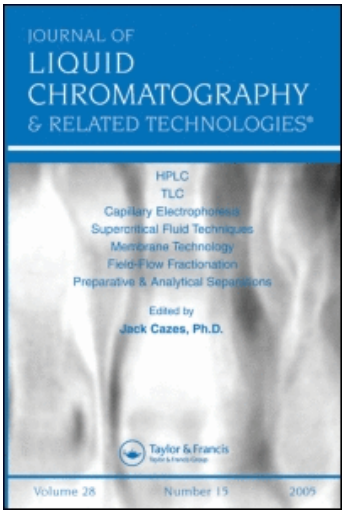

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Tocainide Determination by High-Performance Liquid Chromatography

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TOCAINIDE DETERMINATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Tocainide has been assayed after serum deproteinization with acetonitrile by HPLC. The pH was critical in separating the drug from other interferences in the serum. The method is simple and fast.

INTRODUCTION

Tocainide is one of the recent drugs approved for treatment of ventricular arrhythmias (chemical structure in Fig. 1). The therapeutic concentration of this drug in serum is 3-10 mg/L, with a half-life of about 11 hr (1, 2).

The drug has been assayed after organic solvent extraction (3, 4) or derivatization (5, 6). As in the case of most basic drugs, the recovery with solvent extraction is not very high (3, 4). In addition, solvent extraction or derivatization is time-consuming. Here we describe a method for tocainide assay based on a simple step of serum deproteinization. At the appropriate pH, the drug can be separated from other interferences in the serum. The method is quite simple and fast compared to solvent extraction or derivatization.

MATERIALS AND METHODS

Procedure:

Add 200 µL of serum to 200 µL of acetonitrile, mix and centrifuge. Inject 50 µL of the supernatant directly on column.

Equipment:

A Model 110 A pump (Beckman Instruments, Fullerton, CA) was used to deliver the solvent at a rate of 1.3 mL/min onto Synchropak SCD-100, a C-18 column, 150 mm X 4.6 mm, 5 µm particles, 100 Å pore size (Synchrom, Linden, IN). The column is silanol-deactivated for basic drug analysis. The eluent was detected with a variable wavelength detector, Spectro Monitor II (Laboratory Data Control, Riviera Beach, FL), set at 230 nm, 0.020 mA.

Reagents:

Pump solvent: Acetonitrile 24% in ammonium phosphate buffer 25 mmol/L, pH 7.3.

Stock standard: Tocainide 100 mg/L in acetonitrile.

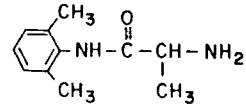
Working standard: Tocainide 10 mg/L. Dilute the stock standard 1:10 in water.

RESULTS AND DISCUSSION

Tocainide has a nonspecific absorption between 230 and 200 nm. The light absorbance of this drug at 230 nm is very low, about 10 times less than that at 200 nm. However, at low wavelengths (about 200 nm) many drugs and endogenous substances interfere in the test and for this reason we chose 230 nm to monitor this test. This wavelength provides sufficient sensitivity for the assay while avoiding many of the interferences from the endogenous compounds in serum (Fig. 1).

The following common drugs did not interfere with the test: theophylline, phenobarbital, phenytoin, tegretol, procainamide, norpace, quinidine.

Because tocainide is a basic compound it tends to bind to the surface of the tubes, causing low and non-reproducible recovery. We found that solvent extraction did not yield reproducible results. Direct serum injections avoided this



TOCAINIDE

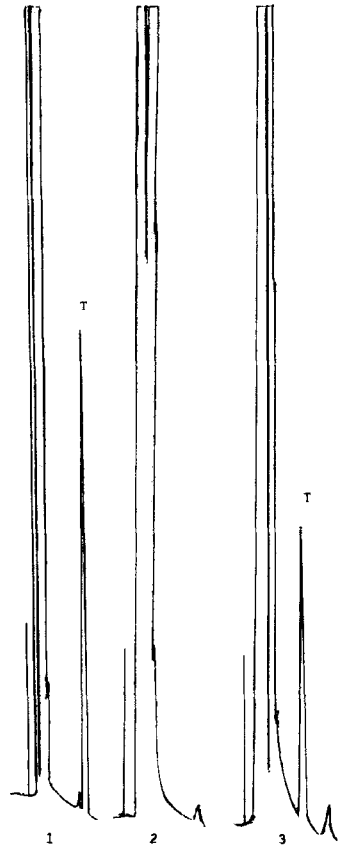


Figure 1: Representative chromatogram of: 1- Standard, (10 mg/L, T = tocainide), 2- Serum from a patient free of tocainide, and 3- Serum from a patient on tocainide. (Retention time for T is 6 min).
Top: structure of tocainide.

problem; however, some of the proteins produced interfering peaks very close to the tocainide peak. Acetonitrile deproteinization avoided all these problems. The average recovery of 10 mg/L in serum relative to water is 93% (n = 6). The test is linear between 2-20 mg/L. This covers the therapeutic range. Values below 1 mg/L are not quantitated but reported as < 1 mg/L. The between-run CV is 4.8% (n = 10).

The capacity factor increases with increase in the pH (Fig. 2). The pH is very critical in separating tocainide from drugs and other endogenous substances present in the serum. At low pH, many compounds interfered in the assay. However, at pH of 7.3, at 24% acetonitrile, the interfering compounds are avoided. Samples from 30 hospital patients free from tocainide (10 on theophylline, 10 on anti-epleptic drugs and 10 hospitalized for miscellaneous disorders), did not have any intereferences which might co-elute with the tocainide peak.

In order to check the purity of the tocainide peak and eliminate the possibility of any interferences we analyzed seven patients receiving tocainide using a different column (CN 2 μm 75 x 4.6, Supelco, Bellefonte, PA) eluted with a different solvent (ammonium phosphate buffer 25 mmol/L, pH

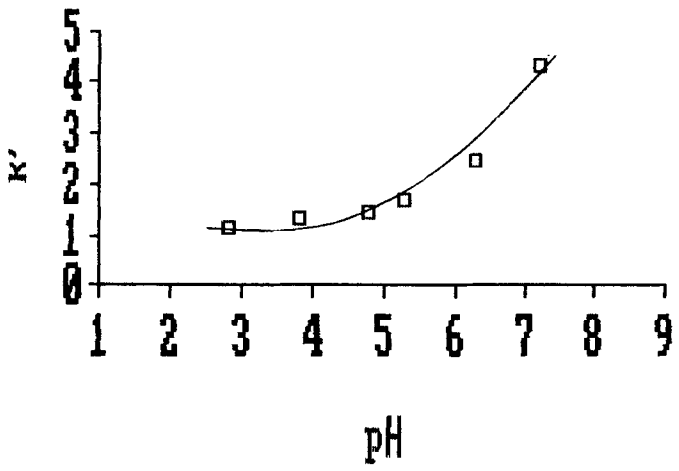


Figure 2: Capacity factor (K') vs pH using 12% acetonitrile.

Tocainide Concentration on Two Columns and Peak Height Ratio at 230/240 nm for 7 Patients

Patient No.	C_{18} Column	CN Column	Ratio 230/240
1	3.4	3.3	0.66
2	3.3	3.5	0.63
3	8.4	8.3	0.62
4	12.2	12.3	0.63
5	4.7	4.9	0.67
6	3.8	4.0	0.62
7	4.3	4.5	0.67

7.1). The results by the two column were similar, indicating the absence of interferences (Table).

In general, the separation was better on the Synchropak column. Futhermore, we analyzed the same patients at two wavelengths, 230 and 240 nm on the Synchropak column. The ratio of tocainide peak at these two wavelengths was essentially the same, indicating also the purity of the tocainide peak (Table).

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