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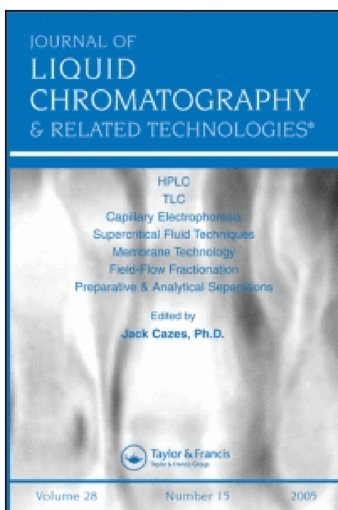

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DETERMINATION OF FLECAINIDE IN HUMAN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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ABSTRACT

A simple, rapid, selective, and sensitive high-performance liquid chromatographic (HPLC) method for the monitoring of plasma flecainide levels in a therapeutic or research environment is described. The drug is first separated from plasma by a single-step extraction with hexane and then quantitated by HPLC with fluorescence detection. Two linear ranges have been established; 100-2000 ng/ml for drug monitoring in clinical management of patients and 3-300 ng/ml for pharmacokinetic studies. The intra-day variation is less than 6%.

INTRODUCTION

Flecainide acetate [R-818, N-(2-piperidylmethyl)-2,5-bis-(2,2,2-trifluoroethoxy)benzamide acetate] is a new antiarrhythmic agent. Its pharmacologic and therapeutic characteristics have been recently documented (1-3). Flecainide acetate is currently undergoing extensive clinical evaluation world-wide and is

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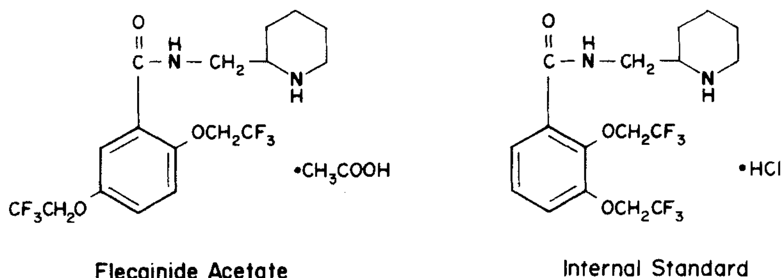

Figure 1. Structure of flecainide acetate.

marketed in West Germany and approved for marketing in the United Kingdom. Plasma level measurements are made to provide better therapeutic management of patients. Existing methods include gas-liquid chromatography (4), fluorometry (5, 6), and high-performance liquid chromatography (7, 8). The chromatographic methods are somewhat complex in procedure and are perhaps best suited for use in a research environment. The fluorometric methods are to be used under well controlled conditions due to their lack of selectivity.

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A new method for the monitoring of flecainide levels in human plasma using a single liquid-liquid extraction step and HPLC-fluorescence detection is described. The method is simple, rapid, selective, sensitive, and especially suitable for therapeutic drug monitoring.

MATERIALS AND METHODS

Reagents

The methanol, acetonitrile, and hexane were HPLC grade. The phosphoric acid was analytical reagent grade. The water was deionized.

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Chromatography System

The analysis was performed on a modular liquid chromatograph equipped with a ConstaMetric III pump (Laboratory Data Control), a Model 710B Intelligent Sample Processor (Waters Assoc., Inc.), a Model SP4100 Computing Integrator (Spectra-Physics), and a Model RF-530 Fluorescence Spectromonitor for high-speed liquid chromatography (Shimadzu Corp.). The excitation and emission wavelengths were 300 and 370 nm, respectively. A μBondapak phenyl column (30 cm x 3.9 mm, Waters Assoc., Inc.) was used. The mobile phase was prepared by mixing 400 ml acetonitrile and 600 ml aqueous 0.06% phosphoric acid. Prior to use the mobile phase was filtered through a Nylon 66 filter with a pore size of 0.45 μm. The flow rate was 2.2 ml/min.

Preparation of Standard Solutions

Standard solutions of flecainide were prepared by diluting a 10 mg flecainide acetate per liter aqueous stock solution. The concentrations used were 3, 5, 10, 25, 50, 100, 200, 300, 600, 1000, 1600, and 2000 ng in 0.5 ml; 3-300 ng standards were used for low range calibration and 100-2000 ng standards for high range calibration. A positional isomer of flecainide, [N-(2-piperidylmethyl)-2,3-bis(2,2,2-trifluoroethoxy)benzamide hydrochloride], was used as the internal standard. An internal standard solution of 1000 ng/0.5 ml or other concentrations were prepared by diluting a 10 mg per liter aqueous stock solution.

Extraction Procedure

Pipet 1 ml unknown human plasma into a 20 x 150 mm glass culture tube with a polyethylene-lined cap; add 0.5 ml deionized water (for calibration standards, add flecainide in

0.5 ml deionized water to one ml blank human plasma), internal standard in 0.5 ml deionized water, 1 ml 1N NaOH, and 10 ml hexane. Cap the tubes, shake on a reciprocal mechanical shaker (tubes in horizontal position) at a speed of ∿250 cycles per minute for 10 minutes and centrifuge for 5 minutes at ∿900 x g. Transfer 9.0 to 9.5 ml of the hexane phase to a 15 ml conical centrifuge tube. Evaporate to dryness at 60°C under N_2. Reconstitute the residue with 200 µl of the mobile phase and inject 50-150 µl into the liquid chromatograph.

Calibration

A least squares line of the peak height ratios (flecainide/ internal standard) versus the flecainide concentrations in the calibration standards was obtained by linear regression. The slope and intercept of the least squares line were used to determine the flecainide concentrations in the unknown samples.

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RESULTS AND DISCUSSION

The separation of flecainide and internal standard from the plasma was achieved by a single hexane extraction, and thus, considerably simplifies all existing procedures for sample preparation. Although the procedure of De Jong et al (7) using protein precipitation is also efficient, it does require a high speed centrifuge for the removal of precipitated proteins. Such a high speed centrifuge might not be available in clinical chemistry laboratories.

The internal standard, a positional isomer of flecainide, was well resolved from flecainide (Fig. 2) and there was no interference with either flecainide or the internal standard by endogenous materials from human plasma. The clean chromatogram

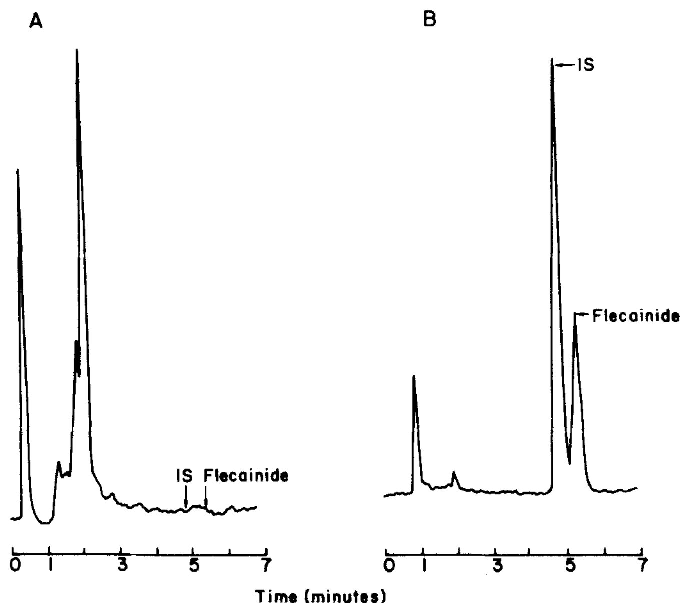

Figure 2. Chromatograms of human plasma samples; A. blank human plasma, 150 µl of total 200 µl of the reconstituted extract was injected; B. blank human plasma spiked with 100 ng flecainide and 5000 ng internal standard, 20 µl injected.

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is largely due to the selectivity of fluorescence detection. Two linear ranges were established; 100-2000 ng/ml for therapeutic monitoring and 3-300 ng/ml for pharmacokinetic studies (Fig. 3). The minimal concentration quantifiable is 3 ng/ml with a one ml sample. At 3 ng/ml, the response was at least 10 times that of the background. The linearity was not tested beyond 2000 ng/ml. In the procedure described, 1000 ng internal standard was used for the high concentration range when a fixed aliquot was injected by the autosampler and peak heights measured by an integrator. However, when a strip chart recorder is used and all peaks are to be contained on scale by manipulating the

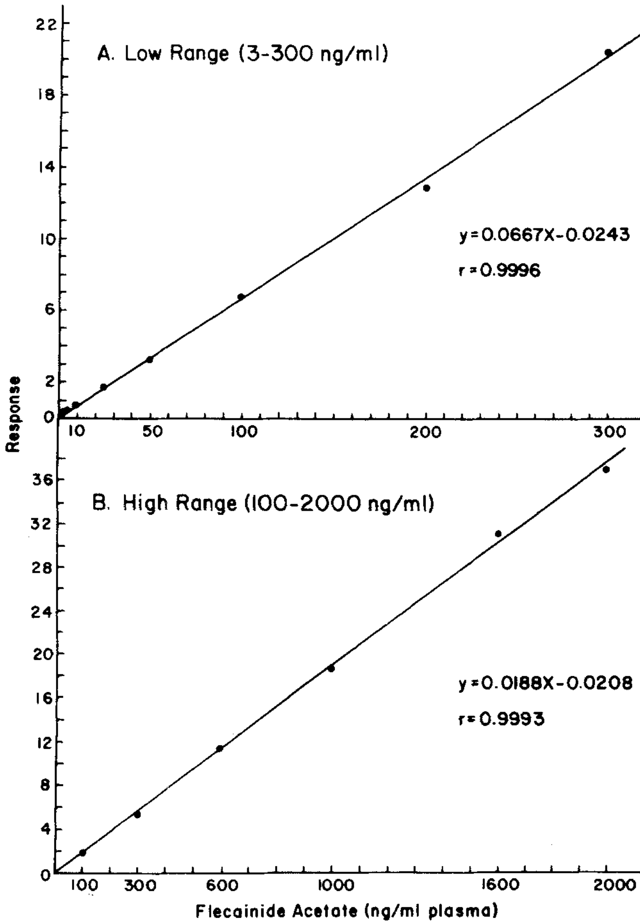

Figure 3. Calibration curves; A. low concentration range, 3-300 ng/ml; B. high concentration range, 100-2000 ng/ml.

size of the aliquot injected or range of the detector, then a larger amount of internal standard should be used -- 2500 to 5000 ng. For the low concentration range, 500 or 1000 ng internal standard is usually used.

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Occasionally, some samples of blank human plasma showed a memory peak at about 12 min. after injection. The peak height was generally small and constituted no significant interference with flecainide in the high concentration range. However, for samples in the 3-25 ng/ml range, the injections should be spaced to avoid this memory peak.

Intra-day precision, expressed as the coefficient of variation (CV), was examined at 5, 10, 25, 50, 100, 200, 300, 600, 1000, and 1600 ng/ml. The CV was less than 4% at all levels except at 5 ng/ml, a CV of 6% was found. Accuracy, expressed as relative error, was +6.0 to -7.9% for concentration levels of 10-1600 ng/ml. At 5 ng/ml, the relative error was larger. The intra-day precision and accuracy data are shown in Table 1.

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Procainamide, N-acetyprocainamide, propranolol, quinidine, lidocaine, salicylic acid, and disopyramide were tested for potential interference in the assay. These drugs were tested

TABLE 1

Precision and Accuracy

Sample Concentration (ng/ml)	Number of Samples	Coefficient of Variation (%)	Relative Error (%)
5	5	5.9	+30.4
10	5	2.9	+ 6.0
25	5	3.6	- 6.7
50	4	2.1	- 6.8
100	5	3.0	- 7.9
200	5	3.5	- 1.0
300	5	1.9	- 3.3
600	5	2.0	- 3.4
1000	3	1.9	- 3.1
1600	4	1.5	+ 0.4

by direct injection into the liquid chromatograph and their retention times were compared with those of flecainide and internal standard. None of these drugs were found to constitute any interference.

Among the existing methods for the quantitation of flecainide levels in human plasma, the GLC method (4), involving derivatization and detection by electron capture, has been most extensively used. More than 10,000 samples have been analyzed by this method in our laboratory. The new method described here was compared with the GLC method by analyzing 53 patient samples in a parallel manner. The results are depicted in Figure 4. The correlation coefficient (r) was 0.9962 and

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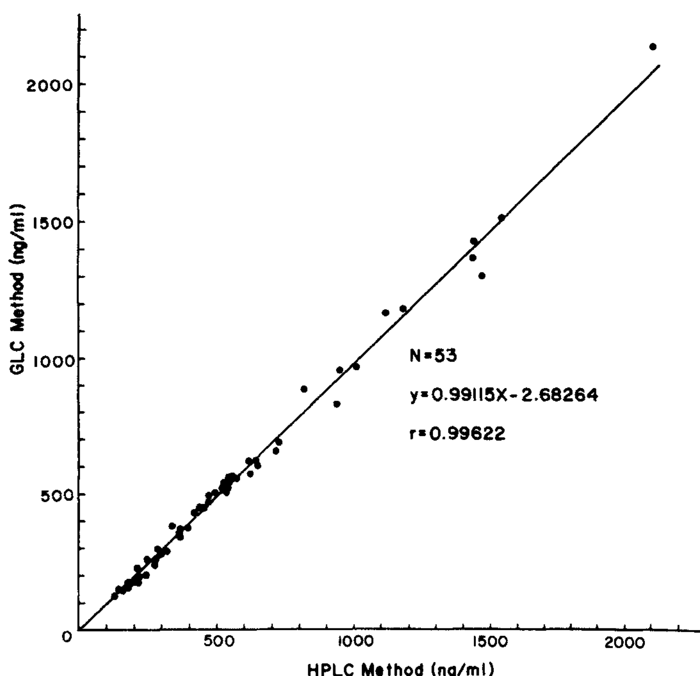

Figure 4. Comparison of the HPLC method using fluorescence detection with an established GLC method for the determination of flecainide in human plasma.

coefficient of determination (r^2) was 0.9924, indicating excellent agreement between the HPLC method and the well established GLC method.

In conclusion, this newly developed HPLC method with fluorescence detection is simple, rapid, sensitive, and selective, and is suitable for monitoring flecainide levels in plasma for the clinical management of patients.

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