Journal of Chromatography, 274 (1983) 341—345
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1600

Note

Determination of aprindine in plasma by gas chromatography—mass spectrometry

TERUO ITO, HIROSHI NAMEKAWA and TAKASHI KOBARI*

Institute of Biological Science, Mitsui Pharmaceuticals, Inc., 1900-1 Togo, Mobara-shi, Chiba-ken, 297 (Japan)

(First received September 13th, 1982; revised manuscript received December 7th, 1982)

Aprindine hydrochloride [N-phenyl-N-(3-diethylaminopropyl)-2-indanylamine hydrochloride] (Fig. 1), an effective antiarrhythmic drug against a variety of supraventricular and ventricular arrhythmias, has been used for several years in Europe [1] and is currently undergoing clinical evaluation in Japan. As this drug has a narrow therapeutic index [1], monitoring of plasma levels of the drug is of great importance in assessing drug therapy.

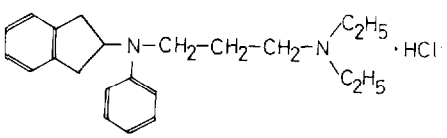

Fig. 1. Structure of aprindine hydrochloride.

Several assay methods using gas chromatography (GC) with a flame ionization detector [2,3] and with a nitrogen-sensitive detector [3,4] as well as high-performance liquid chromatography [5] have been applied for the measurement of aprindine in biological fluids. A radioimmunoassay procedure has also been reported [6].

The present report describes a rapid and sensitive gas chromatography—mass spectrometry (GC—MS) method for the measurement of aprindine concentrations in plasma and its application to the analysis of plasma from subjects who received the drug.

0378-4347/83/$03.00

EXPERIMENTAL

Standards and reagents

Aprindine hydrochloride was supplied by A. Christiaens S.A. (Brussels, Belgium) and amitriptyline hydrochloride was purchased from Kodama Chemicals (Tokyo, Japan). Stock standard solutions of aprindine hydrochloride and amitriptyline hydrochloride were prepared in distilled water and stored at 4°C throughout the experiments. The hydrochloride salt of aprindine was used and the concentrations, when expressed as μg/ml, have been calculated as the salt. All other reagents were of analytical grade and obtained from usual commercial sources.

Instrumentation

An MS-DC 05/06 automated gas chromatograph—JMS-DX300 mass spectrometer coupled with a JMA-3500 data system (JEOL, Tokyo, Japan) was used.

The coiled glass column (2 m × 1.5 mm I.D.) packed with 3% silicone OV-1 on Chromosorb 750 (80—100 mesh) (Nihon Chromato Works, Tokyo, Japan) was used for the gas chromatography. The temperatures of the column and injector were maintained at 260°C and 280°C, respectively. The flow-rate of the carrier gas (helium) was set at 40 ml/min.

The mass spectrometer was operated in the chemical ionization (CI) mode under the following conditions; ionization energy, 200 eV; ionization current, 300 μA; accelerating voltage, 3 kV; reagent gas, ammonia at a pressure of 0.5 torr. Under these conditions the ions selected for monitoring were the quasi-molecular ions, $[M+1]^+$, of *m/z* 323 and 278 for aprindine and amitriptyline, respectively, and the retention times were aprindine 0.84 min and amitriptyline 0.5 min.

Sample preparation and extraction

To a 15-ml screw-cap glass test tube were added in succession human plasma (1 ml), 100 μl of the internal standard solution containing 100 ng of amitriptyline as hydrochloride salt, 1 ml of 0.2 *M* sodium carbonate buffer (pH 10.2) and 6 ml of diethyl ether. The samples were shaken for 15 min. Following centrifugation at 2000 *g* for 5 min, the upper organic phase was transferred to another tube, and the aqueous phase was extracted with 6 ml of ether. The combined organic phase was evaporated to dryness under nitrogen gas flow at 40°C. Then 1 ml of 0.1 *N* HCl and 2 ml of ether were added to the residue, followed by shaking for 5 min. After a brief centrifugation, the organic phase was discarded by aspiration. The aqueous phase was made alkaline with 1 ml of 0.2 *M* sodium carbonate buffer and extracted with 6 ml of ether. The organic phase was evaporated to dryness under nitrogen. The residue was dissolved in chloroform (50—100 μl), and an aliquot (2 μl) was injected into the gas chromatograph—mass spectrometer.

Calibration curve and quantitative analysis

A calibration curve was constructed with every analysis by adding various amounts of aprindine hydrochloride (10—600 ng) and a constant amount of

internal standard (100 ng as salt) to 1 ml of control plasma and analyzing the samples as described above. To assess linearity, the peak area ratio (PAR) of aprindine hydrochloride to the internal standard was plotted against the amount of aprindine hydrochloride added.

The amount of aprindine in each plasma sample from subjects was calculated from a calibration curve on the basis of the PAR value.

Recovery and precision

Recoveries were calculated by comparing the PAR values of spiked plasma standards with those obtained when the internal standard was added to the plasma and the known amounts of aprindine hydrochloride (50, 100 and 300 ng) were added to the aqueous phase prior to the last extraction with ether. The precision of the method was estimated by analyzing spiked plasma standards that had been prepared on three different days.

Application of the method

Five healthy volunteers (male, 58—69 kg, age 20—40 years) each received a 25-mg capsule of aprindine hydrochloride. Venous blood was collected with heparinized syringes at various intervals for 72 h after dosing and centrifuged at 2000 *g* for 15 min. Plasma samples were stored at −20°C until taken for assay.

RESULTS AND DISCUSSION

To obtain suitable GC—MS conditions for the determination of aprindine and amitriptyline, a comparative evaluation of ionization modes [electron impact (EI) and CI modes] was carried out, and the results are shown in Fig. 2. The EI mass spectra of both compounds showed some fragment ions but no M^+ ion (Fig. 2A). The CI spectra of aprindine and amitriptyline using ammonia as a reagent gas, on the other hand, showed $[M+1]^+$ ion with additional small fragment ions (Fig. 2B). The use of diethylamine as a reagent gas gave similar results, while other reagent gases such as methane and isobutane gave no $[M+1]^+$ ion.

To avoid possible interference and to improve the sensitivity with a view to the analysis of low levels of aprindine in plasma, an ion peak with high intensity should be selected for monitoring. For this reason, mass fragmentation by CI using ammonia as a reagent gas and the detection of the $[M+1]^+$ ion seem to be adequate for the determination of the compounds of interest.

The mass fragmentograms of the plasma samples are presented in Fig. 3. The drug-free control plasma extract gave no interfering peaks at *m/z* 323 and 278, selected for monitoring aprindine and amitriptyline, respectively.

The calibration graph obtained from the analysis of plasma samples containing various amounts of aprindine gave a straight line (correlation coefficient, r=0.96) over the concentration range of 50—600 ng/ml.

The overall recoveries from plasma determined in triplicate at three different concentrations of aprindine (50, 100 and 300 ng) were over 89%. The lower detection limit was 5 ng/ml in the case of a plasma volume of 1 ml.

The overall within-day coefficients of variation were 4.4%, 11.2% and 7.7%

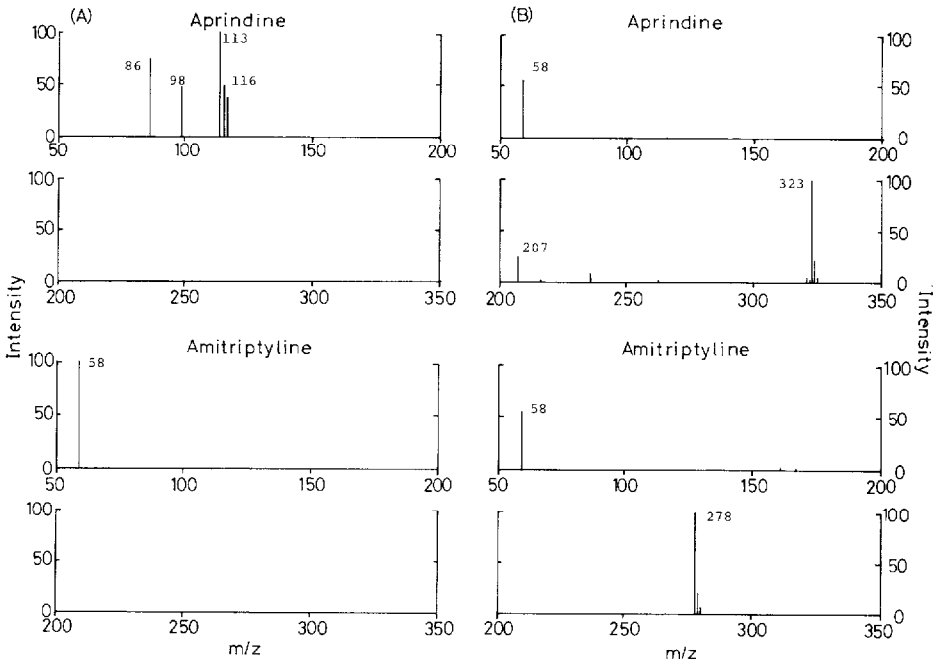

Fig. 2. Mass spectra of aprindine and amitriptyline: (A) electron impact mode; (B) chemical ionization mode with ammonia.

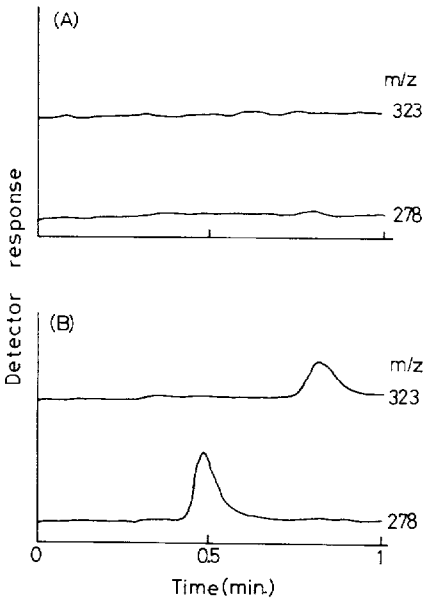

Fig. 3. Chromatograms of (A) plasma blank and (B) plasma standard with aprindine hydrochloride (100 ng) and internal standard (100 ng).

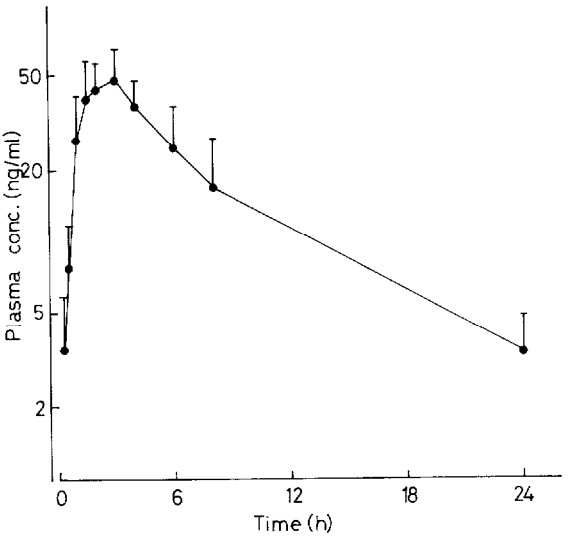

Fig. 4. Plasma concentrations of the unchanged drug after oral administration of aprindine hydrochloride (25 mg) to healthy volunteers. Each point represents the mean ± S.D. from five subjects.

for 100, 200 and 400 ng/ml aprindine, respectively. The overall between-day coefficient of variation ranged from 15.4% to 22.1% at the same concentrations. These wide between-day variations are most likely due to day-to-day changes in ionization reaction conditions and/or column conditions. However, this problem could be overcome by running a calibration curve with every analysis.

The present method is rapid and sufficiently sensitive to be satisfactory for the determination of aprindine in plasma; it is applicable to routine measurement of plasma levels occurring in clinical studies, and one person can analyze 100—150 samples during an average working day.

Application of the method for measuring mean plasma concentrations of aprindine in healthy volunteers who received 25 mg of aprindine hydrochloride orally is illustrated in Fig. 4. Plasma concentrations reached a maximum at about 2 h after dosing, and then declined biexponentially, with a terminal half-life of 8.0 h. These studies will be reported in detail elsewhere.

REFERENCES

1 D.P. Zipes and F.P.J. Troup, Amer. J. Cardiol., 41 (1978) 1005.
2 B.S. Rutherford and R.H. Bishara, J. Pharm. Sci., 65 (1976) 1322.
3 J.F. Nash and R.H. Carmicheal, J. Pharm. Sci., 69 (1980) 1094.
4 F. Andreasen, S. Husted, P. Jakobsen and E.B. Jensen, Acta Pharmacol. Toxicol., 46 (1980) 105.
5 P.-O. Lagerström and B.-A. Persson, J. Chromatogr., 149 (1978) 331.
6 M. Lesne and R. Dolphen, J. Immunol. Methods, 17 (1977) 189.