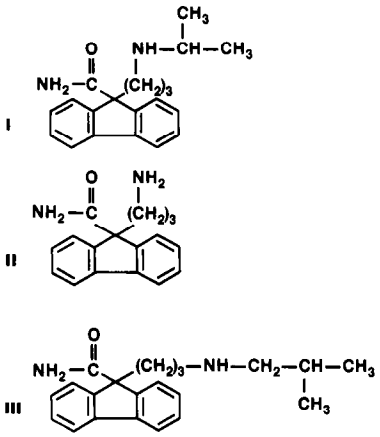


Fig. 1. Structures of indecainide (I), its metabolite (II), and the internal standard (III). I = 9-[3-(isopropylamino)]propyl-9H-fluorene-9-carboxamide; II = desisopropyl indecainide, 9-(3-aminopropyl)-9H-fluorene-9-carboxamide; III = 9-[3-(isobutylamino)]propyl-9H-fluorene-9-carboxamide.

and to study its pharmacokinetics. This report describes a rapid, sensitive, and selective liquid chromatographic (LC) method for the determination of I and its desisopropyl metabolite (II, Fig. 1), in plasma (or serum) and urine samples, using either ultraviolet (UV) or fluorescence detection, as well as the factors affecting the chromatographic separation and the selectivity of detection.

EXPERIMENTAL

Chemicals and reagents

Indecainide hydrochloride, desisopropyl indecainide hydrochloride, and the internal standard, 9-[3-(isobutylamino)]propyl-9H-fluorene-9-carboxamide hydrochloride (III, Fig. 1), were obtained from Eli Lilly and Company (Indianapolis, IN, U.S.A.). All solvents used were distilled in glass. All other reagents were of analytical reagent grade.

Liquid chromatography.

The liquid chromatograph consisted of a Hewlett-Packard Model 1081B pump system equipped with a Model 79841A autoinjector (Hewlett-Packard, Avondale, PA, U.S.A.), a Perkin-Elmer Model 650-10S fluorescence detector, and a Model LC-75 variable-wavelength detector (Perkin-Elmer, Norwalk, CT, U.S.A.). The column was stainless steel, 25 cm × 4.6 mm I.D., packed with 6-μm Zorbax C_8 particles (DuPont, Wilmington, DE, U.S.A.), protected by a guard column (Waters Assoc., Milford, MA, U.S.A.), and packed with Co:Pell ODS (Whatman, Clifton, NJ, U.S.A.). The analytical column temperature was maintained at 35°C. A laboratory computer, Hewlett-Packard Model 1000, was used for peak integration and data calculations.

The eluent was prepared by mixing 600 ml of 0.25 *M* ammonium acetate solution, adjusted to pH 6.0 with acetic acid, with 200 ml of acetonitrile, 160 ml of methanol, and 40 ml of tetrahydrofuran. The eluent flow-rate was 1.5 ml/min. For UV detection, the wavelength was 270 nm, with the sensitivity

set at 0.01 a.u.f.s. For fluorescence detection, the excitation wavelength was 270 nm (slit 10 nm), the emission wavelength was 315 nm (slit 5 nm), and the range was set at 0.3.

Standard solutions

Standards in plasma were prepared to contain 100, 200, 500, and 1000 ng/ml I, and 50, 100, 250, and 500 ng/ml II. The urine standards contained 10, 20, 50, and 100 μg/ml I, and 1, 2, 5, and 10 μg/ml II. The internal standard solution was prepared by dissolving 5 mg of III (hydrochloride) in 1 l of water.

Plasma extraction procedure

To 0.5 ml of plasma sample or standard, placed in a disposable centrifuge tube with a PTFE-lined screw cap, were added 100 μl of the internal standard solution, 1 ml of 0.5 *M* sodium carbonate, and 6 ml of ethyl acetate—hexane (9:1, v/v). The tubes were capped, shaken for 3 min, and then centrifuged (800 *g*) for 5 min. Of the organic phase 5 ml were transferred to a clean tube, 0.1 ml of 1% hydrochloric acid in methanol was added, and the solution was evaporated to dryness under nitrogen. The residue was dissolved in 200 μl of the mobile phase, and 50 μl were injected onto the LC column. The concentrations of the compounds in the samples were determined from their peak height ratios relative to the internal standard and the corresponding least-squares line of the calibration standards.

Procedure for urine samples

To 0.1 ml of urine sample or standard was added 1 ml of the internal standard solution. The sample was vortexed, and 100 μl were injected onto the LC column. If the urine sample contained less than 1 μg/ml I, the extraction procedure detailed for the plasma samples was used.

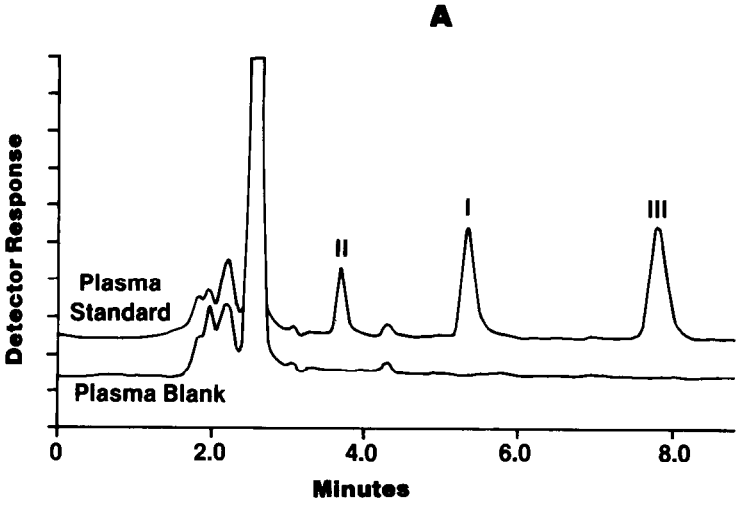
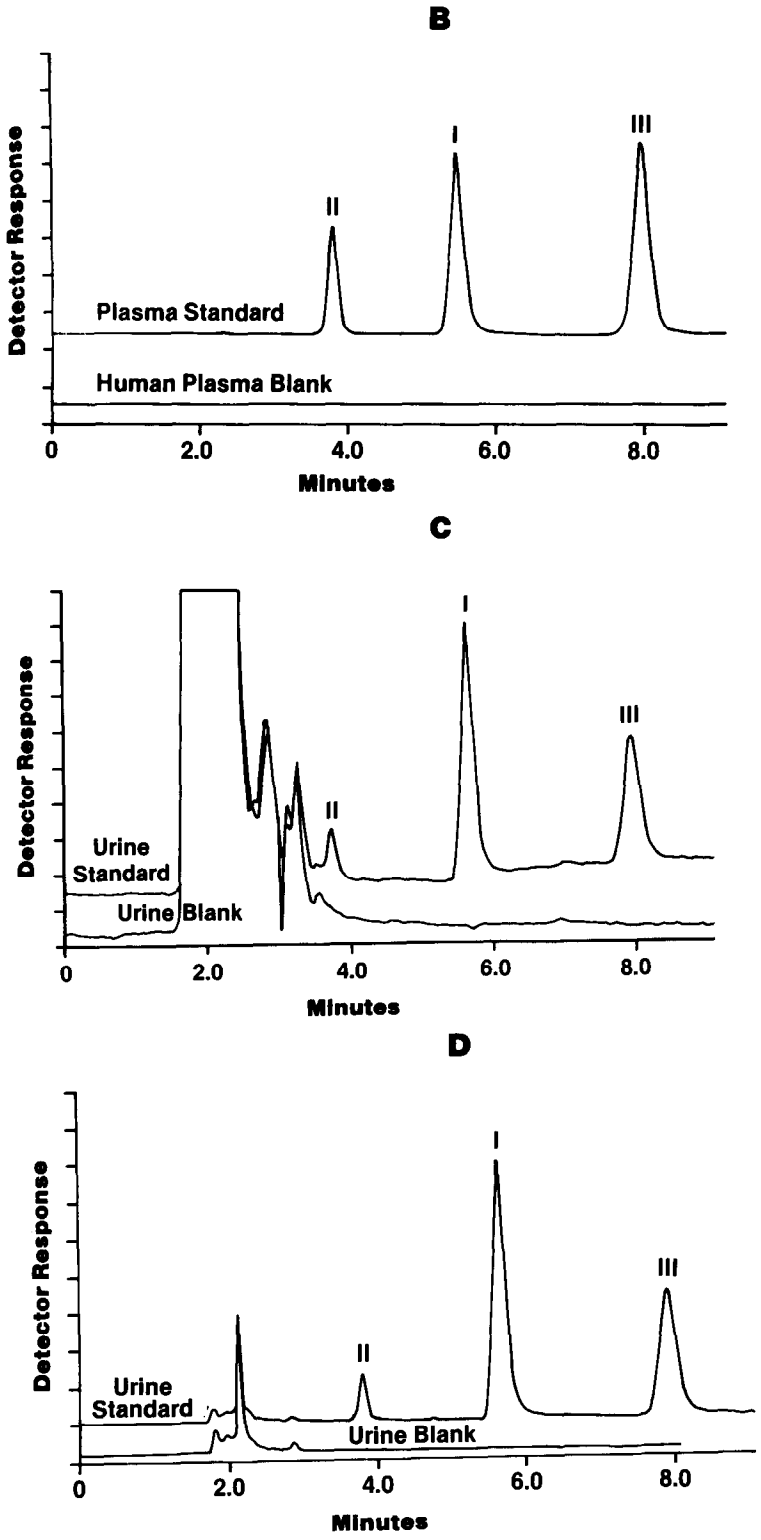


Fig. 2.

(Continued on p. 332)


B
Detector Response
II
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III
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Fig. 2.

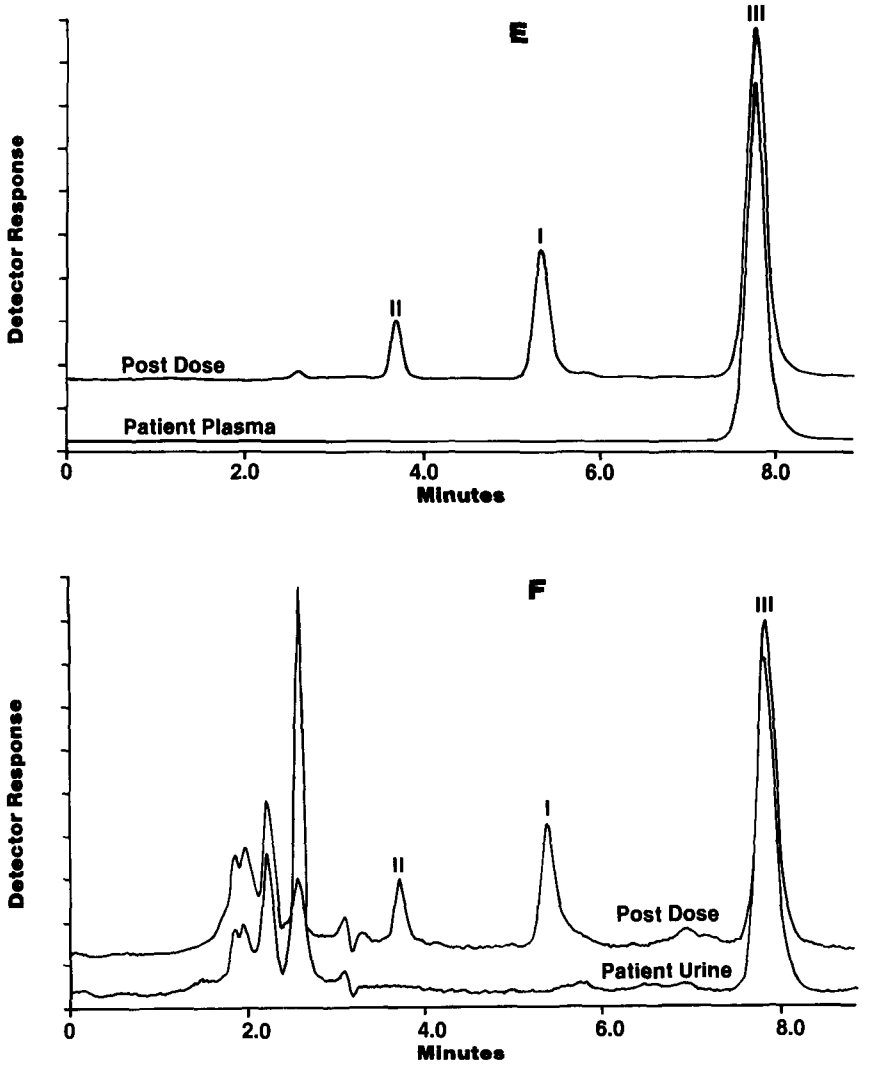

Fig. 2. Chromatograms of plasma and urine samples. (A) Human plasma blank and a standard plasma sample containing 500 ng/ml indecainide (I), 250 ng/ml desisopropyl indecainide (II) and the internal standard (III), using UV detection. (B) The same as A, using spectrofluorescence detection. (C) Human urine blank and a standard sample containing 100 μg/ml I, 10 μg/ml II and the internal standard (III), using UV detection. (D) The same as C, using spectrofluorescence detection. (E) Patients' plasma before dose and following a 50-mg dose of indecainide (spectrofluorescence detection). (F) Patients' urine before dose and following administration of indecainide, 50 mg (spectrofluorescence detection). Chromatographic conditions: column: Zorbax C_8, 25 cm × 4.6 mm I.D.; flow-rate: 1.5 ml/min; eluent: 0.25 *M* ammonium acetate, pH 6.0—acetonitrile—methanol—tetrahydrofuran (60:20:16:4).

RESULTS AND DISCUSSION

Chromatography

Typical chromatograms of plasma and urine samples obtained using the absorbance and fluorescence detectors are shown in Fig. 2A–F. Under the conditions detailed above, the retention times for I, II, and III were 5.3, 3.8, and 7.8 min, respectively. Several factors influencing the elution profile were studied. The combination of methanol, acetonitrile, and tetrahydrofuran was critical, as it has a profound effect on peak symmetry and the overall resolution of the three compounds. The effect of the pH of the aqueous portion of the mobile phase on the retention of the compounds of interest and their resolution from an unknown contaminant found in some patients' plasma is shown in Fig. 3. As the pH was lowered from 7 to 4, the retention time of all three compounds decreased, while the retention time of the unknown contaminant (which absorbed in the UV range but did not fluoresce) was affected to a lesser extent. This resulted in better separation of the

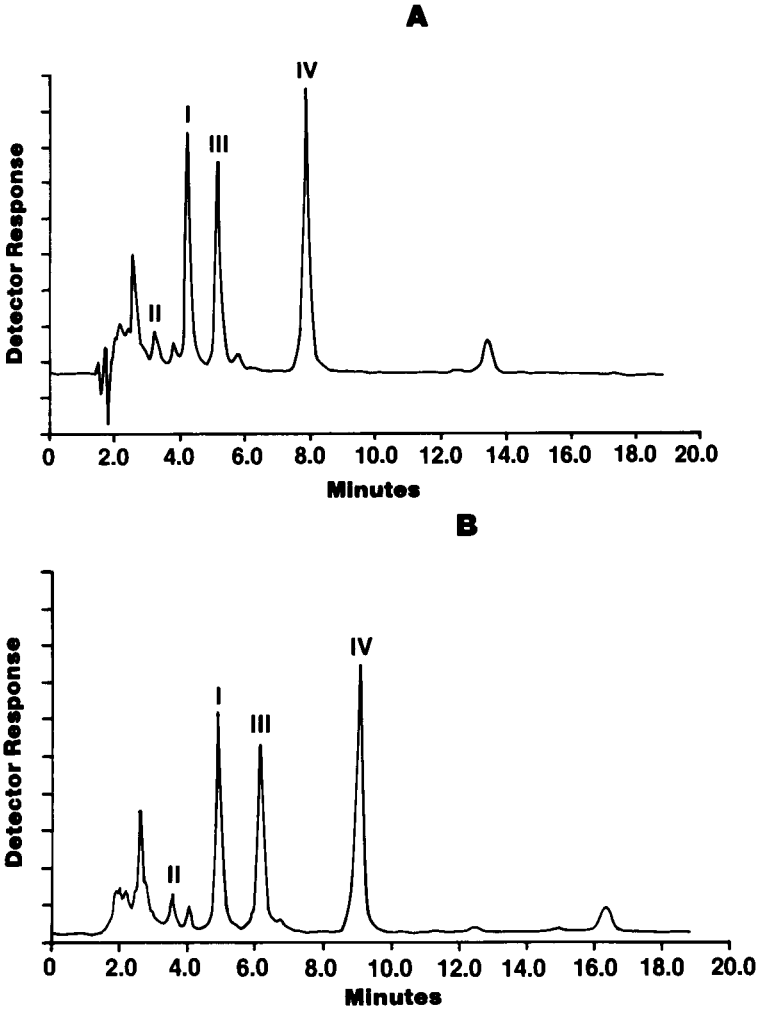

Fig. 3.

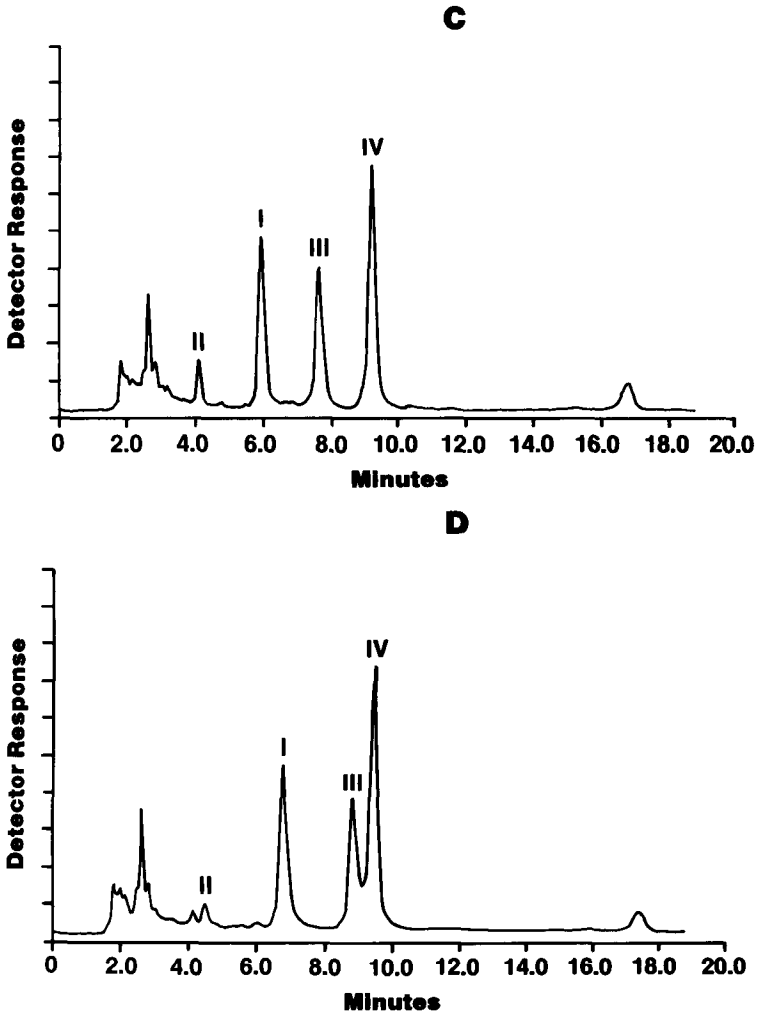

Fig. 3. Effect of mobile phase pH on the chromatographic separation. (A) pH 4; (B) pH 5; (C) pH 6; (D) pH 7. Peaks: I = indecainide; II = desisopropyl indecainide; III = internal standard; IV = unknown contaminant from patients' plasma.

contaminant and III at lower pH values. The resolution was inadequate at pH 7.0. Hence, pH 6.0 was selected since good resolution of I, II, and III as well as endogenous interferences was achieved.

Extraction recovery

Several organic solvents such as hexane, toluene, diethyl ether, butyl chloride, and ethyl acetate were tried for the extraction of I, II, and III from plasma samples. Ethyl acetate gave the best recovery for all three compounds; however, background interference from the plasma was considerably greater, particularly when using UV detection. The addition of hexane (10%) to the ethyl acetate reduced the background from plasma without greatly affecting the recovery of the compounds. The addition of a small amount of methanolic hydrochloride in the evaporation step improved the recovery of the three

TABLE I

SUMMARY OF PRECISION AND ACCURACY DATA OF ASSAY FOR INDECAINIDE AND DESISOPROPYL INDECAINIDE IN PLASMA BY UV DETECTION

		Added (ng/ml)							
		10		60		100		300	
		II	I	II	I	II	I	II	I
Day 1	$\bar{X}$	12.52	11.82	63.38	63.0	106.5	105.5	324.6	320.8
	R.S.D.	5.19	7.58	4.8	3.9	4.0	2.8	0.33	2.5
	n	5	5	5	5	4	4	3	4
Day 2	$\bar{X}$	12.37	12.9	64.2	62.7	105.6	106.4	313.0	311.0
	R.S.D.	4.72	4.1	1.4	1.2	1.5	7.5	1.5	2.2
	n	5	5	5	5	5	5	5	5
Day 3	$\bar{X}$	13.9	12.5	64.7	61.5	104.6	100.0	307.7	298.8
	R.S.D.	5.5	8.9	5.7	3.0	3.0	2.9	2.2	1.8
	n	5	5	5	5	5	5	5	5
Overall precision									
	$\bar{X}$	12.9	12.4	64.1	62.4	105.5	103.9	313.6	309.4
	R.S.D.	7.3	7.6	4.2	2.9	2.8	5.6	2.6	3.6
	n	15	15	15	15	14	14	13	14
Overall accuracy (relative error, %)		29	24	6.8	4	5.5	3.9	4.5	3.1

TABLE II

SUMMARY OF PRECISION AND ACCURACY DATA OF ASSAY FOR INDECAINIDE AND DESISOPROPYL INDECAINIDE IN PLASMA BY FLUORESCENCE DETECTION

		Added							
		10 ng/ml II	20 ng/ml I	60 ng/ml II	120 ng/ml I	200 ng/ml II	400 ng/ml I	600 ng/ml II	1200 ng/ml I
Day 1	$\bar{X}$	10.6	19.9	61.85	120.9	195.8	389.2	599.9	1144.4
	R.S.D.	6.84	4.77	1.35	1.14	2.03	0.73	2.5	0.75
	n	5	5	4	4	5	5	5	5
Day 2	$\bar{X}$	9.52	17.7	60.48	118.2	191.4	389.0	600.7	1162.6
	R.S.D.	7.55	6.65	3.54	2.29	2.7	1.31	2.69	2.27
	n	5	5	5	5	5	5	5	5
Day 3	$\bar{X}$	10.38	18.1	54.6	103.5	194.9	397.6	590.9	1150.0
	R.S.D.	1.58	3.18	4.68	3.91	2.97	2.98	6.86	5.37
	n	5	5	5	5	5	5	5	5
Overall precision									
	$\bar{X}$	10.17	18.59	58.8	113.7	194.0	391.9	597.2	1152.3
	R.S.D.	7.2	7.1	6.4	7.4	2.6	2.1	4.2	3.2
	n	15	15	14	14	15	15	15	15
Overall accuracy (relative error, %)		1.7	−7.05	−2.0	−5.25	−3.0	−2.0	−0.47	−3.97

compounds, particularly of II. This is possibly owing to the reduction of adsorption of the amines to the glass surface. The recovery of known concentrations of I and II from plasma samples containing 100—1000 ng/ml I and 50—500 ng/ml II was determined by comparing the peak heights of the extracted compounds to those obtained from aqueous standard solutions injected directly. The recoveries for the concentration ranges specified were 97.6 ± 3.8% and 83.6 ± 3.8% for I and II, respectively (n = 16). The recovery of III was 97.1 ± 2.5% (n = 16).

Precision and accuracy

The precision and accuracy of the method was determined by spiking blank plasma with I and II at four different concentrations. Five replicates of each plasma sample were assayed on three different days. The concentrations ranged from 10 to 300 ng/ml for UV detection, and from 10 to 1200 ng/ml for fluorescence detection. The results are shown in Tables I and II. The between-

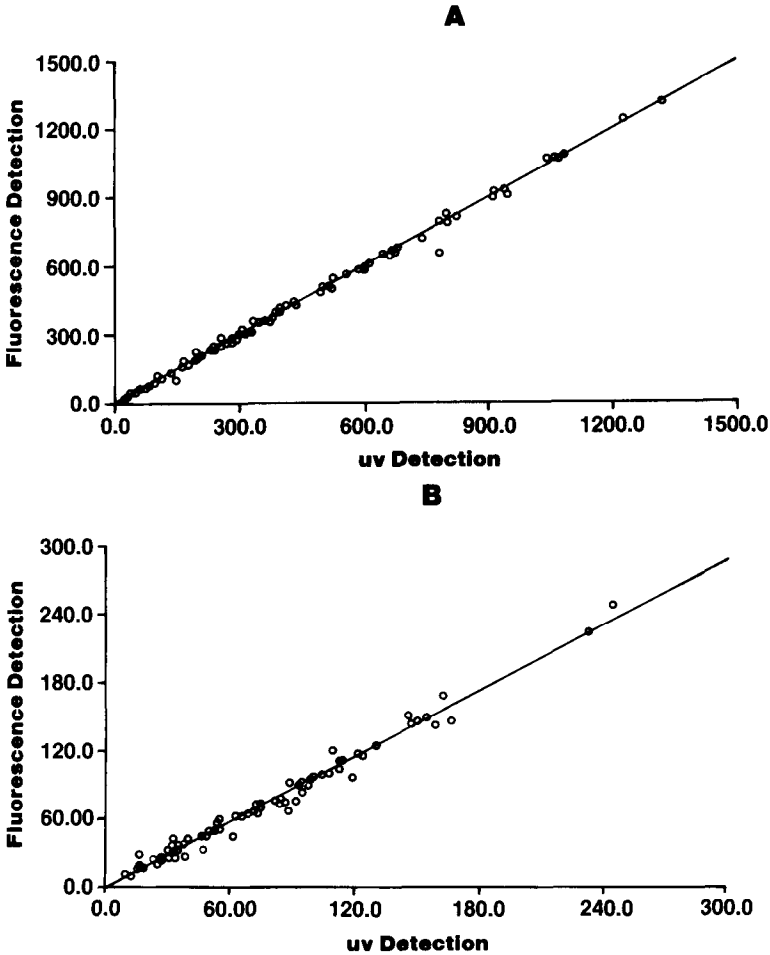

Fig. 4. Correlation between the UV detection and fluorescence detection. (A) Indecainide (ng/ml) in plasma, (B) desisopropyl indecainide (ng/ml) in plasma.

day and within-day precisions were good for both detection methods, ranging from 3.0 to 10.3% using the UV detector, and from 2.0 to 7.2% using fluorescence detection. The overall accuracy, expressed as relative error, ranged from 1.6 to 7.5% using fluorescence detection, and from 3.1 to 6.8% using UV detection, except for the plasma containing 10 ng/ml in which the latter case yielded a relative error of 29%. This indicated that UV detection was subject to marked interferences at lower concentrations.

Comparison between the UV and fluorescence detection

To compare the UV and fluorescence detection of I and II in plasma, the two detectors were connected in series to monitor the effluent from the chromatographic column. About 300 plasma samples from clinical studies were analyzed. The assay results, using the two detectors, were obtained and plotted (Fig. 4). Excellent correlation was obtained for I (r = 0.999). The correlation was equally good for II, with the exception of certain patients' samples where results based on UV detection were significantly higher, showing the susceptibility of the UV detection to interferences. Upon further investigation, the interference was found to be due to the presence of triamterene in these plasma samples. Triamterene co-eluted with II under the chromatographic conditions

TABLE III

RETENTION DATA OF DRUGS TESTED FOR INTERFERENCE IN THE ASSAY OF INDECAINIDE AND DESISOPROPYL INDECAINIDE

Compound	Amount injected (μg)	Retention times* (min)
Acetaminophen	1	2.2
Aprindine	2	ND**
Caffeine	1	2.2
Captopril	0.1	ND
Chlorpheniramine	1	10.1 (tailing)
Clonidine	0.05	ND
Diazepam	0.05	ND
Digitoxin	1	ND
Digoxin	1	ND
Disopyramide	1	ND
Furosemide	2	3.25
Hydralazine	0.2	3.0
Hydrochlorothiazide	0.5	2.75
Lidocaine	2	ND
Methyldopa	0.5	ND
Phenobarbital	5	ND
Procainamide	1	2.2
Propranolol	0.2	12.5***
Quinidine	2	12.8
Secobarbital	1	ND
Theophylline	1	2.2
Triamterene	0.2	4.0

*Detected by UV except where noted.
**ND, not detected.
***Also detected by fluorescence.

discussed, and absorbs at 270 nm, but was not detectable using the spectrofluorometer at the excitation and emission wavelengths specified. However, it was found that if a filter fluorometer was used with the emission cut-off filter of 295 nm, triamterene was also detectable. This resulted in falsely high values of II since the emission wavelength of triamterene is 470 nm. Because triamterene, a diuretic, may be co-administered with indecainide, the use of a spectrofluorometer having excitation- and emission-grating monochromators is the detector recommended when assaying for I and II.

Other drugs that may be administered concomitantly with indecainide were tested for potential interference in the assay. These compounds included analgesics, CNS stimulants, β-blockers, diuretics, cardiac glycosides, and other antiarrhythmic drugs. Solutions of these compounds were injected directly onto the chromatographic column and monitored by both the UV and fluorescence detectors. Table III lists the compounds tested and their retention times. With the exception of triamterene, discussed above, none of the drugs tested interfered in the assay using either detector.

Linearity and sensitivity

The relationship between the concentrations of I and II and their peak height ratios relative to the internal standard, using either detection system, was linear over a wide concentration range (25—1000 ng/ml), with correlation coefficients of 0.998 or better for both compounds. The detection limits varied, depending on the detection mode used. Using the UV detector (270 nm), the limit of detection was 10 ng/ml for I and II; whereas using the spectrofluorometer, the sensitivity of the assay for both compounds was 0.5 ng/ml.

Application of the method in pharmacokinetics studies

Three patients with ventricular arrhythmias were given oral doses of I, ranging from 75 to 125 mg every 8 h over nineteen days in a single blind study

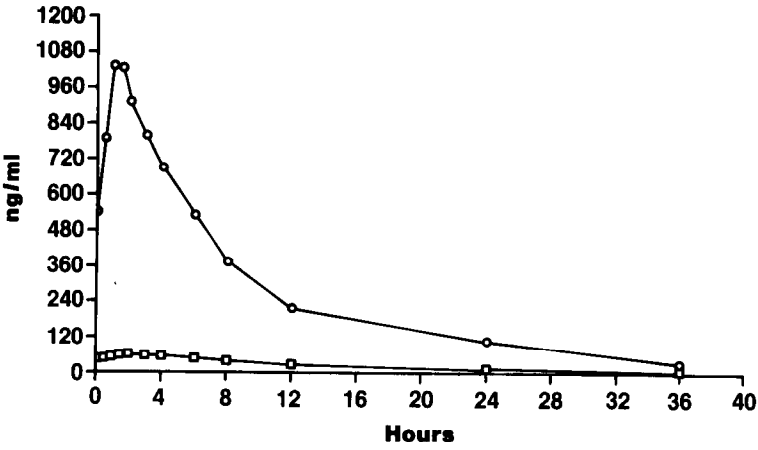

Fig. 5. Mean plasma concentration of indecainide and desisopropyl indecainide following the last 100-mg dose of indecainide in a multiple-dose study. ○, Indecainide; □, desisopropyl indecainide.

with a placebo lead-in period. Blood samples were collected periodically before the morning dose and at pre-determined time intervals up to 36 h following the last dose. Urine was collected, starting on day 19, every 6 h for up to 36 h. The concentrations of I and II in plasma and urine samples were determined by the described method. The plasma profile following the last dose is illustrated in Fig. 5. The results indicated that there was no accumulation of I or II following multiple doses of indecainide hydrochloride. The results also demonstrate the suitability of the method for pharmacokinetic studies.

ACKNOWLEDGEMENTS

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