately 20 ng/ml for the three products. A typical gas chromatogram of a patient treated with lidocaine is shown in Fig. 1.

The method described here is simple and the GLC analysis takes only 6 min. It has a high degree of specificity due to the improved resolution by the capillary column in comparison with the packed column. No interfering chromatographic peaks were noted in plasma samples obtained before the lidocaine therapy was started in several patients.

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