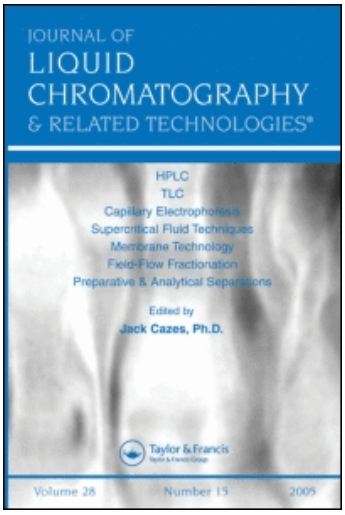

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LIQUID CHROMATOGRAPHIC ANALYSIS FOR FLECAINIDE WITH USE OF A MICRO-BORE COLUMN AND SMALL SAMPLE VOLUME

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ABSTRACT

We describe a simple, isocratic high-performance liquid chromatographic method for measuring the oral antiarrhythmic agent flecainide acetate in serum or plasma. Sample analysis involves a single step extraction followed by chromatography on a microbore reverse phase column with fluorescence detection.

INTRODUCTION

Flecainide acetate (tambocor), a fluorinated derivative of the basic structure of procainamide, has recently been approved in the United States as an oral agent for the treatment of ventricular arrhythmias. The drug is readily absorbed following oral administration and is not subject to significant first pass

metabolism by the liver. Although the drug is biotransformed into two major active metabolites, the unconjugated concentrations of these metabolites in plasma are much less than that of the parent drug and have been shown to be of little clinical significance. Both the desired therapeutic effect and toxicity can be directly related to the concentration of flecainide in plasma. As an aid in optimizing treatment both the electrocardiogram and plasma levels of flecainide should be monitored as guides to avoid potentially toxic concentrations (>1000 μg/L) of this drug.

Analytical methods that have been successfully used to monitor flecainide in plasma include spectrofluorometry (1,2), gas chromatography (3), and more commonly high performance liquid chromatography (4-7). The potential for added specifity exists when the fluorescent properties of flecainide are exploited and coupled with HPLC (6,7). Sample preparations have included acid precipitation without internal standard (4), multiple step organic extraction followed by evaporation to concentrate the sample (5), and solid phase column extraction (6), each followed by chromatography using conventional diameter analytical columns.

We have developed an HPLC procedure which is based upon the use of microbore chromatography and fluorescence detection. A positional isomer of flecainide is used as internal standard. In addition to being both simple and rapid, the assay described here possesses all of the advantages which may be obtained from the

proper use of microbore chromatography, including (a) increased sensitivity, (b) reduced specimen requirement, (c) excellent resolution of components, and (d) significant reduction in solvent usage and flow rates with the resulting increase in the useful life of pump components.

MATERIALS AND METHODS

Reagents

All reagents were "HPLC" grade. Acetonitrile and methyl-t-butyl ether were obtained from Burdick and Jackson Labs (Muskegon MI 49422), and triethylamine obtained from Mallinckrodt (Paris, KY 40361). Flecainide acetate and the internal standard, N-(2-piperidylmethyl)-2,3-bis(2,2,2-trifluoroethoxy)benzamide hydrochloride, were obtained courtesy of Riker Laboratories (St. Paul, MN 55144).

Chromatography

Measurements were made using a Varian Model 5000 liquid chromatograph (Varian Associates, Sunnyvale, CA 94086) equipped with a Kratos FS 970 Spectroflow fluorescence detector (Kratos Analytical Instruments, Ramsey, NJ 07446). A 2.0 mm (i.d.) x 25 cm ASTEC 5 micron microbore octyl column (Advanced Separation Technologies, Whippany, NJ 07981) connected to a 1 cm RP-8 guard column (Brownlee Labs, Santa Clara, CA 95050) was used. The mobile phase was 0.05% triethylamine in acetonitrile:0.1 M sodium acetate, pH 4.5 (40:60 by volume), used at an average flow rate

of 500 µL/min. Chromatographic separation was monitored by fluorescence detection using an excitation wavelength of 300 nm, emission wavelength 370 nm, sensitivity 35, 0.02 µAmps full scale.

Specimen Preparation

A 100 µL plasma sample was pipetted into a 2 mL polypropylene conical microfuge tube. To this was added 20 µL of internal standard (100 mg/L), 50 µL of 0.1 M sodium carbonate, and 100 µL methyl-t-butyl ether. The mixture was vortexed for 30 seconds, followed by centrifugation at 9500 x g for 3 minutes. Then 20 µL of the top ether layer was injected for analysis.

Calculations

Two plasma standards of 400 and 1200 µg/L were used. From this a calibration line was established by determining peak height ratios relative to the internal standard.

RESULTS AND DISCUSSION

Typical chromatograms for standard solutions and patient specimens are shown in Figure 1. We observed that the addition of triethylamine to the mobile phase resulted in both improved resolution as well as sharpened, taller peaks in contrast to mobile phase that did not contain triethylamine. As can be observed in Figure 1, flecainide is well separated from the internal standard peak. Neither peak has any effect on the

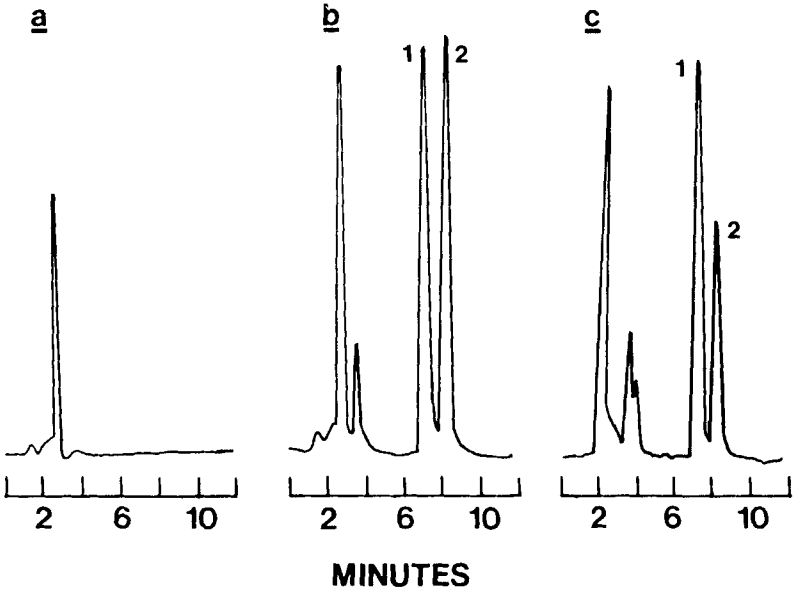

FIGURE 1. Chromatograms of (a) blank plasma, (b) 1000 µg/L flecainide standard, (c) patient sample containing 540 µg/L flecainide. Peak 1, internal standard; peak 2, flecainide.

measured peak height of the other compound of interest. The performance characteristics of this assay are displayed in Table 1 and Figure 2.

No interference from endogenous plasma substances was observed. Additionally, we tested numerous drugs that may be co-administered with flecainide acetate. None of these drugs (Table 2), including those with known potential fluorescent properties, were found to interfere with the assay described here.

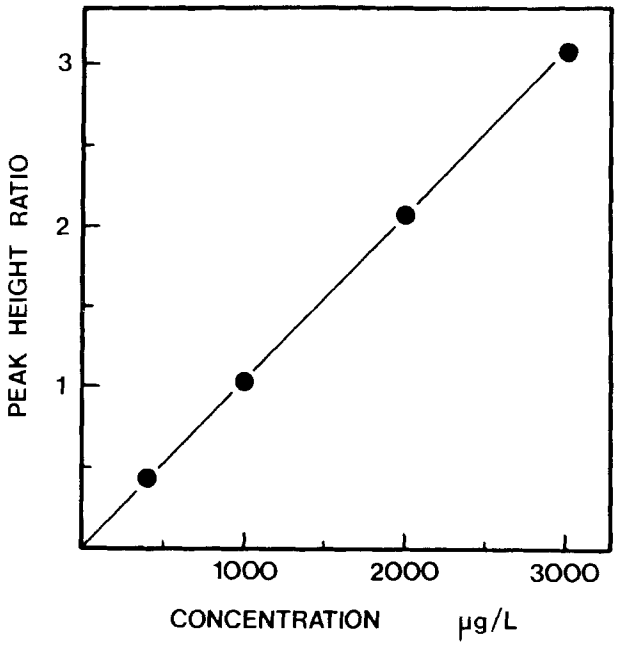

FIGURE 2. Calibration curve demonstrating linearity of assay.

TABLE 1

Assay Characteristics

Precision (CV)			
	Within-Run	400 µg/L	2.4%
		800 µg/L	2.3%
	Between-Run	400 µg/L	3.8%
		800 µg/L	3.5%
Recovery		Flecainide	98 ± 6%
		Int. Std.	97 ± 5%
Sensitivity		20 µg/L	

TABLE 2

Drugs Tested For Interference

N-Acetylprocainamide	Lidocaine
Acetylsalicylic acid	Mephenesin
Amiodarone	Mexiletine
Amitriptyline	Nordiazepam
Caffeine	Nortryptyline
Carbamazepine	Phenobarbital
Chloramphenicol	Phenytoin
Clonazepam	Primidone
Desmethyldoxepin	Procainamide
Desipramine	Propranolol
Diazepam	Protriptyline
Digoxin	Theophylline
Disulfiram	Tocainide
Doxepin	Quinidine
Ethosuximide	Valproic acid
Imipramine	Warfarin
Indomethacin	

Unlike some other HPLC methods for flecainide, the assay described here can be performed with a small sample requirement (100 μL or less). This is a result of the increased sensitivity that is obtained through the use of a microbore column. Although it is possible to modify the assay to use as little as 10 μL of plasma, we recommend the routine use of 100 μL sample for two reasons. First, this 100 μL sample volume is obtainable from nearly any type of patient, and also is a volume that is more readily and reproducibly handled in an average laboratory. Second, with the added sensitivity afforded with the larger 100 μL sample size and the microbore column, a wider full scale response range can be selected and the supply voltage for the

photomultiplier tube also significantly reduced, thereby minimizing noise caused by background light energy as well as maximizing the life of the photomultiplier.

Reagent costs can be substantially decreased by the use of this assay, because only 100 µL of methyl-t-butyl ether is used for the extraction and the chromatographic flow rate is only 500 µL/minute. At this flow rate, only 12 mL of acetonitrile and a total of 30 mL mobile phase would be used per hour. While a lower flow rate is required with the 2 mm diameter analytical column, the 500 µL/minute rate here is sufficient to allow the assay to be performed with the standard size flow cells that are normally present in detectors, with no significant effect of band broadening or diffusion on the quality of the observed chromatography.

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