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Determination of concentrations of flecainide in human serum by high-performance liquid chromatography on a fluorocarbon-bonded silica gel column

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Abstract

An optimized method for the determination of flecainide in serum is presented. Extraction using a solid-phase C_{18} column and chromatography on a stabilized fluorocarbon-bonded silica gel column effectively separate flecainide from an internal standard (a positional isomer of flecainide). The HPLC apparatus and conditions were as follows: analytical column, Fluofix 120N; sample solvent, 20 μl; column temperature, 40°C; detector, Shimadzu RF-5000 fluorescence spectrophotometer (excitation wavelength=300 nm, emission wavelength=370 nm); mobile phase, 0.06% phosphoric acid containing 0.1% tetra-*n*-butyl ammonium bromide–acetonitrile (75:25, v/v); flow-rate, 1.0 ml/min. The standard curves for flecainide were linear in the concentration range examined (10–2000 ng/ml). The regression equation was $y=0.08+0.0078x$ ($r=0.9998$). The minimum detectable amount of flecainide was approximately 5 ng/ml. In the within-day study, the precision coefficients of variation were 2.66, 2.18, 2.54, 2.72, 2.88, 2.24, and 3.29% for the 10, 50, 100, 200, 500, 1000, and 1500 ng/ml standards, respectively. The absolute recovery rates of flecainide at each concentrations were 94–100%. The method described provides analytical sensitivity, specificity and reproducibility suitable for both biomedical research and therapeutic drug monitoring.

Keywords: Fluorocarbon-bonded silica gel column; Flecainide

1. Introduction

Flecainide acetate, (2,5-di(2,2,2-trifluoroethoxy)-*N*-(2-piperidylmethyl-benzamide acetate)) is a class Ic antiarrhythmic agent, available for oral administration (Fig. 1). It is particularly useful for the treatment of ventricular and supraventricular arrhythmias [1–4]. Effective serum levels of flecainide range from 0.2 to 1.0 μg/ml [2,3]. Flecainide is eliminated by both renal and hepatic routes, the former depending on urinary pH [4]. It has a

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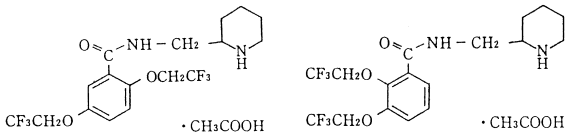

Fig. 1. Structure of flecainide acetate and internal standard.

relatively long half-life, ranging from 12 to 27 h [3,4]. The determination of serum flecainide concentration allows clinicians to distinguish therapy failure from suboptimal dosing in patients with renal failure, and to monitor patient compliance.

Several analytical methods for measuring plasma or serum concentrations of flecainide have been published based on direct detection by gas chromatography [5] or high-performance liquid chromatography (HPLC) with either fluorescence detection or UV detection [6–13]. HPLC methods with fluorescence detection possess adequate sensitivity and specificity. However, the internal standard initially used was a positional isomer of flecainide, *N*-(2-piperidylmethyl)2,3-bis(2,2,2-trifluoroethoxy)-benzamide acetate, and the peak shapes and separation of flecainide from the internal standard were unsatisfactory [7–13].

Recently, a new reversed-phase chromatographic column based on fluorocarbon-bonded silica gel, 1H, 1H, 2H, 2H, 3H, 3H-tridecafluoro-(4,4-dimethylheptyl)silyl (F13/C9)-bonded spherical microporous silica gel, has become commercially available. This column yields better resolution and specific separation than conventional octadecylsilane (ODS) columns for fluorinated compounds and biological materials [14–17].

In this paper, a rapid and sensitive HPLC method is described which measures nanogram concentrations of flecainide and its positional isomer in serum on a fluorocarbon-bonded silica gel column with solid-phase extraction clean-up.

2. Experimental

2.1. Materials

Flecainide acetate and the internal standard, *N*-(2-piperidylmethyl)2,3-bis(2,2,2-trifluoroethoxy)-benzamide acetate (Fig. 1), were obtained from Eizai (Tokyo, Japan). Acetonitrile and methanol were HPLC grade. All other solvents and reagents were of analytical reagent grade. Control human serum was purchased from Kaketuken (Kumamoto, Japan). The 1 ml solid-phase extraction columns containing 100 mg of ODS packing (Bakerbond SPE C_{18}) were manufactured by Baker (Phillipsburg, PA, USA).

2.2. Preparation of solutions

Stock solutions of flecainide (1 mg/ml) and internal standard (1 mg/ml) in methanol were stored in small portion at 4°C for 6 months without detectable decomposition. Working standard solutions of flecainide (20 μg/ml) and internal standard (10 μg/ml) were prepared by appropriate dilutions of their respective stock solutions with distilled water if necessary.

2.3. Apparatus and HPLC conditions

The HPLC apparatus and conditions were as follows: pump, Shimadzu LC-6A liquid chromatograph (Shimadzu, Kyoto, Japan); analytical column, Fluofix 120N (4.6 mm×150 mm I.D., 5 μm; Neos, Kobe, Japan); sample solvent, 20 μl; column temperature, 40°C; detector, Shimadzu RF-5000 fluorescence spectrophotometer (excitation wavelength= 300 nm, emission wavelength=370 nm, slit widths= 10 mm, time interval=0.3 s); mobile phase, 0.06% phosphoric acid containing 0.1% tetra-*n*-butyl ammonium bromide–acetonitrile (75:25, v/v); flow-rate, 1.0 ml/min.

2.4. Extraction procedure

Serum samples (1 ml) were placed in culture tubes, and 0.3 ml of internal standard (10 μg/ml) and 0.2 ml of 0.2 *M* sodium carbonate were added. The samples were vortex-mixed for 10 s and applied to solid-phase extraction columns previously conditioned with 2 ml of methanol, followed by 2 ml of water. The columns were not allowed to dry before the addition of the samples. After passing the sample through the columns, they were rinsed with 2 ml water, followed by 1 ml of 50:50 (v/v) methanol in water. Flecainide and the internal standard were eluted into glass culture tubes with 1 ml of methanol.

The eluted samples were then placed in a 40°C water bath and evaporated to dryness under nitrogen. Each sample was reconstituted with 200 μl of the mobile phase, and 20 μl was injected for HPLC analysis. All procedures were conducted in room temperature.

3. Results and discussion

3.1. Chromatography

Fig. 2 shows the typical chromatograms of flecainide and internal standard separated under the above mentioned conditions.

No interfering peaks appeared when the following drugs were added to the serum: other antiarrhythmic drugs (aprindine, procainamide, lidocaine, mexiletine, propafenone, pildicainide, disopyramide), digoxin, verapamil, diltiazem, isosorbide dinitrate, nicorandil, phenytoin, and diazepam.

For developing a solid-phase extraction procedure for serum sample clean-up, an octyl (C_8) SPE column was initially used [7,8]. However, recovery of flecainide from the serum was unsatisfactory with this system. A selective extraction method was then chosen to separate flecainide from the serum. Solid-phase extraction columns with C_8 and C_{18} solvents were evaluated in this study; the C_{18} column was more effective in eliminating the interfering serum peak and yielded very good recoveries of flecainide and internal standard. Optimization data for solid-phase extraction (C_{18}) of flecainide and internal standard are illustrated graphically in Fig. 3.

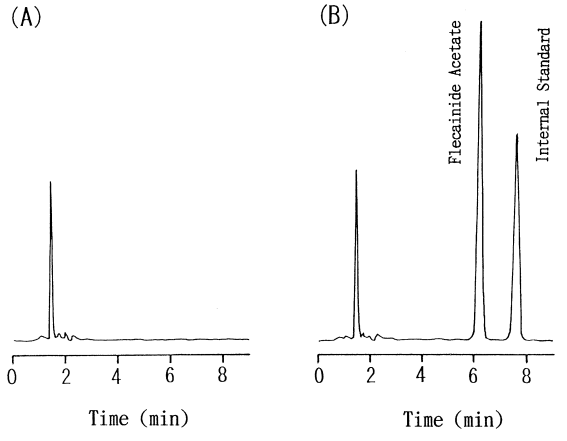

Fig. 2. Typical chromatograms with fluorescence (excitation at 300 nm, emission at 370 nm) detection of 20-μl injections of standard solution. Chromatograms of (A) blank human serum, (B) human serum with 200 ng/ml of flecainide.

3.2. Calibration

Calibration was performed by adding known amounts of flecainide and internal standard to control human serum to yield concentrations in the range of

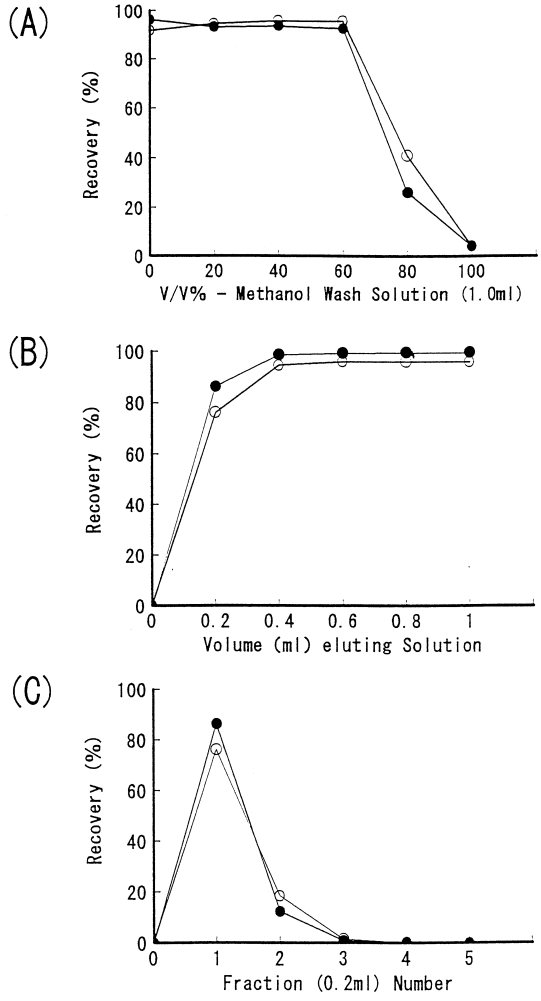

Fig. 3. Optimization data for solid-phase extraction; (A) methanol content of wash solution on recovery; (B) volume of eluting solution on recovery: also effect of elution volume on recovery (C), on the SPE C_{18} cartridge. Sequential 0.2 ml methanol were applied; –●– flecainade, –○– internal standard ($n=2$).

10–2000 ng/ml. The standard curves for flecainide were linear in the concentration range examined. The regression equation was $y=0.08+0.0078x$ ($r=0.9998$). The minimum detectable amount of flecainide was approximately 5 ng/ml with a signal-to-noise ratio of 3:1. Linearity in the above range makes it useful for biomedical research and therapeutic drug monitoring in arrhythmias patients.

3.3. Precision and recovery

The precision of analysis was assessed by five replicate analyses of human serum containing flecainide in concentrations of 10, 50, 100, 200, 500, 1000, and 1500 ng/ml, and then the within-day variations were calculated. The within-day and between-day reproducibility is summarized in Tables 1 and 2. In the within-day study, the precision coefficients of variation were 2.66, 2.18, 2.54, 2.72, 2.88, 2.24, and 3.29% for the 10, 50, 100, 200, 500, 1000, and 1500 ng/ml standards, respectively. In the between-day study, the precision coefficients of variation were 3.15, 2.57, 3.48, 3.21, 3.77, 2.29, and 3.51% for the 10, 50, 100, 200, 500, 1,000, and 1,500 ng/ml standards, respectively.

The absolute recoveries of flecainide and internal standard from the serum were determined by comparing the analyte peak height obtained after extraction of study samples to the peak height of known amounts of unextracted analyte. Analytical absolute recoveries of flecainide standard in serum were determined at concentrations in the range 10–1500 ng/ml. The absolute recovery rates of flecainide at each concentrations were 94–100% ($n=3$ at each concentration). The absolute recovery of internal standard was determined at concentrations described in the extraction procedure. The analytical absolute recovery rate of internal standard was 95–98% ($n=3$).

Table 2
Between-day precision; values represent six replicate samples over a 14-day period

Concentration added (ng/ml)	Concentration found (mean±S.D.) (ng/ml)	Coefficient of variation (%)
10	10.15±0.32	3.15
50	48.55±1.25	2.57
100	98.90±3.44	3.48
200	202.5±6,5	3.21
500	490.2±18.5	3.77
1000	1001±22	2.29
1500	1455±51	3.51

In summary, an optimized method for the determination of flecainide in serum is presented. Extraction using a solid-phase C_{18} column and chromatography on a stabilized fluorocarbon-bonded silica gel column effectively separate flecainide from an internal standard (a positional isomer of flecainide). Performance data validate method linearity within the clinically significant concentration range. Overall precision coefficients of variation range from 2.18 to 3.29%, and extraction recoveries are more than 94%. The method described provides analytical sensitivity, specificity and reproducibility suitable for both biomedical research and therapeutic drug monitoring.

Table 1
Within-day precision and relative error of flecainide in human serum; values represent five replicate samples

Concentration added (ng/ml)	Concentration found (mean±S.D.) (ng/ml)	Coefficient of variation (%)	Relative error (%)
10	10.04±0.27	2.66	+0.40
50	47.01±1.02	2.18	−5.98
100	94.51±2.40	2.54	−5.49
200	193.6±5.3	2.72	−3.20
500	485.3±14.0	2.88	−2.94
1000	976.5±21.9	2.24	−2.35
1500	1431±47	3.29	−4.60

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