DETERMINATION OF DILTIAZEM IN BODY FLUIDS BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION

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原　　報

DETERMINATION OF DILTIAZEM IN BODY FLUIDS BY GAS CHROMATOGRAPHY WITH SURFACE IONIZATION DETECTION

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表面電離検出ガスクロマトグラフィーによる体液中ジルチアゼムの定量

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Summary

Diltiazem, an antiarrhythmic drug, has been tested for its detection by gas chromatography equipped with surface ionization detection (GC-SID). The calibration curve using flurazepam as internal standard was linear between 30 and 500 pg on column. The detection limit was about 20 pg on column (1 ng/ml). Solid phase extraction of the drug with Sep-Pak C_{18} cartridges from whole blood and urine samples gave excellent recovery and relatively clean backgrounds. The sensitivity of the present method is higher than that of any GC and HPLC method so far reported.

Key words: Diltiazem; Antiarrhythmic drug; Gas chromatography (GC); Capillary GC; Surface ionization detection; Sep-Pak C_{18} cartridges

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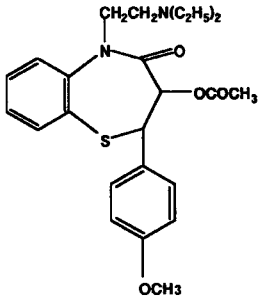
Introduction

Diltiazem is a calcium channel blocker widely used for treatment of angina pectoris and arrhythmias. This calcium antagonist has effects on contractile mechanism of the coronary artery, smooth muscle and heart muscle [1, 2]. Some fatalities caused by the suicidal ingestion of diltiazem were reported [3, 4]. The analysis of the drug was made by high-performance liquid chromatography (HPLC) [5, 6], by gas chromatography (GC) with nitrogen-phosphorus detection (NPD) [7], by GC with electron-capture detection (ECD) [8, 9] and GC-mass spectrometry (MS) [10]. In this brief report, we present a method of GC with surface ionization detection (SID) for diltiazem, which is much more sensitive than those by the GC-NPD and GC-ECD [7−9].

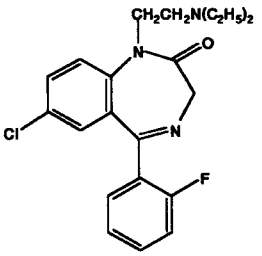
Experimental

Materials

Diltiazem-HCl and flurazepam-2HCl (internal standard, IS) were gratefully gifted from Tanabe Seiyaku Co., Ltd. (Osaka) and Nippon Roche Pharmaceutical Co., Ltd. (Tokyo), respectively. Their chemical structures are shown in Fig. 1. Sep-Pak C_{18} cartridges were purchased from Waters Associates (Milford, MA, USA). Other chemicals used in this study were of analytical grade. Whole blood and urine were obtained from healthy subjects.



Diltiazem



Flurazepam

Fig. 1. Chemical structures of diltiazem and flurazepam (internal standard).

Extraction with Sep-Pak C_{18} cartridges

Sep-Pak C_{18} cartridges were pretreated by passing 10 ml of chloroform/methanol (9 : 1), 10 ml of methanol and 10 ml of distilled water and this procedure was repeated 5 times.

To 1 ml of whole blood or urine, with or without of addition of diltiazem (12.5 ng) and flurazepam (25 ng), 8 ml of distilled water was added. The sample solution was applied onto the pretreated Sep-Pak C_{18} cartridge at a rate less than 5 ml/min. After washing with 20 ml of distilled water, the drugs were eluted with 3 ml of chloroform/ethanol (9 : 1). The organic

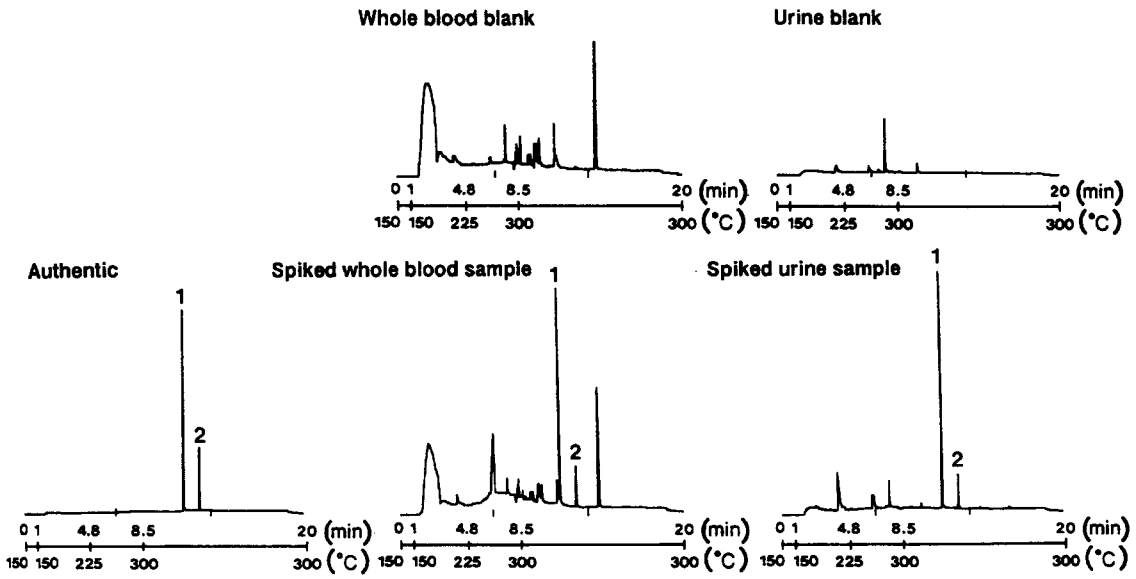


Fig. 2. Capillary GC-SID for diltiazem extracted with Sep-Pak C_{18} cartridges from whole blood and urine samples and for their backgrounds. Numbers 2 and 1 indicate the peaks of diltiazem and flurazepam (internal standard), respectively. The amounts of diltiazem and flurazepam spiked to each 1 ml sample were 12.5 ng and 25 ng, respectively.

phase containing the drugs was dried up under the stream of nitrogen. The resultant residue was dissolved in 100 μl of methanol and a 2-μl aliquot was injected into the GC port.

GC conditions

GC-SID was carried out on a Shimazu GC-14A gas chromatograph (Shimazu Corp., Kyoto). The GC conditions were as follows: column temperature, 150−300 ℃ (1 min hold at 150 ℃ and 20 ℃/min); injection temperature, 230 ℃; flow rate of He-carrier, 3 ml/min. The samples were injected in the splitless mode at a column temperature of 150 ℃ and splitter was opened after 1 min. The SID conditions were the same as described previously [11].

Results and discussion

Gas chromatograms by GC-SID for human urine or whole blood sample in the presence and absence of diltiazem are shown in Fig. 2. Under our analytical conditions, neither peak tailing nor overlapping with impurities was observed. There were no impurity peaks from 9.8 to 14.1 min for both whole blood and urine samples. The retention times of diltiazem and flurazepam was 12.4 and 11.3 min, respectively.

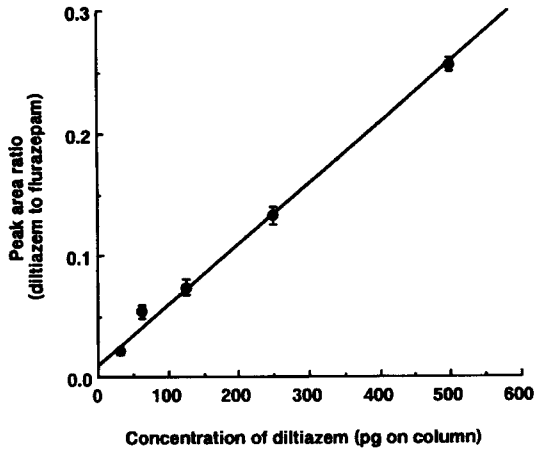


Fig. 3. Calibration curve for diltiazem. The amount of flurazepam used as internal standard was 500 pg on column.

The recovery of diltiazem and flurazepam was 88 % and 111 %, respectively, for urine samples, and 98 % and 129 % for whole blood samples. Although the recovery of internal standard flurazepam was over 100 %, the excess was not considered to be contaminations by impurities.

Figure 3 shows a calibration curve for diltiazem. A standard curve was prepared by adding 250 ng of internal standard flurazepam to each concentration of diltiazem. The equation and regression coefficient were: $y=0.00050\ x + 0.0097$ and $r=0.993$. The detection limit of diltiazem was about 20 pg on column (1 ng/ml).

According to a case report on a suicidal decedent [4], diltiazem levels were 6.7 μg/ml in blood, 5.4 μg/ml in urine and 79 μg/g in the liver. Their levels, of course, far exceed the detection limit of the present GC-SID.

The therapeutic concentrations of diltiazem were reported to be about 20 ng/ml serum 10 h after oral administration of a 60 mg tablet [9] and 8–15 ng/ml plasma 8 h after *i.v.* administration of 20 mg of the drug [8]. Thus, the sensitivity of the present GC-SID sufficiently meets pharmacokinetic studies of this drug also.

This is the first trial to detect diltiazem by GC-SID and to use solid phase extraction with Sep-Pak C_{18} cartridges for the drug. In addition, the sensitivity of the present GC-SID for the drug is higher than that of any GC and HPLC method so far reported [5, 6, 8–10].

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