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Short communication

High performance liquid chromatography for routine monitoring of serum flecainide

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Abstract

We developed a simple, rapid, and selective assay method for determination of serum flecainide by using solid phase extraction and reversed phase high performance liquid chromatography (HPLC) equipped with ordinary octadecylsilyl silica (ODS) column and ultraviolet (UV) detector. Serum samples spiked with the internal standard were treated by a disposable C_{18}-cartridge to extract flecainide. The flecainide and internal standard were separated on ODS column and were detected with an UV detector set at 298 nm. The mobile phase solvent consisting of 0.1 M 1-pentanesulfonic acid sodium salt, acetonitrile, and acetic acid (250:206:2.5 v/v) was used at the flow rate of 1.0 ml/min. The calibration curve for flecainide was linear at the concentration of 50–1500 ng/ml ($r = 0.9998$). The recoveries of flecainide from serum samples were 92–98%. The coefficient of variations (CVs) for intra- and inter-day assay were 1.3–4.8 and 3.2–6.9%, respectively. The method could be applied to routine monitoring of serum flecainide in the patients with tachyarrhythmia. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Flecainide; Therapeutic drug monitoring; High performance liquid chromatography

1. Introduction

Flecainide acetate, a class Ic [Vaughan–Williams] antiarrhythmic agent, has been used for a variety of supraventricular tachycardias [1]. The therapeutic range for serum flecainide has been defined as being 200–1000 ng/ml [2,3]. Trough flecainide concentration greater than 1000 ng/ml produces adverse events such as aggravated ventricular arrhythmias, which may cause death in patients with a history of sustained ventricular tachycardia and left ventricular dysfunction [4]. The serum concentration of flecainide, therefore, is required to remain at 200–1000 ng/ml.

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Several analytical methods, chromatography and immunoassay, for measuring serum flecainide have been published [5–14]. The HPLC method widely used for determining flecainide so far required fluorescence detector [5,7–13] and unusual column such as fluorocarbon column [12,13]. In the present study, we describe a simple HPLC using conventional ODS column and UV detector for routine monitoring of serum flecainide.

2. Experimental

2.1. Chemicals and instruments

Flecainide acetate and the internal standard [*N*-(2-piperidylmethyl)-2,3-bis(2,2,2-trifluoroethoxy) benzamide acetate] were supplied from Eisai (Tokyo, Japan). 1-Pentanesulfonic acid sodium salt was purchased from Wako (Tokyo, Japan). All other chemicals were of HPLC or analytical reagent grade. C_{18}-Cartridges (Extract-Clean C_{18}, 100 mg) used for solid phase extraction were obtained from Alltech Associate (IL, USA).

2.2. Preparation of solutions

The control serum was prepared by using an alternative human serum (Twin-consera H, Nissui, Tokyo, Japan). The serum samples spiked with flecainide acetate at concentrations of 50, 200, 500 and 1000 ng/ml were used as the standard specimens for calibration curve, recovery and assay precision. Standard specimens were stored at -20 °C until use. The internal standard was prepared as the 5 μg/ml solution in distilled water and was stored at 4 °C until analysis.

2.3. C_{18}-Cartrige extraction

Five hundred microliter aliquot of serum was added with 150 μl of internal standard solution and then, was alkalinized by adding 100 μl 0.02 M Na_2CO_3. The mixture was loaded onto C_{18}-cartrige pretreated with 1 ml methanol and followed by 1 ml water. The C_{18}-cartrige was set at Manihold (Alltech) and was subsequently washed with 1 ml water, and 1 ml 50% methanol under the vacuum. Flecainide and internal standard were eluted by treating the cartridge with 1 ml methanol and were collected into glass tubes. The effluents were placed in a dry thermo bath (45 °C) and evaporated to dryness under nitrogen gas. The sample was reconstituted with 100 μl mobile phase solution and the 20 μl aliquot was injected into the HPLC.

2.4. HPLC apparatus and analytical conditions

The HPLC system used in the present study consisted of pump (DP-8020, TOSOH Tokyo, Japan), UV detector (UV-8020, TOSOH), and auto sample injector (AS-8020, TOSOH). ODS column (TSK-GEL, 4.5 i.d. × 250 mm, TOSOH) was maintained at room temperature. The detection wavelength was set at 298 nm. The mobile phase solution consisting of 0.1 M 1-pentanesulfonic acid sodium salt, acetonitrile, acetic acid (250:206:2.5 v/v) was pumped at a flow rate of 1.0 ml/min.

3. Results and discussion

A typical chromatogram for determination of serum flecainide was shown in Fig. 1. The peaks representing flecainide and internal standard were observed at the retention times of 8.4 and 7.9 min, respectively. There was no interfering peak in

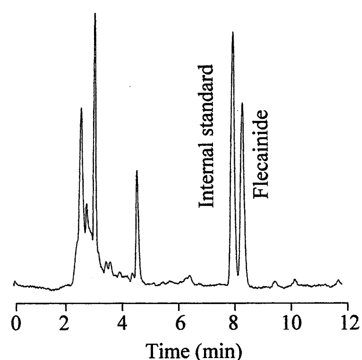

Fig. 1. Typical chromatogram for determination of flecainide. The concentration of flecainide determined was 354 ng/ml.

Table 1
Recovery of flecainide from control serum samples

Concentration (ng/ml)	Recovery ($n = 5$)	
	Mean ± S.D. (%)	CV (%)
50	92.2 ± 5.6	6.1
200	97.5 ± 2.1	2.1
500	94.6 ± 2.3	2.4
100	95.5 ± 2.4	2.5

both control and patient's serum samples. The detection limit of flecainide was 2.5 ng as the injected amount onto the column, which corresponded to 25 ng/ml serum.

The regression curve for determining serum flecainide was linear at the concentration of 50–1500 ng/ml. The equation of the calibration curve calculated by regression analysis was $Y = 334.49X - 5.04$ ($r = 0.9998$), where Y was the serum flecainide concentration (ng/ml) and X, the peak height ratio of flecainide to internal standard.

The recoveries of flecainide examined at concentrations of 50, 200, 500 and 1000 ng/ml serum were more than 92% with the CV values less than 6.1% (Table 1). The assay precision was evaluated by intra- and inter-day validation at concentrations of 50, 200 and 1000 ng/ml (Table 2). For intra-day precision, five sets of each control sample were assayed on the same day. For inter-day assay precision, five sets of each control sample were assayed on 5 different days. The CVs for intra- and inter-day assay were 1.3–4.8 and 3.2–6.9%, respectively. The relative errors (bias) for intra- and inter-day assay were less than 3.4 and 8.7%, respectively.

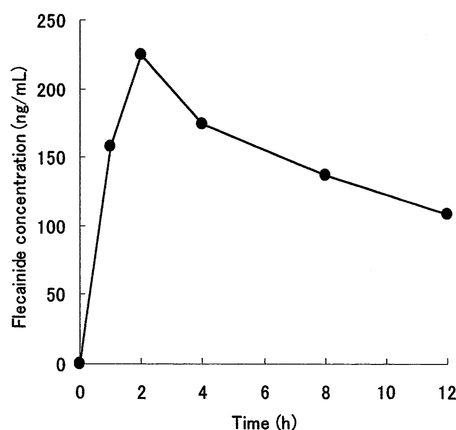

Fig. 2. Blood concentration profile for flecainide after oral administration of 100 mg flecainide in a patient with arterial fibrillation.

We applied present method to the samples obtained from the ten patients with supraventricular tachycardia. There was no interfering peak on the chromatogram, though the patients had received warfarin potassium, ticlopidine hydrochloride, and atenolol concomitantly (data not shown). We conducted a pharmacokinetic study to calculate the parameter for making dosage regimen in one case. As shown in Fig. 2, present HPLC could be applied to illustrating serum concentration profile of flecainide. The calculated pharmacokinetic parameters for this case: AUC, oral clearance, and half-life were 3663 ng h/ml, 27.3 l/h, and 11.9 h, respectively.

Fluorescence detection and/or the special column, such as fluorocarbone column, have been required in a previous paper describing flecainide HPLC [12,13]. The combined use of solid phase extraction and ion-pair chromatography reported so far also have employed fluorescence detection

Table 2
Intra- and inter-day precision for the determination of flecainide

Concentration (ng/ml)	Intra-day ($n = 5$)			Inter-day ($n = 5$)		
	Mean ± S.D. (ng/ml)	CV (%)	Bias (%)	Mean ± S.D. (ng/ml)	CV (%)	Bias (%)
50	51.7 ± 2.5	4.8	3.4	50.4 ± 3.5	6.9	0.8
200	199.7 ± 2.6	1.3	0.2	213.1 ± 6.9	3.2	6.6
1000	997.0 ± 19.3	1.9	0.6	1087.1 ± 52.6	4.8	8.7

because of its high sensitivity [11]. In the present HPLC, we used UV detector instead of fluorescence detector with minor modification. Assay precision of the present method, which was confirmed by the recovery and intra- and inter-day validations, was almost the same as the previous method. The sensitivity was sufficient for routine monitoring of serum flecainide in the therapeutic range of 200–1000 ng/ml, though the UV detection was less sensitive compared with the fluorescence detection. Indeed, we conducted trough level monitoring in ten patients receiving 150–250 mg/day flecainide and determined the concentration of 219–947 ng/ml. No interfering peak on the chromatogram was observed even though the patients were receiving other medications. The present method can be applied to routine monitoring flecainide especially in the hospitals and medical institutions.

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