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Note

# High-performance liquid chromatographic determination of aprindine and its active desethyl metabolite in plasma

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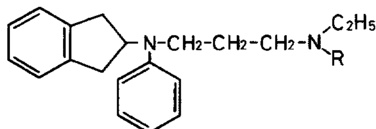
Aprindine [AP, N-phenyl-N-(3-diethylaminopropyl)-2-indanylamine] (Fig. 1), an effective anti-arrhythmic agent, has been used successfully to treat both ventricular and supraventricular arrhythmias in Europe and the United States [1,2] and is currently undergoing review for approval for use in Japan.

Measurement of AP in plasma is important because of its narrow therapeutic index. We have reported the rapid and sensitive gas chromatographic—mass spectrometric (GC—MS) determination of AP in plasma [3]. This method, however, has some disadvantages for general use because it requires an experienced technician for the operation of the expensive instrument. Furthermore, since one of the major metabolites, desethylaprindine (DEAP) (Fig. 1), is reported as active as AP in antagonizing ouabain-induced arrhythmias in dogs [4], a specific assay for both compounds is needed for the appropriate drug therapy.

The present paper reports the development of a simple, accurate and sensitive high-performance liquid chromatographic (HPLC) assay for quantitating AP and DEAP in plasma.




R : $C_2H_5$ Aprindine (AP)
R : H Desethylaprindine (DEAP)

Fig. 1. Structures of aprindine (AP) and desethylaprindine (DEAP).

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## EXPERIMENTAL

### *Standards and reagents*

AP, DEAP and other metabolites were supplied by A. Christiaens (Brussels, Belgium) and amitriptyline hydrochloride was purchased from Kodama Chemicals (Tokyo, Japan). HPLC grade acetonitrile and methanol were from Junsei Chemicals (Tokyo, Japan). All other reagents were of analytical grade and obtained from usual commercial sources. Standard solutions of AP, DEAP and amitriptyline hydrochloride were prepared in distilled water and stored at 4°C throughout the experiments. The hydrochloride salts of AP and DEAP were used and the concentrations were calculated as the salt.

### *Instrumentation*

HPLC was performed on a Shimadzu Model LC-3A liquid chromatograph equipped with a variable-wavelength UV detector (Model SPD-2A). A 30 cm × 4 mm I.D. stainless-steel column packed with 10-μm μBondapak $C_{18}$ particles (Waters Assoc., Milford, MA, U.S.A.) was used and the mobile phase was acetonitrile—0.1 *M* $KH_2PO_4$ (45:55, v/v, pH 3.0). The flow-rate was 2.0 ml/min with a pressure of about 10 MPa, and the detector was set at 259 nm because AP and DEAP gave intense absorbance at around 259 nm. The column temperature was ambient.

### *Procedure*

Extraction was carried out basically according to the previously reported procedure [3] except for the use of 500 ng of amitriptyline hydrochloride as internal standard. After evaporation of the final extract, the residue was dissolved in 100 μl of mobile phase, and a 10-μl sample was injected into the chromatograph.

### *Quantitation*

Spiked plasma extracts were chromatographed, and standard curves were constructed by plotting the ratios of AP and DEAP to internal standard peak areas versus the concentrations of the drug. Calibration standards were chromatographed each day when the unknown samples were analyzed. The concentrations of unknown samples were determined by comparison of the peak area ratios to the standard curves obtained that day.

### *Recovery and precision*

Analytical recoveries of AP and DEAP were determined by comparing the peak areas obtained from spiked plasma standards with those obtained when the internal standard was added to the plasma and known amounts of AP were added to the aqueous phase prior to the last extraction with diethyl ether. The precision of the method was estimated by analyzing spiked plasma standards which had been prepared on different days.

### *Application of the method*

Plasma samples obtained from a patient receiving AP [20 mg t.i.d. (ter in die)] were analyzed by the present method. Plasma samples from patients who

received AP orally at different doses were also analyzed by the present method and compared with the results obtained by the GC-MS method [3].

## RESULTS AND DISCUSSION

Under the described chromatographic conditions, the retention times of AP, DEAP and the internal standard were 4.40, 2.93 and 3.65 min, respectively (Fig. 2), and no overlap with authentic metabolites which were identified in human urine [5] was observed (Table I). The standard curves of AP and DEAP were linear over the plasma concentration range 50—1000 ng/ml ($r^2$ = 0.999 and 0.998 for AP and DEAP, respectively). Recoveries of AP and DEAP from spiked plasma samples in triplicate at two different concentrations (200 and 600 ng) were 95—97% and 88—89%, respectively.

The precision and accuracy of the method are shown in Table II. The within-day and between-day coefficients of variation were <5% and the sensitivity limit of the assay was 20 ng/ml for AP and 50 ng/ml for DEAP, in the case of a plasma volume of 1 ml. However, this sensitivity could be increased by using a larger volume of sample.

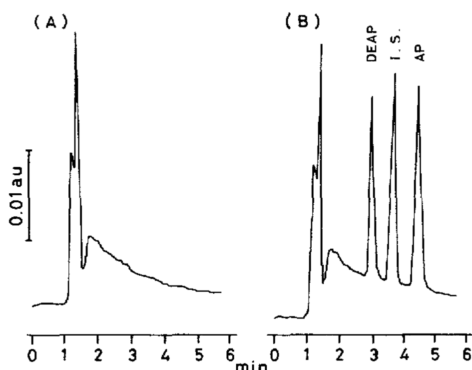


Fig. 2. Chromatograms of (A) a blank plasma extract, (B) an extract of plasma added with aprindine (AP), desethylaprindine (DEAP) (300 ng each) and internal standard (I.S., 500 ng).

TABLE I

RETENTION TIMES OF APRINDINE (AP), ITS METABOLITES AND OTHER ANTI-ARRHYTHMIC AGENTS

| Compound | Retention time (min) |
|---|---|
| Aprindine (AP) | 4.40 |
| Desethylaprindine (DEAP) | 2.93 |
| Desindanylaprindine | 1.82 |
| Desphenylaprindine | 1.31 |
| *p*-Hydroxyaprindine | 1.80 |
| Mexiletine | 1.70 |
| Quinidine | 1.82 |
| Lidocaine | 1.87 |
| Disopyramide | 2.10 |

TABLE II

PRECISION AND ACCURACY OF THE METHOD ($n$ = 9)

| Concentration (ng/ml) | Coefficient of variation (%) | | | |
|---|---|---|---|---|
| | Within-day | | Between-day | |
| | AP | DEAP | AP | DEAP |
| 50 | 3.43 | 4.74 | 3.14 | 4.34 |
| 100 | 4.00 | 4.65 | 3.77 | 4.16 |
| 200 | 3.42 | 3.63 | 2.77 | 4.17 |
| 400 | 3.51 | 3.41 | 3.21 | 3.41 |
| 600 | 2.11 | 2.58 | 2.06 | 2.50 |
| 800 | 2.11 | 3.19 | 1.88 | 3.07 |
| 1000 | 1.49 | 2.80 | 1.40 | 2.69 |

Since AP may be given concurrently with other antiarrhythmic drugs, several were examined to determine whether they would influence the assay. No interference was observed with mexiletine, quinidine, lidocaine and disopyramide (Table I).

The developed method was applied to the analysis of plasma samples from a patient taking AP chronically for the management of premature ventricular contractions (Fig. 3). After multiple oral administration of AP (20 mg t.i.d.) a steady-state plasma AP level was achieved as late as one week with a trough level of 0.4—0.5 µg/ml. During the early period, the DEAP level was not above the threshold sensitivity of the assay. A steady-state plasma level of DEAP was obtained in less than six days with a trough level of 0.08 µg/ml.

The described HPLC procedure was compared to the earlier GC—MS procedure [3]. There was a good agreement between the values obtained by the two methods (Fig. 4). A correlation coefficient ($r^2$) of 0.995 and a slope value of 0.951 were obtained for the samples from the patients, analyzed by the two methods.

The increased sensitivity of the GC—MS procedure [3], which measures

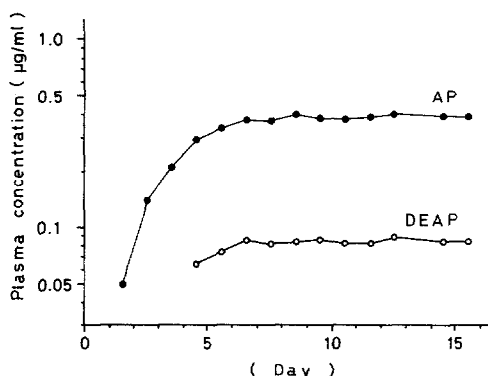


Fig. 3. Time course of plasma aprindine (AP) and desethylaprindine (DEAP) concentrations in a patient after multiple oral doses of aprindine (20 mg t.i.d.). Each value shows the plasma concentration just before the second dose of the day.

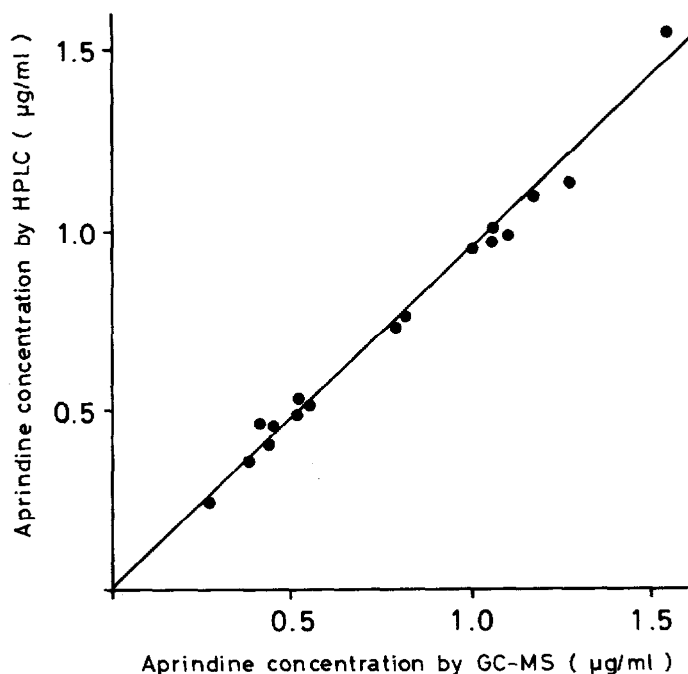


Fig. 4. Plot of plasma aprindrine concentration in patients by GC—MS method [3] versus plasma concentration of aprindine measured by the described HPLC method ($n$ = 17, $r^2$ = 0.995, and slope = 0.951).

concentrations less than 5 ng/ml of plasma, is offset by the ease of the present method, which makes use of technology readily available in most laboratories and is satisfactory for routine clinical analysis. This method could be applicable to urine analysis because no interference substances were encountered in urine samples.

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