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RAPID AND SENSITIVE DETERMINATION OF APRINDINE IN SERUM BY GAS CHROMATOGRAPHY USING A SURFACE IONIZATION DETECTOR

HITOMI KAWANO and NOBUO INOTSUME

Department of Pharmaceutical Services, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto 860 (Japan)

HIROMI ARIMOTO

Analytical Application Department, Shimadzu Corporation, Nishinokyo, Nakagyo, Kyoto 604 (Japan)

TOSHIHIRO FUJII

Division of Chemistry and Physics, National Institute for Environmental Studies, Tsukuba, Ibaraki 305 (Japan)

and

MASAHIRO NAKANO*

Department of Pharmaceutical Services, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto 860 (Japan)

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SUMMARY

A rapid and highly sensitive method for the determination in serum of aprindine, an antiarrhythmic drug, was developed employing gas chromatography with a surface ionization detector. No interfering peak from endogenous substances appeared when an organic phase was directly injected into the system after single extraction from a serum sample. A standard curve obtained was linear up to the serum level of 6 μg/ml, and the limit of sensitivity was 16 pg. The method described is applicable to routine therapeutic monitoring of serum concentrations of aprindine.

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INTRODUCTION

Aprindine, N-phenyl-N-(3-diethylaminopropyl)-2-indanylamine, an effective antiarrhythmic drug, was recently introduced for the management of ventricular and supraventricular arrhythmias [1]. The therapeutic range of aprindine in serum has been reported to be narrow, and its great inter-individual variability in the steady state has also been reported [1]. Moreover, non-linear pharmacokinetic behaviour of aprindine has been demonstrated [2]. Thus, there is a need for a safe and effective clinical method of controlling the serum aprindine level within the therapeutic range.

Several analytical methods have been reported for the measurement of aprindine in biological fluids, including gas chromatography (GC) [3,4], high-performance liquid chromatography (HPLC) [5] and gas chromatography–mass spectrometry (GC–MS) [6], but they are complicated and time-consuming when used to determine serum levels of aprindine.

A surface ionization detector, a new detector for GC, has exhibited an excellent response to compounds with an amino group, especially tertiary amines [7]. The aim of the present study is to develop a rapid and highly sensitive method for the measurement of aprindine concentrations in serum samples, employing a capillary column and a surface ionization detector.

EXPERIMENTAL

Materials

Aprindine hydrochloride was kindly supplied by Mitsui Pharmaceutical (Tokyo, Japan) and imipramine hydrochloride was purchased from Nakarai Tesque (Kyoto, Japan). Distilled and deionized water was used for the preparation of stock solutions of aprindine hydrochloride and imipramine hydrochloride. Organic solvents were distilled before use. Other chemicals were of reagent grade and purchased from Wako Pure Chemicals (Osaka, Japan).

Extraction procedure

To a 100-μl portion of a serum sample in a 1.5-ml glass microtube, 50 μl of a 0.5 *M* sodium hydroxide solution saturated with sodium chloride and 10 μl of an imipramine solution as an internal standard (2.5 μg/ml) were added. Then 500 μl of ethyl acetate–hexane (9:1, v/v) were added, and the samples were mixed for 20 s by a vibration mixer. After centrifugation, an aliquot of the organic phase was injected into the GC system.

Instruments

GC-R1A and GC-16A systems (Shimadzu, Kyoto, Japan) were employed. The GC-R1A system was equipped with a 15 m $\times$ 0.53 mm I.D. flexible fused-silica megabore column (DB-17, film thickness 1.0 μm, J&W Scientific,

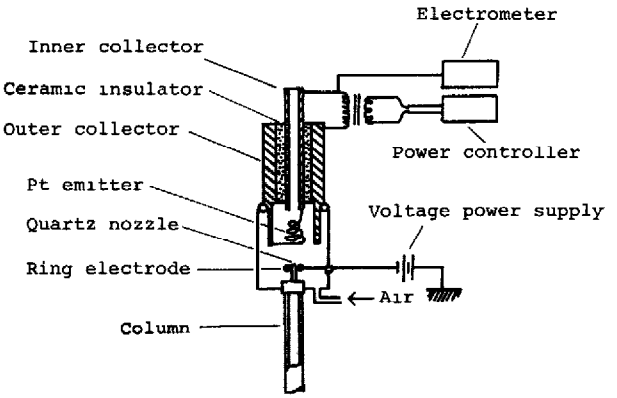

Fig. 1. Schematic diagram of a surface ionization detector.

U.S.A.), and the GC-16A system was equipped with a 30 m×0.31 mm I.D. flexible fused-silica capillary column (DB-1, film thickness 1.0 μm, J&W Scientific). A moving-needle-type solventless sample injector was also employed.

A schematic diagram of the surface ionization detector is shown in Fig. 1. It has an electrically heated platinum filament as an emitter surface, and the process of positive surface ionization on this filament forms the basis of this detector [7]. A flame thermionic detector and a flame ionization detector were also used for comparison.

Helium was used as carrier and make-up gas. The operating temperatures were set as follows: injection port, 270°C; column, 240°C; detector, 270°C.

Standard curves

Known amounts of aprindine (0.05–6 μg/ml) were added to a blank serum. The concentration of aprindine in the serum sample was estimated by the peak-area ratios of aprindine to imipramine. The standard curves were obtained for each set of the serum samples.

Clinical study

A female patient, 14 years old, who had been receiving chronic anti-arrhythmic treatment, participated in the study. Ethical aspects were guided by the Declaration of Helsinki. An informed consent was obtained. The subject took aprindine (20 mg each, two times a day) and metoprolol (40 mg each, three times a day) chronically. At steady state, serum samples were collected at appropriate times and stored at −20°C until analysis.

RESULTS

Gas chromatography

Several organic solvent mixtures, including ethyl acetate–hexane (9:1, v/v), were evaluated in the extraction procedure. The extraction recoveries of

TABLE I

EXTRACTION OF APRINDINE AND IMIPRAMINE WITH VARIOUS SOLVENT EXTRACTION SYSTEMS

Extraction solvent	Recovery (%)	
	Aprindine	Imipramine
n-Hexane	21.1	42.6
Chloroform	52.3	82.4
Diethyl ether	81.7	78.4
Ethyl acetate	102.9	98.5
Ethyl acetate–*n*-hexane (9.1)	91.3	101 6

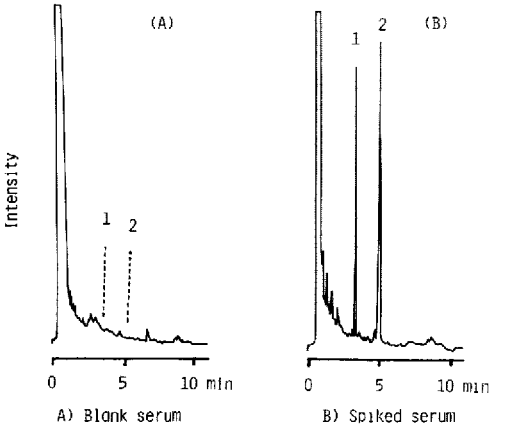

Fig. 2. Chromatograms of (A) a blank serum extract and (B) an extract of a serum spiked with aprindine (100 ng) and imipramine (25 ng) Peaks: 1 = imipramine; 2 = aprindine.

TABLE II

RETENTION TIMES OF APRINDINE, ITS METABOLITE AND OTHER ANTIARRHYTHMIC AGENTS

Compound	Retention time (min)
Aprindine	5.04
Desethylaprindine	4.70
Mexiletine	0.31
Lidocaine	0.75
Metoprolol	1.90
Propranolol	1.50
Disopyramide	8.89
Nifedipine	13.92

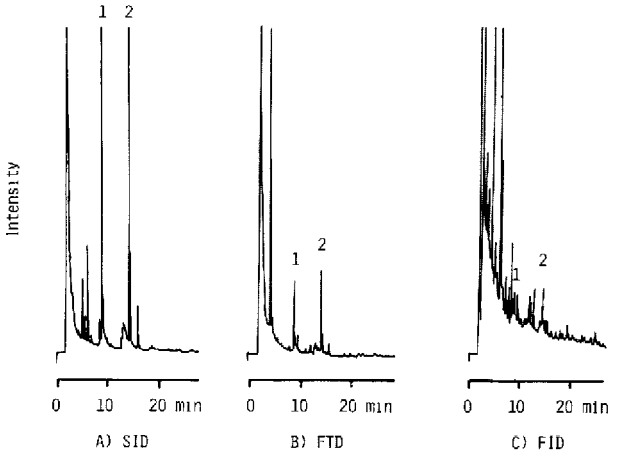

Fig. 3. Gas chromatograms of aprindine and imipramine in a serum obtained with various detectors. (A) Surface ionization detector; (B) flame thermionic detector; (C) flame ionization detector. Peaks: 1 = imipramine (250 pg); 2 = aprindine (160 pg). Sensitivity, $2 \cdot 10^{-11}$ a.u.f.s.

aprindine and imipramine from various solvents are shown in Table I. Ethyl acetate–hexane (9:1, v/v) was used throughout this study because of the difference in relative recoveries of drugs and endogenous substances. GC profiles from the extracts of serum are shown in Fig. 2. No interfering peak from endogenous substances was observed. The retention times of aprindine and imipramine were 5.0 and 3.1 min, respectively. As shown in Table II, good separation of aprindine from its major metabolite and other antiarrhythmic agents was observed. GC profiles of the extract of a spiked serum employing various detectors are shown in Fig. 3. The surface ionization detector gave a better response than the other detectors.

Validation of the method

The minimum detectable limit for aprindine was found to be 16 pg. A standard curve was linear, with a correlation coefficient value of 0.999 in the concentration range 0.05–6 μg/ml. As shown in Table III, recovery values of aprin-

TABLE III

RECOVERY VALUES OF APRINDINE THROUGH THE WHOLE PROCEDURE

Averages of six determinations.

Concentration (μg/ml)	Recovery (%)
0.2	88.3
1.0	90.2
4.0	95.6

TABLE IV

PRECISION AND ACCURACY OF THE METHOD

Concentration added (μg/ml)	Within-day ($n=6$)		Between-day ($n=6$)	
	Concentration found (mean ± S.D.) (μg/ml)	C.V. (%)	Concentration found (mean ± S.D.) (μg/ml)	C.V. (%)
0.2	0.19 ± 0.02	7.14	0.20 ± 0.02	9.59
1.0	0.96 ± 0.02	0.67	1.04 ± 0.03	2.96
4.0	4.12 ± 0.05	0.58	4.01 ± 0.12	4.50

dine in spiked samples were found to be 88, 90 and 96% at aprindine concentrations of 0.2, 1.0 and 4 μg/ml, respectively.

Reproducibility was indicated by the within-day coefficients of variation (C.V.) as well as by the between-day variation values (Table IV). Intra- and inter-day C.V. for quantitation of aprindine were found to be 0.6–7.1 and 3.0–9.6%, respectively, at serum concentrations of 0.2–4.0 μg/ml.

Samples from the patient

The method developed was applied to determine concentrations of aprindine in serum samples from the patient, who was taking aprindine (20 mg each, two times a day) and metoprolol (40 mg each, three times a day). No peak other than those of aprindine and imipramine (I.S.) was observed in GC profiles of serum extracts.

A concentration–time curve of aprindine in serum after oral administration of aprindine (20 mg) is shown in Fig. 4. The disposition pattern was fitted to the two-compartment open model with first-order absorption and lag time.

The concentration range of aprindine at steady state was found to be 0.53–0.75 μg/ml, which is within the previously reported therapeutic range of aprindine [8], and a terminal half-life of aprindine was calculated to be 109 h by a

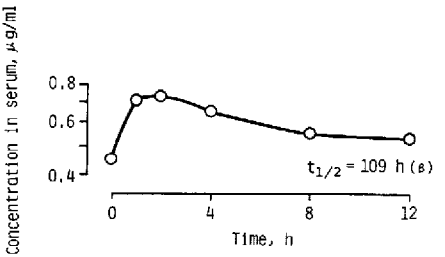

Fig. 4. Concentration–time profile of aprindine in serum from an arrhythmic patient receiving 20 mg each of aprindine twice a day.

TABLE V

PHARMACOKINETIC PARAMETERS OF APRINDINE IN A PATIENT RECEIVING MULTIPLE DOSES OF THE DRUG

Abbreviations. k_a = absorption rate constant; V_{d1} = volume of distribution at the central compartment; F = fraction absorbed; k_{12} = transfer rate constant from the central to the peripheral compartment; k_{21} = transfer rate constant from the peripheral to the central compartment; k_{13} = elimination rate constant from the central compartment

Parameter	Value
k_a (h^{-1})	0.68
V_{d1}/F (l)	36.6
k_{12} (h^{-1})	0.55
k_{21} (h^{-1})	0.057
k_{13} (h^{-1})	0.075
Lag time (h)	0.17

log-linear regression analysis. Pharmacokinetic parameters obtained by a non-linear regression analysis are shown in Table V.

DISCUSSION

The surface ionization detector has been reported to show an extremely high response to aliphatic tertiary amines, such as lidocaine, amitriptyline and imipramine [7]. The precision and accuracy of the method described were sufficient for routine monitoring of serum aprindine levels. The serum level in a patient receiving 20 mg each of aprindine twice a day reached 0.5–0.7 μg/ml at steady state. Aprindine, like phenytoin, is known to be eliminated with non-linear kinetics at relatively high serum concentrations, and its therapeutic range is reported to be narrow, 0.25–1.25 μg/ml [8]. As great inter-individual variations are reported for drugs that are eliminated in a non-linear fashion, frequent monitoring of serum aprindine concentrations is necessary to make aprindine therapy safe and effective.

Monitoring of a free level, i.e. a protein-unbound concentration, of drugs has received great attention in recent years because of its better correlation with a clinical response [9]. Aprindine has been reported to exhibit high extents of protein binding with a bound fraction of 94–97% [2], and is mainly bound to α_1-acid glycoprotein (AAG) [10]. Because an AAG level changes rapidly as the clinical condition of the patient changes, the free fraction of aprindine can be variable. The method described is so sensitive that free levels of aprindine can be determined. Clinical application of the present assay procedures will make aprindine therapy safer and more effective.

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