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Note

Simple method for the measurement of tocainide and lignocaine in blood plasma or serum using gas—liquid chromatography with flame ionisation detection

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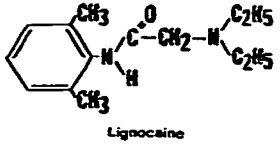
Tocainide [2-amino-N-(2,6-dimethylphenyl)propanamide; Fig. 1] is an orally-effective analogue of lignocaine, and may have advantages over this latter compound in the chronic treatment of ventricular arrhythmias [1—3]. Published high-performance liquid chromatographic methods for the measurement of plasma tocainide concentrations require long extraction times (5—15 min) [4—7], solvent evaporation [5—7], derivatisation following a multiple extraction [6] or relatively large (1 ml) sample volumes [4,5,7]. The gas-liquid chromatographic (GLC) methods described [1,8,9] also require derivatisation following solvent extraction and evaporation steps.



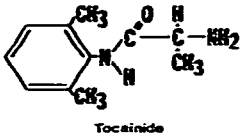


Fig. 1. Structural formulae of tocainide and lignocaine.

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The method described here involves the extraction of only 100 μl of plasma or serum at an alkaline pH with 50 μl of chloroform containing an internal standard, followed by the direct analysis of a portion of the resulting extract using GLC with flame ionisation detection [10]. This technique can be used to measure plasma concentrations of both lignocaine and tocainide simultaneously and has been found to be suitable for the measurement of these drugs at the concentrations attained during therapy.

EXPERIMENTAL

Materials and reagents

Lignocaine and tocainide hydrochlorides were supplied by Astra (Watford, Great Britain) and were used as aqueous solutions containing 1.00 g/l free base of each drug.

The internal standard, *n*-eicosane (C_{20}), was obtained from Koch-Light (Colnbrook, Great Britain) and was used as a 10 mg/l solution in chloroform (analytical reagent grade). γ-Glycidoxypropyltrimethoxysilane (A-187) was obtained from H.S. Chromatography Packings (Bourne End, Great Britain). Tris(hydroxymethyl)aminomethane (analytical reagent grade) was used as a 2 mol/l aqueous solution (Tris solution).

TABLE I

TABLE OF RETENTION TIMES RELATIVE TO *n*-EICOSANE

Compound	Relative retention time
Norpseudoephedrine	0.10
Brompheniramine	0.12
Nicotine	0.12
Pseudoephedrine	0.12
Mexiletine	0.13
Ephedrine	0.14
2,7-Dimethylquinoline [cf. ref. 10]	0.16
Diethylpropion	0.20
Nikethamide	0.20
Chlorphentermine	0.25
Pethidine	0.40
Tocainide	0.45
Monoethylglycinexylidide	0.62
Diphenhydramine	0.63
Ethoheptazine	0.63
Caffeine	0.65
Lignocaine	0.71
Oxprenolol	0.71
n-Eicosane	1.00
Procainamide	2.18
Disopyramide	5.21
Quinidine*	11.3—12.9

*Tailing peak, retention times measured at 1.0 g/l and 10 mg/l, respectively.

Gas—liquid chromatography

A Pye Series 204 dual-column gas chromatograph equipped with flame ionisation detectors was used and integration of peak areas was performed using a Hewlett-Packard 3352 data system. The detector oven temperature was 250°C and the column oven temperature was 230°C. Injection block heaters were not used. The nitrogen (carrier gas) flow-rate was 40 ml/min, and the oxygen and hydrogen inlet pressures were 1.03 and 1.38 bar, respectively, giving flow-rates of approximately 400 and 40 ml/min. The column, a glass tube 2.1 m × 4 mm I.D., was silanised by immersion in 2% dichlorodimethylsilane in toluene for 1 h, rinsed with methanol and dried at 100°C. The column was packed with 3% (w/w) OV-101 on 80—100 mesh Supelcoport purchased ready-prepared from Chromatography Services (Merseyside, Great Britain), and was conditioned at 250°C with nitrogen flow (40 ml/min) for 15 h. Subsequently, daily injections of 5—10 μl of A-187 were performed to maintain the packing in a deactivated form [10].

The retention times of lignocaine, tocainide and some other compounds on this system, measured relative to the internal standard, are given in Table I.

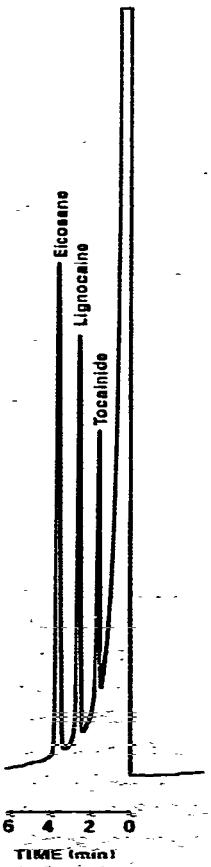


Fig. 2. Chromatogram obtained on analysis of standard solution in chloroform containing lignocaine and tocainide (both 10 mg/l); 5-μl injection. The *n*-eicosane concentration was 10 mg/l. Chromatographic conditions as given in text, except that a 1.5 m × 4 mm I.D. column was used.

The chromatogram of a standard chloroform solution containing tocainide and lignocaine is illustrated in Fig. 2.

Sample preparation

Plasma or serum (100 μl), Tris solution (20 μl) and internal standard solution (50 μl) were added to a small Dreyer test tube (Poulten, Selfe and Lee, Wickford, Great Britain). Hamilton gas-tight luer-fitting glass syringes (1.0 and 2.5 ml, respectively) fitted with Hamilton repeating mechanisms and stainless-steel needles were used for the addition of these latter volumes. The contents of the tube were vortex-mixed for 30 sec and the tube was centrifuged for 2 min at 9950 *g* in an Eppendorf centrifuge 5412 (Anderman, East Molesey, Great Britain). Subsequently, a portion of the extract was obtained by drawing 5 μl of air into a gas chromatographic syringe and passing the syringe needle through the aqueous layer into the chloroform. The air was expelled, and a 2—5 μl portion of the organic phase was taken up into the syringe and injected onto the gas chromatographic column using a syringe fitted with an 11.5-cm needle.

The extraction was performed in duplicate and the mean results taken.

Instrument calibration

Standard solutions containing lignocaine or tocainide at concentrations of 2.0, 5.0, 10 and 20 mg/l free base were prepared in heparinised human plasma by dilution of the appropriate 1 g/l aqueous solution. On analysis of these solutions, the calibration graphs of peak area ratio (drug/*n*-eicosane) against drug concentration were linear, with zero intercept, across the range of the standards. The calibration gradient (peak area ratio/plasma drug concentration) was normally 0.126 l/mg (lignocaine) or 0.065 l/mg (tocainide).

In practice, the instrument was calibrated using a 5.0 mg/l plasma standard, and the calibration confirmed by the analysis of an internal quality control sample containing lignocaine or tocainide (10.0 mg/l) prepared from an independent stock solution.

RESULTS AND DISCUSSION

Recovery studies

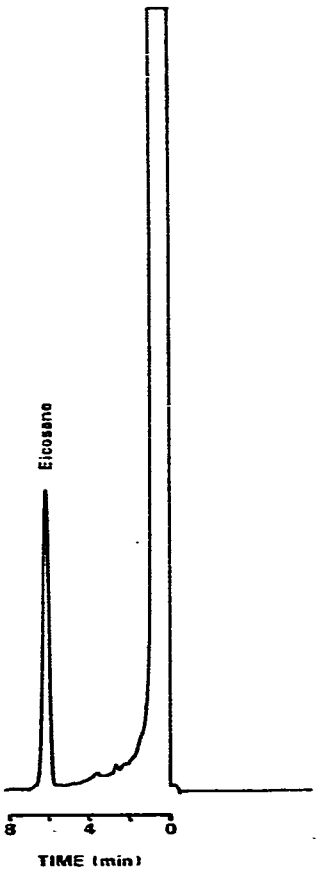
Standard solutions containing either lignocaine or tocainide hydrochlorides at a concentration equivalent to 1.00 g/l free base were prepared in chloroform or chloroform—ethanol (95:5, v/v), respectively. Standard solutions in chloroform were prepared by dilution from the stock solution, each containing the appropriate drug at concentrations equivalent to 2.0, 5.0 and 10.0 mg/l together with *n*-eicosane (10 mg/l). Calibration graphs of peak area ratio drug/*n*-eicosane against drug concentration were prepared using these solutions and the mean apparent recoveries of drug from the heparinised human plasma solutions were found to be 98.7 ± 6.8% (S.D.) and 99.7 ± 7.3% (S.D.) for tocainide and lignocaine, respectively ($n = 4$ at each concentration) after taking into account the 2:1 concentration inherent in the extraction.

The effect of a high concentration of lignocaine on the recovery of tocainide from plasma standards and vice versa was investigated by the preparation of

appropriate standard solutions. The presence of lignocaine at a concentration of 20 mg/l did not significantly affect the tocainide concentration measured at 25 mg/l [without lignocaine 25.62 ± 1.21 (S.D.) mg/l; with lignocaine 24.97 ± 1.09 mg/l] or at 2 mg/l (without lignocaine 2.40 ± 0.08 mg/l; with lignocaine 2.31 ± 0.11 mg/l). Analogous results were obtained when tocainide (20 mg/l) was added to solutions containing lignocaine (18 and 2 mg/l, respectively).

Selectivity

No endogenous sources of interference have been observed [10] and a chromatogram obtained on analysis of an extract of drug-free human plasma



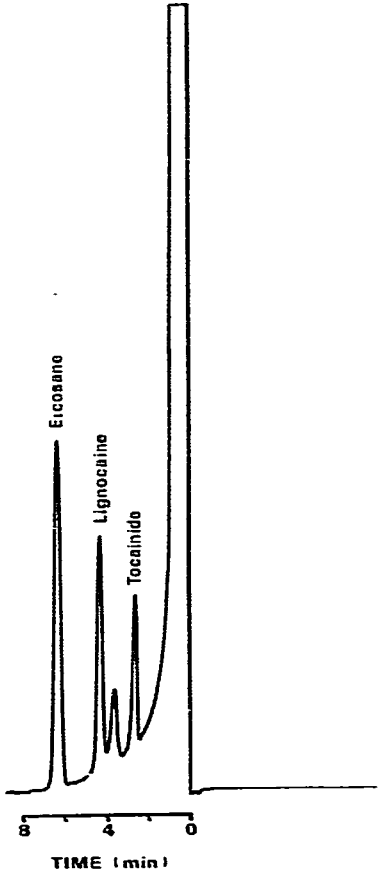


Fig. 3. Chromatogram obtained on analysis of an extract of a plasma sample from a patient receiving neither lignocaine nor tocainide; 5-μl injection. The *n*-eicosane concentration was 10 mg/l. Chromatographic conditions as given in text.

Fig. 4. Chromatogram obtained on analysis of an extract of a plasma sample from a patient receiving both lignocaine and tocainide (1 mg/min intravenously and 600 mg twice daily orally, respectively); 5-μl injection. The sample was drawn during lignocaine infusion and 2 h after the last dose of tocainide. The *n*-eicosane concentration was 10 mg/l, and the tocainide and lignocaine concentrations were found to be 8.8 and 4.9 mg/l, respectively. The peak eluting at a retention time of 0.65 relative to *n*-eicosane was caffeine. Chromatographic conditions as given in text.

is shown in Fig. 3. The chromatogram obtained on analysis of a plasma specimen from a patient under treatment with both tocainide and lignocaine is illustrated in Fig. 4. No interfering peaks were found when this and other specimens from patients receiving tocainide alone were analysed without the addition of the internal standard.

Of other drugs studied which were both extracted and chromatographed under the conditions of the assay (Table I), only the β-blocker drug oxprenolol presents a potential source of interference. However, the plasma concentrations attained during therapy of this latter drug are very low (less than 0.5 mg/l) [11], and interference from this source is unlikely to prove serious. Other compounds, including propranolol, tricyclic antidepressants and benzodiazepine drugs, all elute after the internal standard and, during normal therapeutic administration, are not present in sufficient concentration to be detected by this method. Monoethylglycinexylidide, a metabolite of lignocaine, is extracted under the conditions of the assay but co-elutes with caffeine and cannot, therefore, be measured.

Reproducibility

For tocainide, the intra-assay coefficient of variation (C.V.) was 5.40% at 2.0 mg/l (n = 10) and 3.12% at 10.0 mg/l (n = 10). The inter-assay C.V. was 3.87% at 10.0 mg/l (n = 10).

For lignocaine, the intra-assay C.V. was 5.23% at 2.0 mg/l (n = 10) and 3.20% at 10.0 mg/l (n = 10). The inter-assay C.V. was 3.81% at 10.0 mg/l (n = 10).

Limit of sensitivity

The limit of accurate measurement of the method was 0.2 mg/l for both drugs. A 0.2 mg/l plasma standard gave a mean value of 0.22 ± 0.03 mg/l (S.D.) (lignocaine) and 0.17 ± 0.01 mg/l (S.D.) (tocainide) (n = 5 in both instances). The concentrations of both drugs attained during normal therapy are well above this limit [10,12].

External quality control

Tocainide solutions prepared in heparinised human plasma and with weighed-in tocainide free-base values in the range 0.56—8.48 mg/l were supplied by Astra Chemicals and analysed by the present method. There was good correlation between the results obtained [mean = 4.12 ± 3.39 (S.D.) mg/l] and the weighed-in tocainide value [mean = 4.35 ± 3.45 (S.D.) mg/l] (r = 0.997; n = 5). Linear regression analysis using the weighed-in value as the independent variable revealed a gradient of 0.98 and an intercept on the Y-axis of —0.15 mg/l.

A quality control specimen prepared internally from an external stock-solution was used in the lignocaine assay [10].

CONCLUSIONS

The method described here has been found to be suitable for the simultaneous measurement of the plasma concentrations of both tocainide and lignocaine attained during therapy. Neither extract concentration nor derivatisa-

tion steps are required, and no sources of interference have been identified. Only 200 μl of specimen are required for an analysis, in duplicate, which can be completed together with the analysis of a quality control specimen within 30 min.

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