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Assay of Free and Total Tocainide by High Performance Liquid Chromatography (HPLC) with Ultraviolet (UV) Detection

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ABSTRACT: A rapid high performance liquid chromatographic (HPLC) method for the determination of tocainide, using *N*-(2,6-dimethylphenyl)-2-amino-butanimide as an internal standard, was developed. A methylene chloride extraction involving salting out at pH 9.0 was employed. An 85:15 mixture of 0.025*M* monobasic potassium phosphate at pH 3.0 and acetonitrile was used as the mobile phase. The separation and quantitative analysis of tocainide was performed on a mixed phase column with a 1.0-mL/min flow rate and detection at 210 nm. Separation of tocainide from some of its metabolites required the use of heptane sulfonic acid as an ion-pairing reagent. For the free-drug assay, the specimen was centrifuged through an Amicon Centrifree filter before being processed.

KEYWORDS: toxicology, tocainide, chromatographic analysis, high performance liquid chromatography

Tocainide, 2-amino-*N*-(2,6-dimethylphenyl) propanimide (I), has been established as an effective anti-arrhythmic agent [*1-3*]. Tocainide is reported to have a half-life of 12 to 14 h and over 90% bioavailability [*1,3*]. Because this drug has a low therapeutic index, monitoring of its therapeutic concentrations is desirable. To date, four cases of tocainide overdosage have been reported, three of which were fatal [*4-7*].

Plasma tocainide has been assayed by both gas-liquid chromatographic (GLC) and high performance liquid chromatographic (HPLC) methods [*8-10*]. Tocainide has been determined by GLC with a nitrogen-selective detector after Schiff base formation [*11*]. Tocainide determination by HPLC has also included fluorescence detection with dansyl chloride derivitization. Several different HPLC columns have been examined in previous reports. In our hands, these published methods provided results (1) that lacked adequate sensitivity and precision or (2) that involved extraction or derivitization procedures that were difficult and time-consuming, or (3) both.

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The following report describes a method that simplifies the extraction procedure, eliminates the derivitization step, increases the sensitivity and precision of the assay, and significantly reduces analysis time.

Materials and Methods

Reagents and Materials

Tocainide hydrochloride (HCl) and the *N*-(2,6-dimethylphenyl)-2-aminobutanimide HCl internal standard were obtained from Merck Sharp and Dohme Research Lab. The following reagents were obtained as analytical grade from Fisher Scientific: sodium bicarbonate, sodium carbonate, monobasic potassium phosphate (KH_2PO_4), phosphoric acid, and 1-heptanesulfonic acid (HSA). Methanol and acetonitrile were obtained from Mallinckrodt, and methylene chloride was obtained from J. T. Baker Chemical Co.; all were HPLC grade. The extracting solvent was prepared fresh daily by adding 200 μL of a 12.5-μg/mL stock methanol solution of *N*-(2,6-dimethylphenyl)-2-aminobutanimide HCl to each 5-mL volume of methylene chloride. A 2:1 sodium bicarbonate:sodium carbonate mixture was prepared for salting out the drug from the aqueous matrix and for pH control of the extraction. The 15-μg/mL tocainide control was obtained from UTAK Laboratories, Inc.

Instrumentation

For HPLC: A Varian (part 03-912054-42) PTHAA-5 column (15 cm by 4 mm, spherical phase, silica support) was used. Chromatographic separation was performed on a Varian 5000 Liquid Chromatograph equipped with a UV-50 Variable Wavelength Detector and a CDS 401-Data System.

Chromatographic Conditions

For HPLC: The Varian UV-50 spectrophotometer was set at 210 nm. The mobile phase was 85 parts 0.025*M* KH_2PO_4 (adjusted to pH 3.0 with concentrated phosphoric acid and filtered through a 0.45-μm Millipore filter) and 15 parts acetonitrile, to which 0.81 g of heptane sulfonic acid was added to each litre of mobile phase to give a final concentration of this ion-pairing reagent of 0.004*M*. The flow rate of the mobile phase was 1.0 mL/min; the system was perfused with mobile phase for 15 min before the first injection to achieve system equilibration.

Preparation of Standards

Standards and controls were made from a 100-μg/mL methanol stock solution of tocainide by measuring into screw-cap-equipped tubes the equivalent quantities of tocainide to give the following standards: 0.0, 1.0, 2.5, 5.0, 7.5, 10.0, 15.0, and 20.0 μg/mL. The methanolic solutions were evaporated to dryness with a stream of air at room temperature. Each tube was then reconstituted with 10 mL of plasma.

Extraction Procedure

Pipet 0.5 mL of serum or plasma into a screw cap tube. Add 3/4 Coors No. 01 scoop (approximately 0.5 g) of the sodium bicarbonate:sodium carbonate mixture to adjust the pH to approximately 9.0 and vortex each tube for 10 to 15 s. Add 5.0 mL of the extraction solvent to each tube. Shake the tubes on a mechanical shaker (Eberbach) at high speed for 15 min. Next, centrifuge the tubes at 2000 rpm for 5 min. Aspirate and discard the upper aqueous

layer from each tube. Filter the organic layer using ashless 9-cm 5C Micro Filtration Systems filter paper and evaporate it to dryness using a stream of air at room temperature. Reconstitute the residue with 100 μL of the mobile phase and inject onto the HPLC. For the free drug assay, centrifuge the specimen through an Amicon Disposable Centri-free Micropartition System filter (Amicon, Cat. No. 4104) before processing the specimen as described above.

Drug Interference Study: Stock 1-mg/mL methanolic solutions were prepared for each drug tested (Table 2), and a sufficient quantity was pipetted into screw-cap tubes and evaporated to dryness with air so that on reconstitution with plasma the final concentration of each was 10 μg/mL. The drug-spiked plasma was then processed as described above.

Results and Discussion

Figure 1 is a typical chromatogram for a 15-μg/mL tocainide control monitored at 210 nm. At this wavelength, an increase in detectable limits of three- to four-fold was achieved as compared to monitoring at 225 nm, which was the wavelength used in a previously published procedure [9]. The excellent resolution of the drug from interfering plasma constituents by the PTHAA-5 analytical column permitted monitoring at this more sensitive wavelength. Detection at 210 nm resulted in a maximum noise range limit of 0.65 μg/mL, which allowed for measurement not only of total tocainide with its established therapeutic range of 4 to 10 μg/mL, but of free tocainide as well.

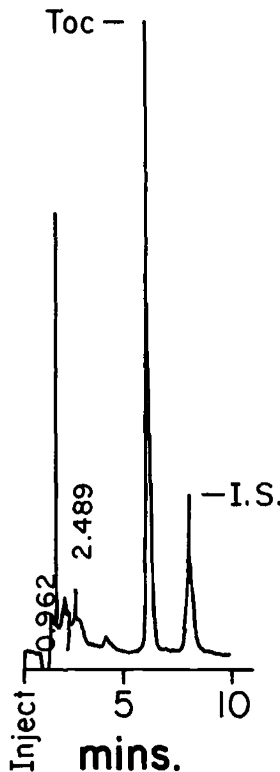

FIG. 1—*Chromatogram of 15-μg/mL UTAK tocainide control.*

The proposed procedure has a within-day coefficient of variation (CV) of 9.9% ($n = 5$), and 6.2% ($n = 4$) at concentrations of 2.5 and 7.5 μg/mL, respectively. The day-to-day CV for the procedure at these two concentrations was 8.2% ($n = 8$) and 11.8% ($n = 8$), respectively.

Standard curves between 1 and 20 μg/mL were linear, with a correlation coefficient of 0.9993. Figure 2 shows a typical standard curve; Table 1 gives typical standard curve calculations.

Figure 3*a* and *b* shows typical results from an ion-pairing study. Figure 3*a*, containing no ion-pairing reagent in the mobile phase, has many interfering peaks from plasma constituents. Figure 3*b*, however, containing 0.004*M* HSA as the ion-pairing reagent, demonstrates the almost total elimination of the interfering peaks.

A drug interference study was also performed. Table 2 lists all drugs tested for interference with tocainide and its internal standard. None were found to interfere.

On a series of five patient serum samples, mean total tocainide was 9.6 μg/mL, with a range of 4.5 to 18.1 μg/mL, and mean free tocainide was 2.4 μg/mL, with a range of 1.6 to 3.2 μg/mL. The mean percent free tocainide was 24.8%.

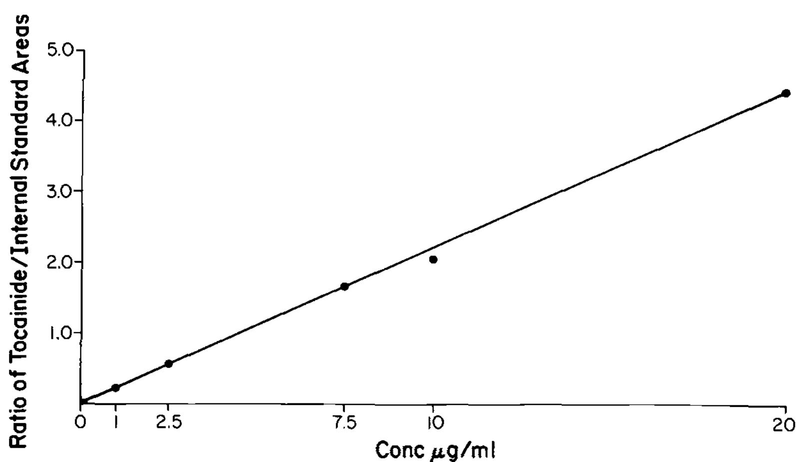

FIG. 2—*Linear-linear plot of ratio of tocainide/internal standard areas versus concentration* ($y = 0.219x - 0.0051$, $r = 0.9993$).

TABLE 1—*Standard curve calculations for tocainide by HPLC* ($r = 0.9993$).

Sample Concentration, μg/mL	Area Ratio of Drug/I.S.	Area Ratio Concentration
0.0	...	...
1.0	0.2240	0.2240
2.5	0.5585	0.2234
7.5	1.693	0.2257
10.0	2.063	0.2063
20.0	4.418	0.2209

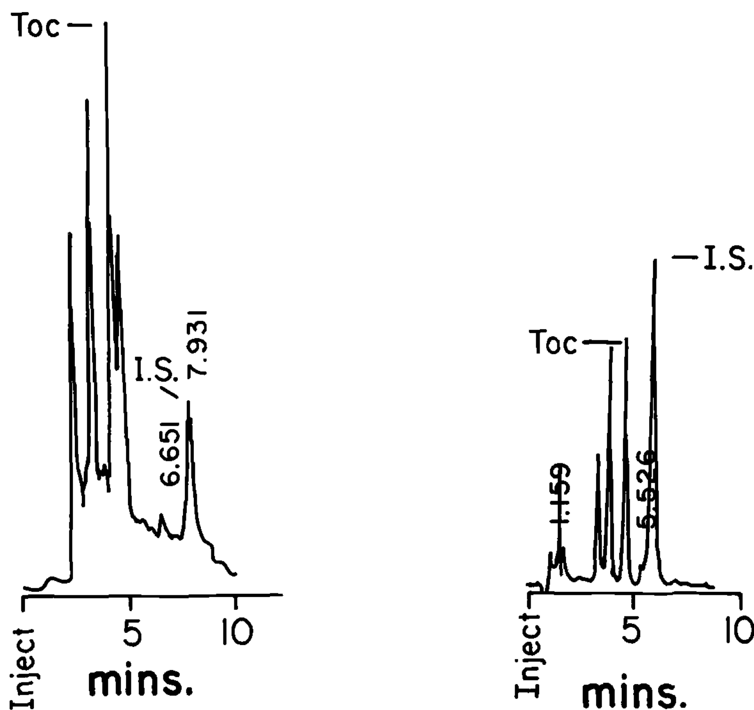

FIG. 3—(a) *Chromatogram of patient specimen without ion-pairing reagent in mobile phase, and* (b) *with ion-pairing reagent in mobile phase.*

TABLE 2—*List of drugs found not to interfere with tocainide or its internal standard, or both, at a concentration of 10 μg/mL.*

Acetaminophen	Lidocaine	Primidone
Atropine	Lidocaine metabolites	Procainamide
Chlorzepate	Meprobamate	Procaine
Clonazepam	Methocarbamol	Pseudoephedrine
Dibucaine	Methylphenidate	Quinine
Diphenoxylate	Nicotine	Salicylamide
Disopyramide	Nordiazepam	Tetracaine
Ethinamate	Phenacetin	Theobromine
Hydralazine	Phenylephrine	Theophylline
Hydroxyzine	Phenylpropanolamine	Trazodone
		Tybamate

Conclusion

The high performance liquid chromatographic determination of tocainide in biological specimens described in this report is accurate and precise and provides increased sensitivity and reduced analysis time when compared to previously reported methods. The procedure is practical and useful for monitoring plasma concentrations of tocainide following therapeutic or toxic dosages.

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