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Note

High-performance liquid chromatographic assay for tocainide in human plasma: comparison with gas—liquid chromatographic assay

PHILLIP A. REECE* and PHILIP E. STANLEY

Department of Clinical Pharmacology, The Queen Elizabeth Hospital, Woodville, S.A. 5011 (Australia)

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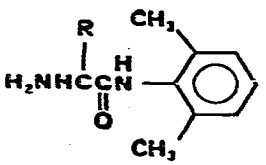
Tocainide (Astra Pharmaceutical Products, Sydney, Australia) (I) is currently undergoing clinical trial in this country and may become established as useful alternative therapy in the treatment of arrhythmias. Plasma levels of the drug have been assayed by gas–liquid chromatographic (GLC) methods [1—3]



I: R=CH_3
II: R=H
III: R=CH_2CH_3

which have involved lengthy sample extraction and derivatization steps. A high-performance liquid chromatographic (HPLC) assay employing dansylation and fluorescence detection has been reported [4] and recently a simplified HPLC assay involving UV detection of the underivatized drug has also been reported [5].

In the present study a precise HPLC assay for tocainide has been developed and is compared with a published GLC assay [3]. Advantages of the new HPLC method over published methods are discussed.

EXPERIMENTAL

Reagents

All reagents were analytical grade and aqueous solutions were prepared using glass-distilled water. Dichloromethane was Nanograde from Mallinckrodt,

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St. Louis, MO, U.S.A. Specially purified acetonitrile (210 nm cut-off, Unichrom from Ajax Chemicals Melbourne, Australia) was used for HPLC. Tocainide hydrochloride (I) and the internal standards (II and III) were generously provided by Astra Pharmaceutical Products.

Standards

Stock solutions of tocainide hydrochloride (I) and the internal standards (II and III) were prepared in water (200 μmol/l each) and were stable for at least one month at 4°C. The tocainide solution was used to prepare the appropriate plasma standards for each assay run. Peak area ratios of tocainide to the internal standard were determined for plasma standards and unknowns, and quantification was performed by reading unknown values from a plotted standard curve.

HPLC tocainide assay

A 1-ml aliquot of plasma was pipetted into a 15-ml glass stoppered tube. The internal standard solution (300 μl) was added, followed by 2 ml of 1.0 *N* sodium hydroxide—0.6 *M* sodium tetraborate (pH 10), and 5 ml of dichloromethane; the mixture was then shaken on a horizontal shaker for 10 min at 100 opm. The phases were separated by centrifugation (1100 *g* for 10 min) and the aqueous layer was removed by vacuum aspiration and discarded. The organic layer was poured into autosampler tubes (diSPo tubes from Scientific Products, State College, PA, U.S.A.) (75 mm × 12 mm) and evaporated under a stream of pure nitrogen at 45°C. The tubes were removed from the heating block as soon as they were dry and the residue was reconstituted in 0.5 ml of mobile phase [1.5 m*M* phosphoric acid—acetonitrile (95:5)]. Fifty microlitres of this solution were injected into the chromatograph.

High-performance liquid chromatography

A chromatograph (Spectra-Physics Model SP 8000) equipped with a ternary solvent system, helium degass and automatic data reduction facilities was used. Files for the instrument operation and integration were stored on disc (Spectra-Physics Model SP 4010 disc module). The reversed-phase column used measured 300 mm × 4 mm and was packed with alkyl phenyl bonded to 10-μm silica (μBondapak/Phenyl from Waters Assoc., Milford, MA, U.S.A.). Column oven temperature was 50°C. The mobile phase was automatically mixed by the instrument and consisted of 1.5 m*M* aqueous phosphoric acid—acetonitrile (95:5) at a flow-rate of 1 ml/min. The instrument was operated in the constant-flow mode and all solvent lines from the column to the detector were carefully thermally insulated. The column effluent was monitored at 230 nm with a variable-wavelength UV detector (Schoeffel Model 770). The detector absorbance setting was 0.04, an attenuation of 2 was used (0.08 a.u.f.s.) and time constant 9.0 sec. Samples were injected automatically using a 50-μl sample loop and an autosampler (Spectra-Physics Model 8010).

Recovery and reproducibility

Recovery of the HPLC assay was determined at concentrations of 10, 20, 40 and 60 μmol/l in plasma by comparison of the tocainide peak area with that

obtained for an aqueous solution containing a known concentration of tocainide (200 μmol/l) injected directly into the chromatograph.

Intra-assay reproducibility of the HPLC and GLC assays was determined at concentrations of 20, 40 and 60 μmol/l by assaying five plasma samples at each concentration. This was repeated using the HPLC assay where 1 *N* sodium hydroxide replaced the 1 *N* sodium hydroxide—0.6 *M* sodium tetraborate. Inter-assay reproducibility was determined by assaying a single plasma sample containing added tocainide (40 μmol/l) in each assay run. Five determinations were made using the HPLC assay and its modification and twenty determinations using the GLC assay.

Comparison of HPLC and GLC assays

Thirty-one venous blood samples were collected from eight patients at steady-state on oral tocainide and plasma levels of the drug assayed by the HPLC and a published GLC method [5].

Steady-state plasma levels

Blood samples were taken hourly over the 8-h dosage interval from each of three subjects taking oral tocainide (600 mg, 8-hourly) at steady-state and assayed for tocainide by the HPLC method.

Interference by other drugs

Samples of the drugs and metabolites listed in Table I were dissolved in mobile phase (200 μmol/l of each) and injected into the high-performance liquid chromatograph. The retention times were obtained if a peak was observed.

TABLE I

RETENTION TIMES OF DRUGS AND METABOLITES IN THE HPLC ASSAY FOR TOCAINIDE

Drug	Retention time (sec)
Tocainide (I)	520
Internal standard (II)	434
Internal standard (III)	670
N-Acetylprocainamide	360
Caffeine	> 1000
Carbamazepine	> 1000
N-Desisopropylpropranolol	> 1000
Dihydroquinidine	> 1000
Disopyramide	> 1000
Ethosuximide	506
4-Hydroxypropranolol	> 1000
Lidocaine	730
Phenobarbitone	> 1000
Phenytoin	> 1000
Primidone	967
Procainamide	391
Propranolol	> 1000
Quinidine	900
Theobromine	467
Theophylline	556

RESULTS AND DISCUSSION

The use of an alkyl phenyl reversed-phase column at 50°C with 1.5 m*M* phosphoric acid—acetonitrile (95:5) as mobile phase resulted in an efficient separation of tocainide, the internal standard and plasma peaks (Fig. 1).

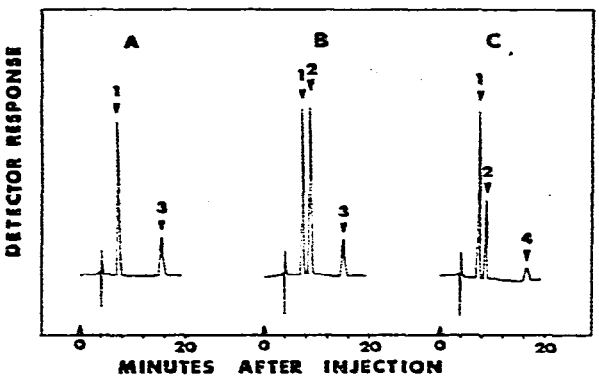

Fig. 1. Chromatograms obtained for the tocainide HPLC assay of (A) blank plasma, (B) plasma standard containing tocainide (60 μmol/l), and (C) plasma from a patient at steady-state containing tocainide (28 μmol/l). Peaks: 1 = internal standard, 2 = tocainide, and 3 and 4 = plasma peaks.

Recovery (69%) was linear over the concentration range 10—60 μmol/l (2—11.5 μg/ml). Monitoring the effluent at 230 nm allowed sufficient sensitivity for the detection of low plasma levels of tocainide with a detection limit of 1 μmol/l (0.2 μg/ml) (determined at a peak height of twice the noise level after injecting one-tenth of the total plasma extract). The assay was simple and rapid to perform requiring approximately 2 h for the processing of twenty patient samples and standards to the injection stage. The retention times of other drugs and metabolites directly injected into the chromatograph are listed in Table I. An alternative internal standard III was not used because of potential interference by lidocaine which had a similar retention time.

Using sodium hydroxide—borate buffer excellent inter- and intra-assay precision was obtained for the HPLC method (Table II). When the sodium hydroxide—borate buffer was replaced by sodium hydroxide in the HPLC assay, both inter- and intra-assay precision were considerably poorer (Table II)

TABLE II

COEFFICIENTS OF VARIATION (CV%) FOR THE TOCAINIDE ASSAYS

		HPLC*	HPLC**	GLC
Intra-assay CV%:	60 μmol/l	1	8	3
	40 μmol/l	3	6	3
	20 μmol/l	3	6	3
Inter-assay CV%:	40 μmol/l	5	19	15

*Using 1 *N* sodium hydroxide—0.6 *M* borate buffer.
**Using 1 *N* sodium hydroxide only.

without a change in recovery. Although the intra-assay reproducibility of the GLC tocainide assay was excellent, the inter-assay variation was poor. The relatively poor correlation obtained (Fig. 2) ($r^2 = 0.81$) for the HPLC and GLC assays is probably a reflection of the high inter-assay variability of the GLC method. The plasma level—time courses obtained for the HPLC assay of plasma samples from three patients taking tocainide (600 mg, 8-hourly) at steady-state are shown in Fig. 3. The method reported has adequate sensitivity and reproducibility for pharmacokinetic studies in man.

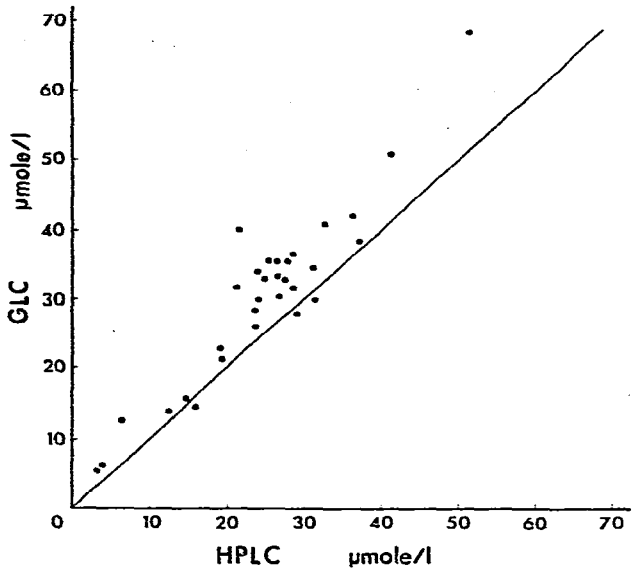

Fig. 2. Comparison of HPLC and GLC tocainide assays for 31 plasma samples taken from eight patients at steady-state. $r^2 = 0.847$, slope = 1.147, intercept = 1.491.

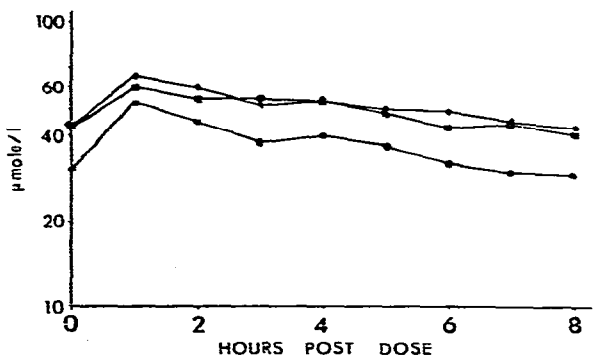

Fig. 3. Steady-state tocainide plasma levels obtained for the HPLC assay of samples from three subjects administered oral tocainide (600 mg, 8-hourly).

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