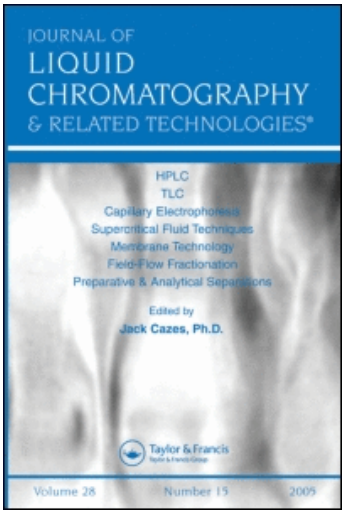

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Thomas Annesley[a]; Keith Matz[a]
[a] University of Michigan Medical Center, Ann Arbor, Michigan

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LIQUID CHROMATOGRAPHIC ANALYSIS FOR FLECAINIDE WITH USE OF A MICRO-BORE COLUMN AND ULTRAVIOLET DETECTION

Thomas Annesley and Keith Matz
University of Michigan Medical Center
1500 E. Medical Center Drive
Ann Arbor, Michigan 48109

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 simple organic extraction followed by chromatography on a microbore reverse phase column with ultraviolet detection at 298 nm.

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.

Methods that have been used to monitor flecainide in plasma include spectrofluorometry (1,2), gas chromatography (3), and more commonly high performance liquid chromatography (4-7). Sample preparations for HPLC analysis 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. Fluorometric detection is usually required in order to achieve the required sensitivity.

We have developed an HPLC procedure which is based upon the use of microbore chromatography and ultraviolet 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 increased sensitivity, which allows for the use of ultraviolet detection in this assay.

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 Kratos Spectroflow 400 pump equipped with a Kratos 783 Spectroflow 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) was used. The mobile phase was 0.05% triethylamine in acetonitrile:0.1 M sodium acetate, pH 7 (45:55 by volume), used at an average flow rate of 400 µL/min. Chromatographic separation was monitored by ultraviolet detection at a wavelength of 298 nm, 0.010 absorbance full scale.

For fluorescent detection a Kratos FS 970 Spectroflow detector was used. Chromatographic peaks were monitored at an excitation wavelength of 300 mm, emission wavelength 370 mm (6).

Specimen Preparation

A 250 µL serum or plasma sample was pipetted into a 2 mL polypropylene conical microfuge tube. To this was added 20 µL of internal standard (200 mg/L), 100 µL of 0.1 M sodium carbonate, and 1 mL methyl-t-butyl ether. The mixture was vortexed for 60 seconds, followed by centrifugation at 9500 x g for 3 minutes. The upper ether layer was transferred to a second microfuge tube and evaporated to dryness under air. The dried tube was reconstituted with 50 µL of acetonitrile: water (50:50), and a 20 µL injection volume was used 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 measured peak height of the other compound of interest. The performance characteristics of this assay are displayed in Table 1. Analytical recoveries for flecainide acetate and internal standard were 104% and 103% respectively. The assay is linear to 2000 μg/L, the highest concentration that we evaluated.

No interference from endogenous substances in plasma was observed. A peak with absorption capabilities at 298 nm is present in plasma, but this component elutes prior to both the internal standard and flecainide. Additionally, we tested numerous drugs that may be co-administered with flecainide acetate. None of the drugs listed in Table 2 were found to interfere with the assay described here.

The increased sensitivity obtained from the use of the microbore column, plus the taller, sharper peaks achieved through the addition of triethylamine, allows the assay to be performed with a 250 μL sample volume, a specimen size that is easily obtained from nearly any type of patient. This can be contrasted

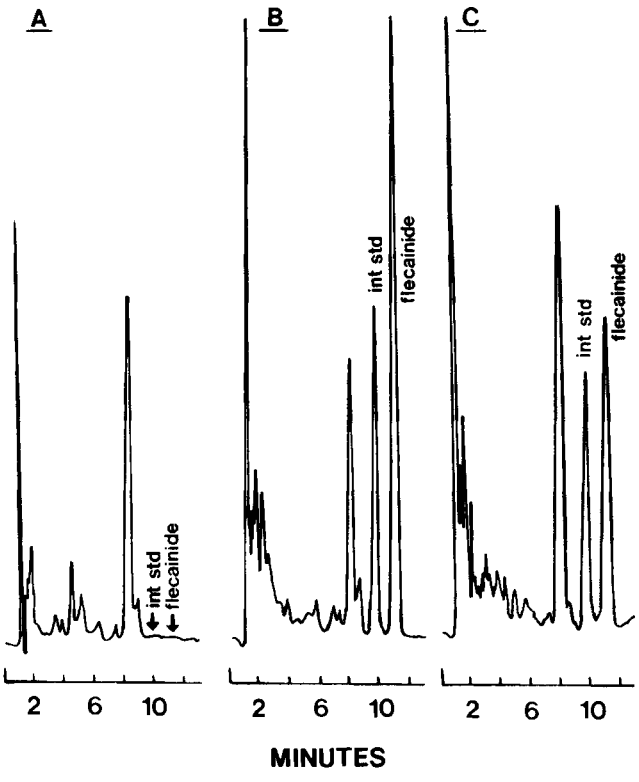

FIGURE 1. Chromatograms of A) serum blank, B) 1000 μg/L standard, C) patient specimen containing 700 μg/L flecainide.

to the 1 ml sample volume used in a number of proposed ultraviolet and fluorescent HPLC assays. Chang and co-workers (6) have successfully developed as assay using ultraviolet detection, using a standard diameter phenyl column, but reported interference from diazepam under the experimental conditions. This interference could be resolved using an octyl column, but the resulting peak shapes were not satifactory. We have found that the addition of triethylamine circumvented this problem. Like Chang et al (6), we observed that interference from diazepam was

TABLE 1

Assay Characteristics

Precision (CV)		
Within-Run	400 µg/L	2.6%
	1000 µg/L	2.9%
Between-Run	400 µg/L	4.4%
	1000 µg/L	3.5%
Sensitivity	80 µg/L	

TABLE 2

Drugs Tested For Interference

N-Acetylprocainamide	Lidocaine
Acetylsalicylic	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	

not a problem with the octyl microbore column chosen for our assay.

Reagent costs can be substantially decreased by use of this assay, because disposable extraction columns are not used and only 1 ml of methyl-t-butyl ether is used for the extraction. At the average chromatographic flow rate of 400 µL/minute only 10 ml of acetonitrile and a total of 24 ml of mobile phase would be used per hour. While a lower flow rate is required with the 2 mm diameter analytical column, the flow rate 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.

We compared the results obtained from the HPLC-UV assay proposed here with the results obtained using fluorescence detection (6). As demonstrated in Figure 2, results obtained

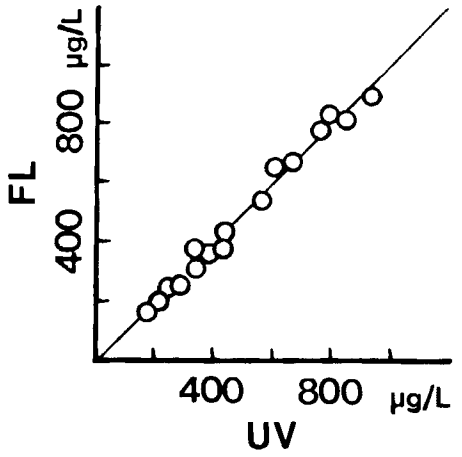

FIGURE 2. Comparison of results obtained using ultraviolet and fluorescent detection assays for flecainide. n = 18, r = 0.987, y = 0.97x + 10.

using both modes of detection were not only comparable, but the microbore HPLC-UV assay described here was sensitive enough to monitor blood concentrations of flecainide covering the entire accepted therapeutic range.

The retention times of flecainide and the internal standard are sensitive to changes in pH. Decreases in the pH of the mobile phase result in decreased retention. However, the retention of the endogenous component and diazepam are not readily affected by changes in pH. Thus minor adjustments in pH can be made in order to optimize chromatography as the analytical columns age or when a new column is used.

In summary, we report here a rapid, sensitive HPLC-UV assay for determining flecainide acetate in small volumes of serum. Because this assay successfully utilizes ultraviolet detection, it is possible for this assay to be performed in the many laboratories that have basic HPLC instrumentation but do not possess flow-cell fluorescence detectors.

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