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SIMPLE GAS–LIQUID CHROMATOGRAPHIC METHOD FOR THE MEASUREMENT OF MEXILETINE AND LIGNOCAINE IN BLOOD-PLASMA OR SERUM

DAVID W. HOLT, ROBERT J. FLANAGAN, ANGELA M. HAYLER and MARY LOIZOU
Poisons Unit, Guy's Hospital, London, SE1 9RT (Great Britain)
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SUMMARY

A simple method has been developed for the measurement of mexiletine and lignocaine in blood-plasma or serum at the concentrations attained during therapy. A relatively small (200 μl) sample volume is made basic and extracted with 50 μl of chloroform containing internal standards, and the extract is analysed directly by gas–liquid chromatography with flame-ionisation detection on two separate columns. The instrument calibrations are linear and pass through the origin of the graphs. Neither solvent transfer nor evaporation steps are used in the extraction procedure, which takes less than 3 min to complete, and no interference from either endogenous sample constituents or other drugs has been observed.

INTRODUCTION

Mexiletine [1-methyl-2-(2,6-xylyloxy)ethylamine] and lignocaine (α-diethylamino-2′,6′-dimethylacetoanilide) are used to treat cardiac dysrhythmias of ventricular origin and the plasma concentrations normally attained during therapy are within the range 0.75–2.00 mg/l (ref. 1) and 2–5 mg/l (ref. 2) respectively. Since it is frequently difficult to establish the optimum dose of either drug, plasma or serum measurements can be of clinical value.

Published methods for mexiletine assay required at least 1 ml of sample[3–9], multiple-extraction steps[3–5], solvent evaporation[3–9] and derivatisation of the drug prior to gas–liquid chromatographic (GLC) analysis[3–7,9]. In addition to flame-ionisation detection (FID)[3,6], both electron-capture[4,5,9] and nitrogen-selective detection[7,8] have been employed. Only one of these methods[8] suggested the analysis of both mexiletine and lignocaine in a single extract, but different GLC conditions were required for each compound. Additional GLC methods for the measurement of lignocaine have been described[10–16], but only one[13] did not involve a solvent evaporation step, whilst several required either back extraction[11,13,16] or derivatisation of the drug[14] in addition to a sample volume in the range 0.5–2.5 ml. A liquid chromatographic method has been described[18], but a sample volume of 1 ml and a solvent evaporation step were required.

The method described here involves the extraction of 200 μl plasma with 50 μl of internal standard solution in chloroform, at an alkaline pH, followed by the GLC–FID analysis of a portion of the organic phase[19]. The method can be used to measure both mexiletine and lignocaine in a single extract by injection of separate portions of the extract onto different column systems, and is suitable for the measurement of these drugs at the concentrations encountered during therapy.

EXPERIMENTAL

Materials and reagents

Mexiletine hydrochloride and a control sample of this drug in human plasma (equivalent to 1.27 mg/l mexiletine base) were obtained from Boehringer Ingelheim (Bracknell, Great Britain). Lignocaine hydrochloride and an aqueous solution of this compound (equivalent to 260 mg/l lignocaine base) were obtained from Astra Chemicals (Watford, Great Britain). This latter solution was used to prepare a quality control sample in heparinised human plasma at a concentration equivalent to 3.91 mg/l lignocaine. The internal standards, 2,7-dimethylquinoline (DMQ) (Aldrich, Gillingham, Great Britain) and eicosane (Koch-Light Labs., Colnbrook, Great Britain) were used at concentrations of 10 and 15 mg/l, respectively, in a single chloroform solution. γ-Glycidoxypropyltrimethoxysilane (A-187) was obtained from H.S. Chromatography Packings (Bourne End, Great Britain). Chloroform and tris-(hydroxymethyl)aminomethane (Tris) were analytical-reagent grade; the latter compound was used as a 2 mol/l aqueous solution.

Apparatus

Hamilton repeating mechanisms fitted with 1.0- and 2.5-ml Hamilton gas-tight luer-fitting glass syringes (Field Instruments, Richmond, Great Britain) were used to dispense volumes of 20 and 50 μl, respectively. Everett stainless-steel needles (No. II serum) were fitted to these syringes. Dreyer tubes (Poulten, Selfe and Lee, Wickford, Great Britain) and an Eppendorf Model 5412 centrifuge[19] (Anderman, East Molesey, Great Britain) were used in the extraction.

Gas–liquid chromatography

A Pye Series 104 dual column gas chromatograph equipped with flame-ionisation detectors was used. Integration of peak areas was performed using a Hewlett-Packard 3352 data system. The detector oven temperature was 250° and the column oven temperature was 195° (mexiletine) and 212° (lignocaine); injection block heaters were not employed. The nitrogen (carrier gas) flow-rate was 60 ml/min and the flame was supplied by oxygen and hydrogen at inlet pressures of 10 and 15 p.s.i., respectively, giving flow-rates of approximately 200 and 60 ml/min.

Coiled glass columns of internal diameter 4 mm were silanised by immersion in 2% dichlorodimethylsilane in toluene for 1 h, and were subsequently rinsed in methanol and dried at 100°. For mexiletine, a 2.1-m column was packed with 10% Apiezon L–2% potassium hydroxide on 80–100 mesh Chromosorb W AW and for lignocaine, a 2.8-m column was packed with 3% OV-101 on 80–100 mesh Supelcoport. Both packings were purchased ready-prepared from Chromatography Services (Merseyside, Great Britain). The packed columns were conditioned at 220° (mexiletine)

and 250° (lignocaine) with a nitrogen flow of 60 ml/min for 15 h. The OV-101 column was treated by injection with a total of 50 μl of A-187 and, thereafter, fortnightly on-column injections of 5–10 μl of this compound were made to maintain the column in a deactivated form[19].

The relative retention times of mexiletine and some other compounds on the Apiezon L–potassium hydroxide system and of lignocaine on the OV-101 system are shown in Tables I and II, respectively. The chromatography of a chloroform solution containing both mexiletine and DMQ is shown in Fig. 1 and that of lignocaine and eicosane in Fig. 2.

TABLE I

RETENTION DATA OF MEXILETINE AND SOME OTHER COMPOUNDS ON THE APIEZON L–POTASSIUM HYDROXIDE COLUMN SYSTEM

Compound	*Retention time (relative to DMQ)*
Nicotine	0.63
Norpseudoephedrine	0.65
Pseudoephedrine	0.67
Ephedrine	0.68
Chlorphentermine	0.70
Mexiletine	0.73
Phenmetrazine	0.94
Diethylpropion	1.01
Nikethamide	1.06
Lignocaine	4.94
Eicosane	9.11

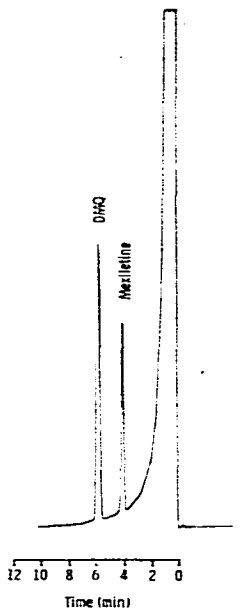
TABLE II

RETENTION DATA OF LIGNOCAINE AND SOME OTHER COMPOUNDS ON THE OV-101 COLUMN SYSTEM

Compound	*Retention time (relative to eicosane)*
Mexiletine	0.25
DMQ	0.26
Pethidine	0.46
Caffeine	0.61
Monoethylglycinexylidide	0.62
Diphenhydramine	0.67
Ethoheptazine	0.74
Lignocaine	0.74
Oxprenolol	0.79
Aminopyrine	0.83
Orphenadrine	0.87
Brompheniramine	0.95
Chlorpheniramine	1.50

Extraction procedure

Sample (200 μl), Tris solution (20 μl) and internal standard solution (50 μl) were added to a Dreyer tube. The contents were vortex mixed for 30 sec and the tube



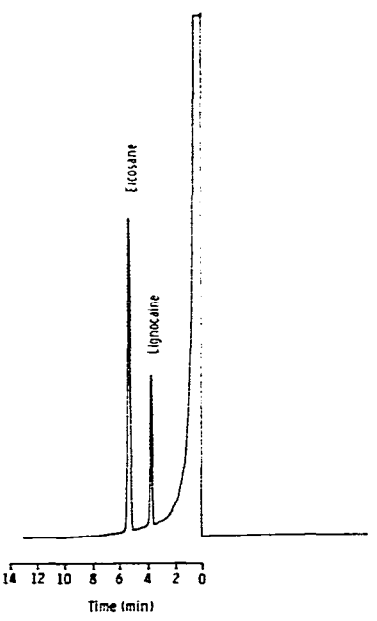


Fig. 1. Chromatogram obtained on analysis of a solution of mexiletine (10 mg/l) and DMQ (10 mg/l) in chloroform on the Apiezon L–potassium hydroxide column system; 3-μl injection.

Fig. 2. Chromatogram obtained on analysis of a solution of lignocaine (8 mg/l) and eicosane (15 mg/l) in chloroform on the OV-101 column system; 3-μl injection.

was centrifuged for 2 min at 9950 *g*. A 3–5-μl portion of the chloroform phase was obtained by drawing 5 μl of air into a gas chromatographic syringe and passing the syringe needle through the basic layer into the chloroform. The air was expelled and a portion of the extract was taken up into the syringe for injection onto the appropriate column.

The extraction was performed in duplicate and the mean result taken. If the difference between the duplicates was greater than 10%, the analysis was repeated.

RESULTS AND DISCUSSION

Mexiletine

Instrument calibration. Standard solutions containing mexiletine at concentrations of 0.5, 1.0, 2.0, 3.0, 4.0, 8.0 and 16.0 mg/l were prepared in heparinised human plasma by dilution of an aqueous solution of mexiletine hydrochloride equivalent to 1 g/l mexiletine. A calibration graph of peak area ratio (mexiletine/DMQ) against mexiletine concentration was linear, with zero intercept, across the range of these standards. The calibration gradient (peak area ratio/plasma drug concentration) was normally 0.185 l/mg. In practice, the instrument was calibrated using a 2.0 mg/l plasma standard, and this calibration was confirmed by the analysis of an internal quality control sample containing mexiletine (1.5 mg/l) prepared from an independent stock solution.

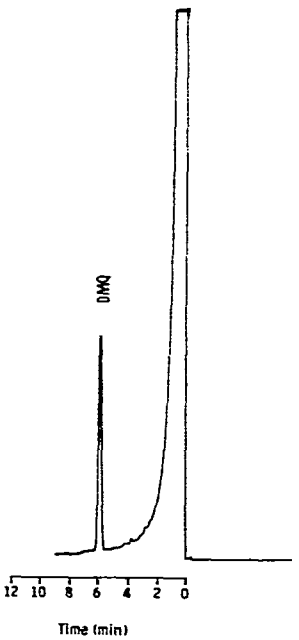
Reproducibility and accuracy. The intra-assay coefficient of variation (CV) was

5.1% at 0.8 mg/l ($n = 10$) and 4.3% at 4 mg/l ($n = 10$). The inter-assay CV was 5% at 1.5 mg/l ($n = 24$).

The mean concentration of mexiletine in the external quality control sample was 1.23 ± 0.07 (S.D.) mg/l ($n = 18$), 96.8% of the weighed-in value.

Specificity. No interference has been observed in extracts of mexiletine free plasma; a chromatogram from such an analysis is illustrated in Fig. 3 and the analysis of a sample from a patient receiving mexiletine is shown in Fig. 4. None of the common antiarrhythmic drugs (*e.g.* lignocaine, disopyramide, procainamide, quinidine, verapamil and phenytoin) interferes with this assay (Table I).

Sensitivity. The limit of sensitivity was 0.1 mg/l; a 0.1 mg/l plasma standard gave a mean value of 0.096 mg/l ± 0.003 (S.D.) ($n = 5$).



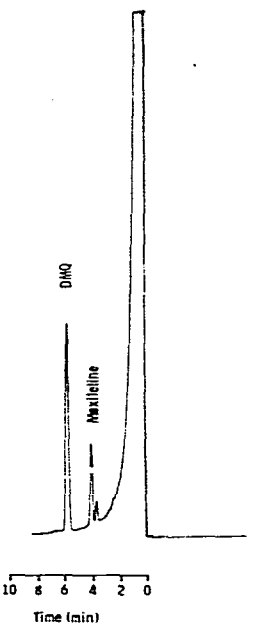


Fig. 3. Chromatogram obtained on analysis of an extract of drug-free human plasma; 3-μl injection. The DMQ concentration was 10 mg/l.

Fig. 4. Chromatogram obtained on analysis of an extract of plasma from a patient receiving mexiletine; 2.5-μl injection. The DMQ concentration was 10 mg/l and the plasma mexiletine concentration was 1.0 mg/l.

Lignocaine

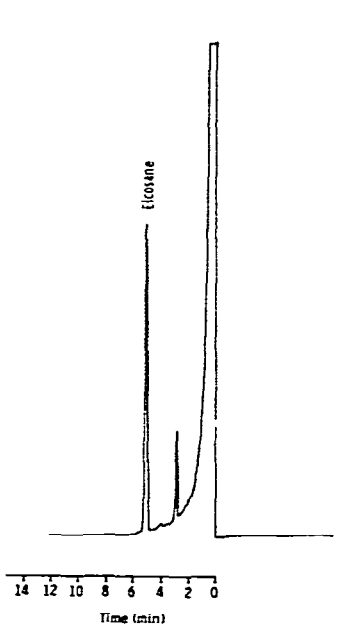
Instrument calibration. Standard solutions containing lignocaine at concentrations of 0.5, 1, 2, 4, 8, 16 and 32 mg/l were prepared in heparinised human plasma by dilution of an aqueous solution of lignocaine hydrochloride equivalent to 1 g/l lignocaine. A calibration graph of peak area ratio (lignocaine/eicosaine) against lignocaine concentration was linear, with zero intercept, across the range of these standards. The calibration gradient (peak area ratio/plasma drug concentration) was normally 0.088 l/mg.

For the routine calibration of the instrument, a 3.0 mg/l plasma standard was analysed and the calibration was confirmed by analysis of the 3.91 mg/l quality control sample.

Reproducibility and accuracy. The within-assay CV of the assay was 6.1% at 2 mg/l ($n = 10$) and 5.6% at 9 mg/l ($n = 10$). The inter-assay CV was 2% at 4 mg/l ($n = 8$). The mean concentration of lignocaine in the external quality control sample was 3.95 mg/l ± 0.15 (S.D.) ($n = 10$), 101% of the expected value.

Specificity. No compounds which could interfere with this assay have been observed in extracts of lignocaine-free plasma; a typical chromatogram illustrating such an analysis is shown in Fig. 5 and the analysis of a plasma sample from a patient receiving lignocaine is shown in Fig. 6. None of the common antiarrhythmic drugs interferes with this assay (Table II). The major metabolite of lignocaine, monoethylglycinexylidide, was not resolved from caffeine on this system.

Sensitivity. The limit of sensitivity was 0.2 mg/l; a plasma standard containing this amount gave a mean value of 0.21 mg/l ± 0.02 (S.D.) ($n = 5$).



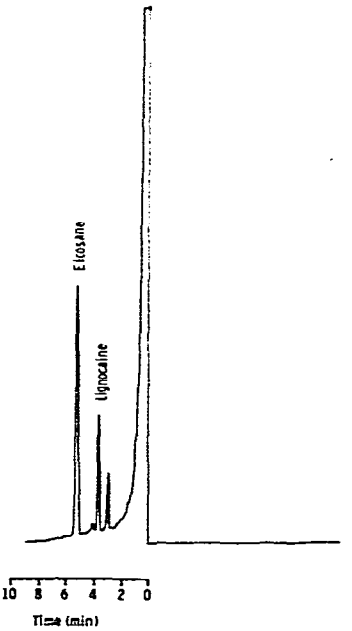


Fig. 5. Chromatogram obtained on analysis of an extract of lignocaine-free human plasma; 4-μl injection. The eicosane concentration was 15 mg/l and the peak eluting at retention time 0.56 relative to eicosane was caffeine.

Fig. 6. Chromatogram obtained on analysis of an extract of plasma from a patient receiving lignocaine; 3-μl injection. The eicosane concentration was 15 mg/l and the plasma lignocaine concentration was 3.4 mg/l. The peak eluting at retention time 0.56 relative to eicosane was caffeine.

CONCLUSIONS

For the results of plasma cardioactive drug measurements to be of clinical relevance rapid assays are needed and, since patients may receive both mexiletine and

lignocaine during acute coronary care, the measurement of both compounds in a single extract is advantageous. Furthermore, if several analyses are to be performed on a single sample, the assay of choice should require only a small volume of blood.

The method described here satisfies these criteria—total analysis time for calibration standards, quality control and patient samples is less than 30 min, only 200 μl plasma are required and, using a mixed internal standard solution, both drugs can be measured in a single extract.

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REFERENCES

1 R. G. Talbot, R. A. Clark, J. Nimmo, J. M. M. Neilson, D. G. Julian and L. F. Prescott, *Lancet*, ii (1973) 399.
2 J. Koch-Weser, *5th Int. Congr. Pharmacol.*, 3 (1972) 69.
3 R. J. Perchalski, B. J. Wilder and R. H. Hanmer, *J. Pharm. Sci.*, 63 (1974) 1489.
4 J. J. Cereghimo, B. T. Wilder, H. J. Kupferberg, W. D. Yonekawa, R. J. Perchalski, R. E. Ramsey, B. G. White, J. K. Penry and L. D. Smith, *Epilepsia*, 16 (1975) 665.
5 S. Willox and B. N. Singh, *J. Chromatogr.*, 128 (1976) 196.
6 M. A. Kiddie, R. B. Royds and T. R. D. Shaw, *Proc. Brit. Pharmacol. Soc.*, (1973) 674P.
7 J. G. Kelly, J. Nimmo, R. Rae, R. G. Shanks and L. F. Prescott, *J. Pharm. Pharmacol.*, 25 (1973) 550.
8 I. D. Bradbrook, C. James and H. J. Rogers, *Brit. J. Clin. Pharmacol.*, 4 (1977) 380.
9 A. Frydman, J. P. Lafarge, F. Vial, R. Rulliere and J. M. Alexandre, *J. Chromatogr.*, 145 (1978) 401.
10 J. B. Keenaghan, *Anaesthesiology*, 29 (1968) 110.
11 F. Reynolds and A. H. Beckett, *J. Pharm. Pharmacol.*, 20 (1968) 704.
12 J. M. Strong and A. J. Atkinson, *Anal. Chem.*, 44 (1972) 2287.
13 N. Benowitz and M. Rowland, *Anaesthesiology*, 39 (1973) 639.
14 K. K. Adjepon-Yamoah and L. F. Prescott, *J. Pharm. Pharmacol.*, 26 (1974) 889.
15 T. R. Irgens, W. M. Henderson and W. H. Shelver, *J. Pharm. Sci.*, 65 (1976) 608.
16 R. L. Nation, E. J. Triggs and M. Selig, *J. Chromatogr.*, 116 (1976) 188.
17 G. Caille, J. le Lorier, Y. Latour and J. G. Besner, *J. Pharm. Sci.*, 66 (1977) 1383.
18 R. F. Adams, F. L. Vandemark and G. Schmidt, *Clin. Chim. Acta*, 69 (1976) 515.
19 A. M. Hayler and R. J. Flanagan, *J. Chromatogr.*, 153 (1978) 461.