Journal of Chromatography, 416 (1987) 365–369
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3581

Note

Determination of flecainide by gas chromatography–mass spectrometry

E. HOWARD TAYLOR*

Department of Pathology, University of Arkansas for Medical Sciences, 4301 W. Markham, Little Rock, AR 72205 (U.S.A.)

ELEANOR E. KENNEDY

Department of Medicine, University of Arkansas for Medical Sciences, 4301 W. Markham, Little Rock, AR 72205 (U.S.A.)

and

ALEX A. PAPPAS

Department of Pathology, University of Arkansas for Medical Sciences, 4301 W. Markham, Little Rock, AR 72205 (U.S.A.)

(First received September 17th, 1986; revised manuscript received January 14th, 1987)

Flecainide acetate (Tambocor®, Riker Labs., St. Paul, MN, U.S.A.) (Fig. 1) is a fluorinated benzamide derivative recently approved for oral treatment of ventricular arrhythmias. Flecainide is well absorbed; about 50% of a dose is metabolized in the liver with a mean plasma elimination half-life of 13 h in healthy adults but may be prolonged in older patients or in cases of cardiovascular or renal disease [1]. Flecainide is generally well tolerated, with such symptoms as dizzyness and visual blurring occurring in less than 10% of patients [2]. Unfortunately, flecainide results in worsening of ventricular arrhythmias in 11–13% of patients [2, 3]. Arrhythmia recurrence during flecainide therapy may be due to either inadequate dosing or adequate dosing resulting in a paradoxical worsening of arrhythmia.

Methods for analysis exist which involve pentafluorobenzamide derivatization and detection by gas chromatography (GC) with electron-capture detection (ECD) [4] and column liquid chromatography with fluorescence detection [5–7] since absorbance does not give sufficient response for quantitation less than 50 μg/l [7].

0378-4347/87/$03.50

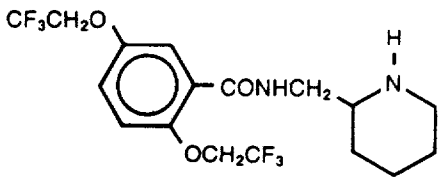

Fig. 1. Structure of flecainide.

We describe here the rapid extraction of flecainide with a solid-phase extraction and determination by gas chromatography–mass spectrometry (GC–MS) without the need for derivatization.

EXPERIMENTAL

Reagents and standards

Flecainide acetate (Tambocor) and the internal standard [N-(2-piperidylmethyl)-2,3-bis(2,2,2-trifluoroethoxy)benzamide hydrochloride] were gifts from Riker Labs. Methanol and acetonitrile were HPLC grade and water was deionized. Sodium carbonate was AR grade. Disposable C_{18} preparatory minicolumns (Analytichem International, Harbor City, CA, U.S.A.) are used and inserted into the Vac-Elut extraction system (also Analytichem International).

Dilute the flecainide acetate stock solution, 20 mg in 100 ml methanol, with water to yield aqueous standards of 1000, 10 000 and 50 000 μg/l. Prepare serum-based standards by dissolving 100 μl of aqueous standard with 900 μl of drug-free serum to yield working standards of 100, 1000, and 5000 μg/l. Dissolve the internal standard in methanol to give a stock concentration of 86 mg/l. Prepare the working internal standard solution by diluting the stock solution with water to 8.6 mg/l.

Sample extraction

The extraction method is a solid-phase extraction based on the procedure of Chang et al. [7]. Activate the appropriate number of Bond-Elut C_{18} extraction columns by washing them under vacuum with two volumes of methanol followed by two volumes of water. Combine 1 ml of patient serum or standard, 500 μl of water, 100 μl of working internal standard and 200 μl of 0.2 *M* sodium carbonate (21.2 g/l). Vortex for 5 s and apply to extraction columns. Aspirate the sample through the column. Turn off the vacuum once all the liquid is through the bed of the column packing and add the rest of the sample. Turn on the vacuum to pull the sample through the column. Wash the columns with two volumes of water followed by two volumes of acetonitrile. Release the vacuum. Elute the drug by adding 500 μl of methanol and collect the eluate. Evaporate the eluate under a stream of nitrogen at 60°C. Reconstitute the residue with 25 μl of methanol and inject 1 μl of this into the gas chromatograph–mass spectrometer.

GC–MS assay

We used instrumentation from Hewlett-Packard (Palo Alto, CA, U.S.A.), consisting of an HP 5890 gas chromatograph with a 5988A mass-selective detector

TABLE I

ABUNDANCE OF MASSES OF FLECAINIDE AND THE INTERNAL STANDARD

Mass	Abundance (% of base peak)	
	Flecainide	Internal standard*
56	3.71	4.86
83	2.51	–
84	100.00	100.00
85	6.02	6.53
96	3.34	4.46
97	12.38	12.33
98	–	2.07
107	2.54	2.83
135	2.82	–
149	–	2.71
207	–	4.23
209	1.22	–
301	4.14	–

*Internal standard differs only in the position of the trifluroethoxy group on the aromatic ring.

set for electron impact and a 5970B workstation with a 7946 55-megabyte disk drive for data collection. For GC separation we used a 12 m × 0.2 mm I.D. fused-silica capillary column coated with a 0.33-μm film of cross-linked methyl silicone (HP column No. 19091A-102). The flow-rate of helium through the column was 0.8 ml/min with a split (10:1) injection liner, with a total flow-rate of 60 ml/min measured with a bubble flowmeter at the exit port. The head pressure was approximately 60 KPa. The linear velocity of carrier gas through the column was 43 cm/min. A 1-μl sample was injected with the following GC–MS settings: injection port temperature, 250°C; initial oven temperature, 100°C with a 1-min initial hold; oven temperature program step 1, 50°C/min to a final temperature of 220°C; oven temperature program step 2, 20°C/min; final temperature, 280°C with a 5-min hold. We used an initial solvent delay of 3.5 min. The detection employed selected-ion monitoring (SIM) of masses 84, 97 and 56. The electron multiplier voltage was 1652 V. The temperatures of the transfer line, ion source and analyzer manifold were 280, 200 and 100°C, respectively. The ion source pressure was approximately $1 \cdot 10^{-6}$ Torr. Operating parameters for the mass spectrometer were adjusted with the HP "autotune" provided with the mass spectrometer with perfluorotributylamine (PFTBA) as the calibration standard.

RESULTS AND DISCUSSION

Analytical method

The abundance of masses for flecainide and the internal standard are shown in Table I. The three most abundant masses are 84 (base peak), 97 and 56. Quantitations were derived by recording mass 84. The mass 84 most likely represents the piperidine ring (see structure in Fig. 1). Fig. 2 shows SIM of masses 84 and 97 from a patient receiving flecainide acetate. The drug and the internal standard

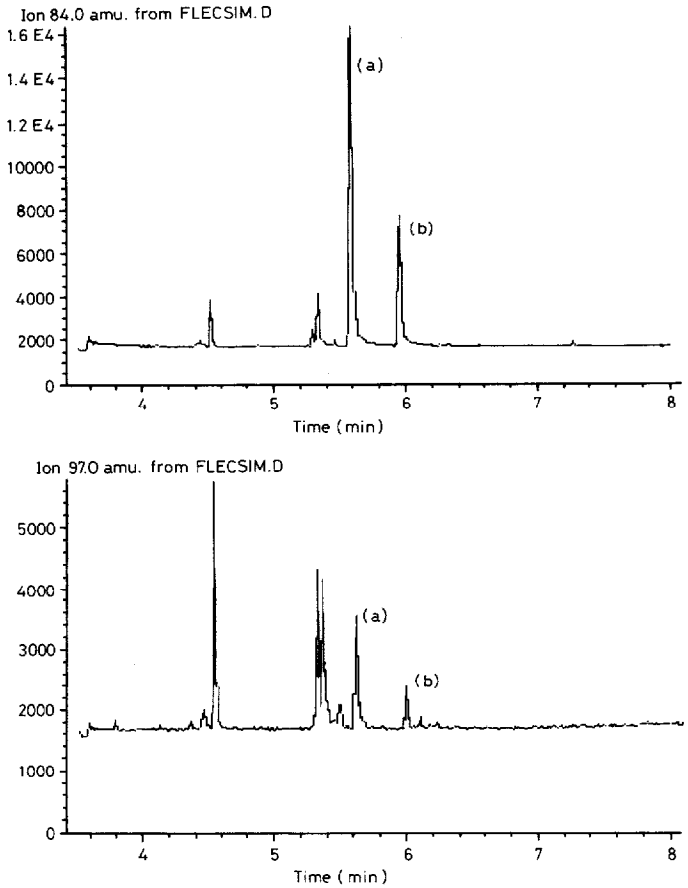

Fig. 2. Selected-ion monitoring of *m/z* 84 (top) and *m/z* 97 (bottom) from a patient shown to have 293 μg/l flecainide acetate. Peaks: a = flecainide; b = internal standard.

are well resolved. Previous analysis of ten different serum blanks showed no interference from serum components. Many commonly prescribed drugs which include a number of other antiarrhythmic drugs do not interfere with the assay. A standard plot for concentrations of 100, 1000 and 5000 μg/l against peak-area ratio was linear ($y = 0.000556x - 0.018$; $r = 0.9998$). The recovery and precision of the method are shown in Table II. The recovery for samples spiked with 250 μg/l flecainide acetate was excellent and precision also good with a within-run coefficient of variation (C.V.) of 4.6% and a day-to-day C.V. of 8.1%. Samples spiked within 25 μg/l could easily be detected with this method with a C.V. of 9.8% ($n = 4$).

Clinical interpretation

The patient whose chromatogram is depicted in Fig. 2 was having a recurrence of ventricular tachycardia at the time the specimen was obtained, and was taking oral flecainide acetate at 100 mg twice daily. His flecainide acetate serum level was 293 μg/l. A therapeutic range of 100–1000 μg/l flecainide acetate has been

TABLE II

PRECISION OF FLECAINIDE ACETATE FROM SERUM

	Added (μg/l)	n	Found (mean $\pm$ 1S.D.) (μg/l)	Recovery (%)	Coefficient of variation (%)
Within-day*	250	4	250.2 $\pm$ 11.5	100.0	4.6
Day-to-day**	250	12	244.7 $\pm$ 19.8	97.9	8.1

*The four samples were previously analyzed and found to be free of flecainide acetate.
**The twelve serum samples were analysed over three different runs.

suggested [3], not for the free base flecainide. We have continued to follow this convention for expressing our data. Proarrhythmic effects appear to occur more commonly at levels higher than 1000 μg/l, although there are exceptions [8]. Unless changes in electrocardiographic or electrophysiologic parameters suggest a strong flecainide effect, a cautious increase in dosage to 150 mg twice daily could be undertaken in this case. Otherwise, the patient would be subjected to a trial of another antiarrhythmic agent or considered for surgery or implantable antitachycardia device.

REFERENCES

1 G.J. Conard and R.E. Ober, Am. J. Cardiol., 53 (1984) 41B.
2 Flecainide Ventricular Tachycardia Study Group, Am. J. Cardiol., 57 (1986) 1299.
3 J. Morganroth and L.N. Horowitz, Am. J. Cardiol., 53 (1984) 89B.
4 J.D. Johnson, G.L. Carlson, J.M. Fox, A.M. Miller, S.F. Chang and G.J. Conard, J. Pharm. Sci., 73 (1984) 1469.
5 J.W. de Jong, J.A.J. Hegge, E. Harmsen and P.Ph. de Tombe, J. Chromatogr., 229 (1982) 498.
6 S.F. Chang, T.M. Welscher, A.M. Miller and R.E. Ober, J. Chromatogr., 272 (1983) 341.
7 S.F. Chang, A.M. Miller, A.M. Fox and T.M. Welscher, Ther. Drug Monit., 6 (1984) 105.
8 H.J. Duff, D.M. Roden, R.J. Maffucci, B.S. Vesper, G.J. Conard, S.B. Higgins, J.A. Oates, R.F. Smith and R.L. Woosley, Am. J. Cardiol., 48 (1981) 1133.