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Note

Rapid and inexpensive high-performance liquid chromatographic method for the quantification of flecainide in serum

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Flecainide acetate [2,5-di-(2,2,2-trifluoroethoxy)-N-(2-piperidylmethyl)-benzamide acetate; Tambacor[®]] has been shown to markedly suppress ventricular and supraventricular rhythm disorders [1-7]. Effective serum levels of flecainide range from ca. 0.2 to 1.0 µg/ml [1,3,6-8]. Although the antiarrhythmic efficacy of flecainide acetate is not correlated directly with its serum level, the determination of the drug is recommended where drug therapy is ineffective in order to differentiate failure of therapy from suboptimal dosing, where adverse effects occur, or in order to check a patient's compliance.

The aim of this study was to develop a high-performance liquid chromatographic (HPLC) assay for flecainide acetate which is rapid and accurate in the therapeutic range and inexpensive, allowing easy drug monitoring in clinical practice.

EXPERIMENTAL

Chemicals and reagents

Flecainide acetate was provided by Kettelhack Riker Pharma (Borken, F.R.G.) and the internal standard *p*-chlorodisopyramide [4-diisopropylamino-2-(4-chlorophenyl)-2-(2-pyridyl)butyramide] by Albert Roussel Pharma (Wiesbaden,

F.R.G.); acetonitrile, hydrochloric acid, triethylamine, dichloromethane and sodium hydroxide were obtained from Merck (Darmstadt, F.R.G.); 1-octanesulphonic acid (PIC B-8 reagent) came from Waters Assoc. (Eschborn, F.R.G.).

Instruments

The system consisted of a Model 721 system controller, a 510 HPLC pump, a Wisp 710B injector block and a Lambda-Max Model 481 variable-wavelength detector, all from Waters Assoc. The absorbance spectrum of flecainide acetate (dissolved in 0.01 M hydrochloric acid) was established using a DU 50 spectrophotometer (Beckman, Munich, F.R.G.).

Chromatographic conditions

The analysis was performed using a Shandon ODS reversed-phase column (125 mm \times 4.6 mm I.D., particle size 5 μm) (Grom, Ammerbuch, F.R.G.). The mobile phase consisted of 55% A (994 ml water, 5 ml PIC B-8 reagent, 1 ml triethylamine) and 45% B (acetonitrile). The flow-rate was 1.0 ml/min. The column effluent was monitored at 222 nm, using a detector range of 0.02 a.u.f.s. and a chart speed of 0.5 cm/min. The injection volume was 200 μl .

Standards

Two stock standard solutions were prepared, containing 10 $\mu\text{g}/\text{ml}$ flecainide acetate and internal standard in 0.01 M hydrochloric acid, respectively. The solutions were stable for at least two months if stored at 4 °C. A standard working solution was obtained by combining aliquots of the stock solutions and diluting with hydrochloric acid to concentrations of 1.0 $\mu\text{g}/\text{ml}$ for either flecainide acetate or the internal standard.

Sample collections

Serum was obtained by centrifugation at 900 g for 10 min. Twenty-two serum samples from normal volunteers (twelve male, ten female) were pooled (normal pool). Twenty-four serum samples from patients (sixteen male, eight female) treated with cardiovascular drugs, such as acebutolol, atenolol, betaxolol, metoprolol, pindolol, sotalol, digoxin, nifedipine, verapamil, amiodarone, mexiletin, propafenone, quinidine, phenprocoumon, acenocoumarol, acetylsalicylic acid, dipyridamol, sulfinpyrazone, spironolactone, furosemide, hydrochlorothiazide and triamterene, were pooled in four pools (patient pools). Twenty-six serum samples from twenty-three patients (sixteen male, seven female) treated with flecainide (150–400 mg/day) were analysed individually.

Extraction procedure

A 2.0-ml volume of dichloromethane, 1.0 ml of serum, 100 μl of 1 M sodium hydroxide and 100 μl of the stock solution of the internal standard were introduced into a glass centrifuge tube (Kästner, Tübingen, F.R.G.). The tube was closed with a stopper (from Sarstedt, Nürmbrecht, F.R.G.), vortexed for 60 s and centrifuged at 3600 g for 10 min. Subsequently the serum phase was discarded. Then 1.0 ml of the dichloromethane phase was transferred to a clean glass tube

and evaporated to dryness at room temperature under a stream of nitrogen. The residue was redissolved in 500 μl of 0.01 *M* hydrochloric acid.

Linearity test

The linearity of the chromatographic procedure was tested for flecainide acetate and the internal standard by analysing five standard solutions with the concentrations 1.0, 0.75, 0.5, 0.25 and 0.1 $\mu\text{g}/\text{ml}$ for each of the components. The linearity test of the entire procedure was done with the same standard solutions. The amount of each standard required to reach concentrations in the range 0.1–1.0 $\mu\text{g}/\text{ml}$ was introduced into a glass centrifuge tube, and 1.0 ml of the normal pool serum was added. Subsequently the spiked serum samples were extracted and subjected to the chromatographic analysis.

Recovery rates

The recovery rates were determined by comparing the peak areas of flecainide acetate and internal standard obtained by analysing a spiked serum sample (100 μl of each stock solution standard plus 1.0 ml of the normal pool) with the peak areas obtained by direct injection of the standard working solution (1.0 $\mu\text{g}/\text{ml}$ flecainide acetate and internal standard).

Quantification

The determination of the concentration of flecainide in the serum was based on calibration graphs obtained from standard solutions (0.1, 0.25, 0.5, 0.75, 1.0 $\mu\text{g}/\text{ml}$). The lower limit of quantification was 0.05 $\mu\text{g}/\text{ml}$. Since the calibration graph was linear and the calibration was stable for three months, the calculation was performed with the 1.0 $\mu\text{g}/\text{ml}$ standard only, using the following formula:

concentration of flecainide ($\mu\text{g}/\text{ml}$)

$$= \frac{\text{peak area of flecainide}}{\text{peak area of standard}} \times \frac{100}{\text{recovery rate of internal standard}}$$

Method of Boutagy

In addition to our procedure we tried to use the one of Boutagy et al. [19] exactly as described and also using several modifications in order to determine plasma flecainide levels. We tried the following modifications: (I) the diethyl ether extract was evaporated to dryness under nitrogen and the residue was redissolved in 0.01 *M* hydrochloric acid (no use of 0.1 *M* phosphoric acid) and injected; (II) during the last extraction step only 1.5 ml of diethyl ether were removed and after evaporating the remaining diethyl ether under nitrogen an aliquot of the aqueous phase (0.1 *M* phosphoric acid) was injected; (III) after freezing of the aqueous phase (2 *M* sodium hydroxide) the diethyl ether was removed, evaporated to dryness and then redissolved in 0.01 *M* hydrochloric acid (no use of 0.1 *M* phosphoric acid) and injected; (IV) after freezing of the aqueous phase (2 *M* sodium hydroxide) the diethyl ether was added to 0.1 *M* phosphoric

acid and (after vortexing and centrifugation) again the aqueous phase was frozen and injected after discarding of the diethyl ether.

RESULTS

The linearity test showed, for the entire range tested, a linear relationship between concentration and peak area for the standards as well as for the entire procedure. The recovery rates (mean \pm S.D.) were $99.5 \pm 2.3\%$ for flecainide and $102.7 \pm 3.4\%$ for the internal standard (mean \pm S.D. of ten determinations; the

TABLE I

SERUM LEVELS OF FLECAINIDE ACETATE FROM PATIENTS TREATED CHRONICALLY WITH FLECAINIDE

Patient No.	Oral dose of flecainide acetate per day (mg)	Serum level of flecainide acetate ($\mu\text{g}/\text{ml}$)		Serum level per body surface ($\mu\text{g}/\text{ml m}^{-2}$)	
		Trough	After 3 h	Trough	After 3 h
1	150	0.49	0.57	0.27	0.31
2	200	0.48	0.72	0.21	0.32
3	200	0.16	0.48	0.08	0.23
4	200	0.23	0.35	0.13	0.19
5	200	0.09	0.17	0.05	0.09
6	200	0.56	0.62	0.25	0.27
7	200	0.57	0.61	0.29	0.31
8	200	0.78	0.92	0.45	0.54
9	200	0.59	0.89	0.32	0.49
10	200	0.20	0.40	0.10	0.20
11	200	0.54	0.75	0.33	0.45
12	200	0.13	0.34	0.07	0.19
13	200	0.35	0.50	0.16	0.24
14	200	0.25	0.38	0.12	0.19
15	200	0.39	0.54	0.24	0.34
16	200	0.34	0.49	0.18	0.27
17	200	0.27	0.31	0.15	0.17
Mean \pm S.D.		0.37 ± 0.20	0.50 ± 0.21	0.20 ± 0.11	0.28 ± 0.12
18	300	0.14	0.68	0.20	0.32
19	300	0.23	0.35	0.12	0.19
20	300	1.00	1.19	0.54	0.65
21	300	0.63	0.88	0.30	0.41
22	300	0.39	0.53	0.26	0.35
23	300	0.32	0.46	0.17	0.25
24	300	0.48	0.64	0.27	0.36
25	300	0.54	0.69	0.29	0.38
Mean \pm S.D.		0.50 ± 0.24	0.68 ± 0.26	0.27 ± 0.13	0.36 ± 0.14
26	400	0.33	0.61	0.18	0.33

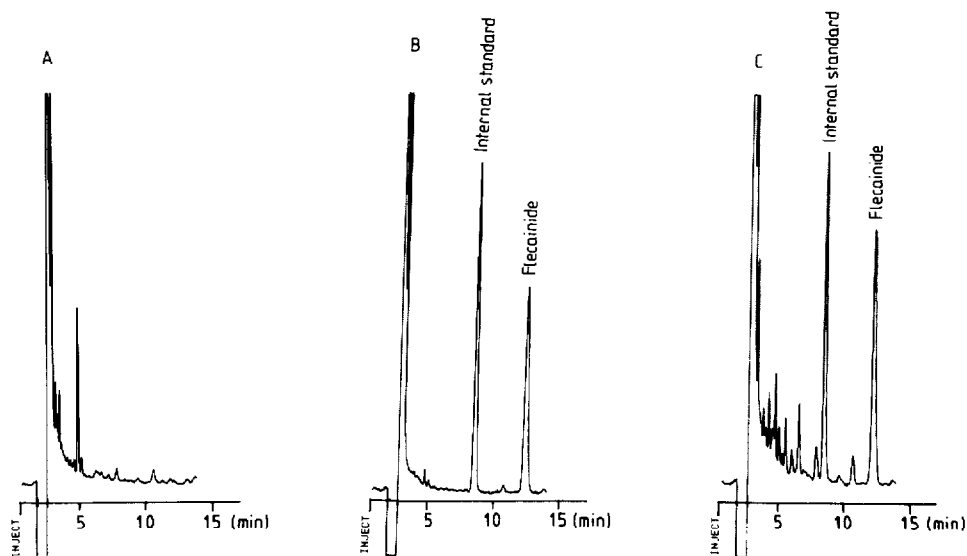


Fig. 1. Chromatograms of a blank from the normal pool (A), of a standard working solution (B) and of the serum of a patient receiving flecainide (C).

Present METHOD
(Univ. of Tübingen)

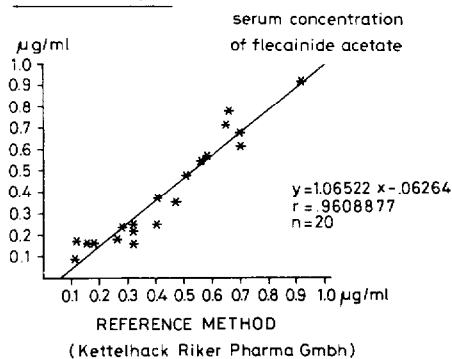


Fig. 2. Comparison of serum concentrations of flecainide acetate determined both with the present HPLC method and the method of Becker [9].

technical error of the apparatus was less than 1% for flecainide and the internal standard).

We did not observe any cardiovascular substances as interfering peaks in the patients' pools. The individual levels of flecainide (trough level and 3 h after drug intake) are shown in Table I. A typical chromatogram obtained from the analysis of a serum sample of a patient receiving flecainide is shown in Fig. 1. A comparison of the results from twenty serum samples analysed either in the laboratories of Kettelhack Riker (by a HPLC method already published [9], with replacement of UV detection by fluorescence detection) and in our laboratory (by the present HPLC method) is shown in Fig. 2. In Table II the serum levels of one serum sample determined repeatedly during one year (storage at -20°C) are shown. The UV spectrum of flecainide acetate is displayed in Fig. 3.

TABLE II

STABILITY OF SERUM FLECAINIDE DURING STORAGE AT -20°C

Duration of storage (weeks)	Serum flecainide level ($\mu\text{g/ml}$)
0	0.80
0.5	0.76
1	0.85
2	0.89
4	0.81
6	0.83
11	0.85
36	0.83
52	0.84

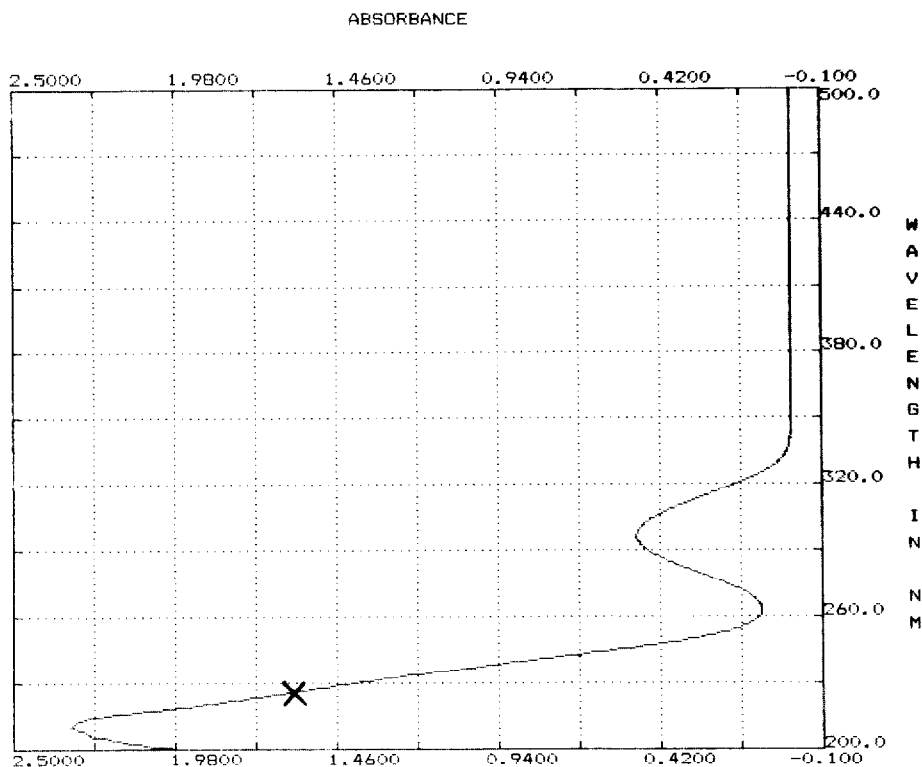


Fig. 3. UV spectrum of flecainide acetate (the cross is at 222 nm).

Using the method of Boutagy et al. we had severe problems [“clotting” of both aqueous phases (2 M sodium hydroxide and 0.1 M phosphoric acid) after nearly complete removal of the diethyl ether phase in the large majority of samples]. The recovery rates (mean \pm S.D.) for flecainide were $81.7 \pm 2.6\%$ for modification

I, $82.6 \pm 16.6\%$ for modification II, $73.1 \pm 9.2\%$ for modification III and $79.6 \pm 5.1\%$ for modification IV (mean \pm S.D. of nine determinations each).

DISCUSSION

Direct fluorometric [10,11], gas chromatographic (GC) [12] or HPLC methods with either fluorescence detection [13–17] or UV detection [9,16,18,19] have been used for the determination of serum or plasma levels of flecainide acetate. The main disadvantage of direct fluorometric methods [10,11] is a lack of specificity due to interference from other drugs. GC methods [12] are specific and highly sensitive but rather time-consuming, and require equipment not routinely available in clinical laboratories. HPLC methods with fluorescence detection [13–17] show adequate sensitivity and specificity, although many laboratories do not possess a fluorescence detector. In addition, the method of De Jong et al. [13] lacks an internal standard, has only a relatively moderate recovery of ca. 65% and requires an expensive and sophisticated spectrofluorometer and cutting a commercial column. The method of Chang et al. [16] also has a relatively moderate recovery of ca. 65–70%, whereas the recovery was not reported by Bhamra et al. [14] or Chang et al. [15]. In contrast, Plomp et al. [17] demonstrated complete recovery, using a bonded-phase extraction column procedure identical with that of Chang et al. [16]. HPLC methods with UV detection [9,16,18,19] are sensitive and specific means for determination of serum or plasma flecainide acetate levels, although the method of Chang et al. [18] has a relatively moderate recovery of ca. 67% and is rather time-consuming owing to a complicated liquid–liquid extraction procedure. Similar extraction recoveries (concentration range 3–25 ng/ml, 57–67%, concentration range 50–1600 ng/ml, 73–79%) were reported by Chang et al. in another paper [16]. Becker [9] reported a recovery of ca. 80% using a bonded-phase extraction column procedure.

UV detection is performed at 298 or 308 nm in the above-mentioned studies [9,16,18], probably in order to minimize spectral interference, though the maximum absorbance peak of flecainide acetate occurs at ca. 213 nm. Boutagy et al. [19] demonstrated detection at a wavelength as low as 214 nm with a recovery of 89–95% for flecainide and of 86% for the internal standard. However, in our hands the extraction procedure of Boutagy et al. [19] revealed severe problems and after some necessary modifications the recovery was comparatively low. Because of those facts and of some minor disadvantages (diethyl ether extraction, varying quality of different μ Bondapak C₁₈ reversed-phase columns, use of a precolumn) we consider our method to have marked advantages. Besides, no data about patients' plasma flecainide levels were given by Boutagy et al. [19]. To allow detection at a wavelength near the maximum absorbance the mobile phase has to be adapted as mentioned above. Using the chromatographic conditions described, flecainide acetate could be detected at 222 nm at a low detector range of 0.02 a.u.f.s., thus improving the signal-to-noise ratio compared with detection at a higher wavelength. A recovery rate of 99.5% for flecainide acetate was obtained due to our extraction procedure. In addition the present extraction procedure is rapid and inexpensive (e.g. no use of a bonded-phase extraction column). No

cardiovascular substance or endogenous substance was observed as an interfering peak, although many substances absorb at 222 nm. The presently reported HPLC method for the assay of flecainide acetate serum levels is rapid, sensitive, specific, inexpensive and can be performed in every laboratory possessing an HPLC system with a variable-wavelength UV detector without need for additional equipment. Thus, this method is suitable for monitoring therapeutic and toxic serum levels of flecainide acetate. Serum samples can be stored at -20°C for at least a year without deterioration of the drug. An excellent correlation with an established HPLC method is demonstrated, and mean trough serum levels of flecainide acetate are demonstrated to be $0.37 \pm 0.20 \mu\text{g/ml}$ (200 mg of flecainide orally per day) or $0.50 \pm 0.24 \mu\text{g/ml}$ (300 mg of flecainide orally per day) in patients on chronic flecainide therapy using the reported HPLC method.

In conclusion, this newly developed HPLC method is suitable for the clinical management of patients, especially because of its simplicity, rapidity and accuracy.

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