Journal of Chromatography, 336 (1984) 403—409
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2318

Note

Quantitation of cibenzoline in human plasma by gas chromatography—negative-ion chemical-ionization mass spectrometry

BO H. MIN and WILLIAM A. GARLAND*

Department of Pharmacokinetics, Biopharmaceutics and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, NJ 07110 (U.S.A.)

(First received May 21st, 1984; revised manuscript received July 16th, 1984)

Cibenzoline (I, Ciprolan®, Fig. 1), is currently under clinical investigation for use as a cardiac antiarrhythmic agent [1, 2]. Recently, gas chromatography with electron-capture detection [3] and high-performance liquid chromatography (HPLC) [4] methods for I have been reported. This paper reports a gas chromatographic—mass spectrometric (GC—MS) assay for I which uses a simple sample work-up, and which is more sensitive than existing assays for I. The method features the use of a $^{15}N_2$-stable isotope analogue of I, compound II (Fig. 1), as the internal standard, and detection in the GC effluent by selected-ion monitoring of the negative-ion chemical-ionization (NICI) generated (M − 2HF)$^-$ ions of the pentafluoropropionyl (PFP) derivatives of I and II.

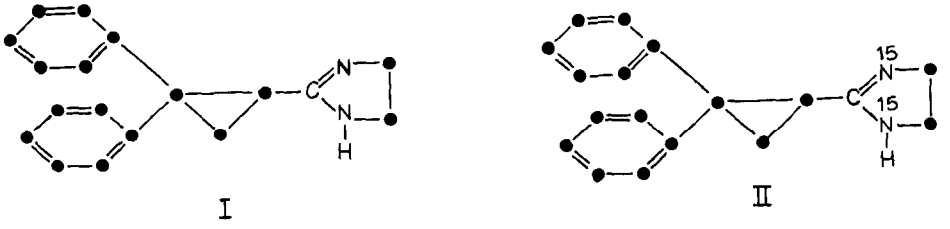

Fig. 1. Chemical structures of cibenzoline (I) and its $^{15}N_2$-stable isotope analogue (II).

EXPERIMENTAL

Chemicals

Compound I was supplied as the free base by Dr. P. Sorter (Chemical Research Department, Hoffmann-La Roche, Nutley, NJ, U.S.A.). Compound II

0378-4347/84/$03.00

was supplied as the butanedioate salt by Dr. C. Perrry (Isotope Synthesis Group, Hoffmann-La Roche). Nanograde ethyl acetate, methylene chloride, acetonitrile, methanol and benzene were obained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). Pentafluoropropionic anhydride (PFPA) was purchased from Pierce (Rockford, IL, U.S.A.). The anhydride was stored under nitrogen at −4°C to prevent decomposition. Stock solutions (μg/ml) of I and II were prepared in acetonitrile. Aliquots of the stock solutions were diluted with acetonitrile to give a series of working solutions containing 100 ng/ml II and either 0 ng/ml I (solution A), 10 ng/ml I (solution B), 50 ng/ml I (solution C), 100 ng/ml I (solution D), 250 ng/ml I (solution E) or 500 ng/ml I (solution F). All stock solutions were stored at −4°C when not in use.

Equipment

All test tubes and pipettes were washed with detergent, rinsed with distilled water, dried and siliconized by immersion for 15 min in a 1% aqueous solution of Prosil 28® (PCR Research Chemicals, Gainesville, FL, U.S.A.) prior to rinsing with distilled water and sonication with dichloromethane and methanol.

A Finnigan 3200 mass spectrometer was used with a Finnigan 9500 gas chromatograph. A glass GC column (120 cm × 2 mm I.D.) was packed with 3% SP-2250 on 80—100 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). Methane at a pressure of 1.2 kg/m^2 was used as GC carrier gas (ion source pressure of 67 Pa). The temperature of the injection block, column and GC—MS transfer line were 275°C, 265°C and 210°C, respectively. The continuous dynode electron multiplier was operated at −1.2 kV, and the conversion dynode was operated at +2.5 kV. Modifications to the mass spectrometer to permit the detection of negative ions have been described [5].

Selected-ion monitoring measurements at *m/z* 368 and *m/z* 370 were made using a Finnigan PROMIM® peak monitor. The responses were recorded on a multichannel chart recorder (Rikadenki KA-41). Both channels were operated at gain of 10^{-8} A/V, 100 msec dwell time and a filter setting of 0.5 Hz. The recorder was operated at a chart speed of 2 cm/min.

Procedure

Calibration curve samples were prepared in duplicate by fortifying 1-ml aliquots of control plasma with 100 μl of either solution A, B, C, D, E or F to give final concentrations of 10 ng/ml II and either 0, 1, 5, 10, 25 or 50 ng/ml I, respectively. All experimental plasma samples were fortified with 100 μl of solution A (10 ng of II).

Extractions were performed with C_{18} bonded-phase disposable columns (Cat. No. 607101) using a vacuum manifold from Analytichem (Harbor City, CA, U.S.A.). Column washings and eluents were drawn through the columns by a vacuum of approx. 400 Pa. The columns were washed with methanol (2 × 1 ml), distilled water (2 × 1 ml), and 0.02 *M* phosphate buffer, pH 11 (2 × 1 ml) before the plasma samples (1 ml) were applied to the columns. The columns were washed with 0.02 *M* phosphate buffer, pH 11 (2 × 1 ml) and acetonitrile (1 ml). Compounds I and II were then eluted with methanol (2 × 1 ml). The methanol was transferred to 75 × 10 mm disposable culture tubes, and was evaporated to dryness at 40°C under a stream of nitrogen.

The residues were reconstituted with 50 μl of ethyl acetate followed by 10 μl of 10% PFPA in ethyl acetate. The tubes were tightly capped and allowed to stand for 60 min at room temperature. Following evaporation of the derivatizing agent under a stream of nitrogen, the residues were reconstituted in 25—50 μl of ethyl acetate just prior to analysis of 1 to 5 μl of this solution by GC—NICI-MS.

The slope (m) and intercept (b) values from a linear least-squares regression analysis of the observed m/z 368 to m/z 370 ion ratio (R) versus amount (ng) added data from the analysis of the calibration curve samples were used to calculate the amount (x) of I in an experimental sample from the measured R using the equation $x = (R - b)/m$. The concentration of I in an experimental sample was calculated by dividing the amount found by the volume of plasma analyzed.

Determination of extraction recoveries

In order to determine the extraction efficiency, pooled human plasma was fortified with [^{14}C]cibenzoline (10 ng, $5.8 \cdot 10^5$ dpm/ml). Aliquots of 1 ml were applied directly to the C_{18} disposable columns, which were washed first with 2 × 1 ml of 0.02 *M* phosphate buffer, pH 11 and finally with 1 ml of acetonitrile. The [^{14}C]cibenzoline was eluted with 2 × 1-ml aliquots of methanol. The samples were collected and counted for radioactivity.

RESULTS AND DISCUSSION

The electron-capture NICI mass spectra of the PFP derivatives of I and II are shown in Fig. 2. The spectra consist principally of $(M - 2HF)^-$ ions. The loss of 2HF has been previously observed in the NICI mass spectra of PFP derivatives of other amines [6, 7].

Typical selected-ion current profiles from the assay are shown in Fig. 3.

Calibration curves were linear for concentrations between 1 and 50 ng/ml (correlation coefficients greater than 0.99). Mean intra- and inter-assay precisions were 2.1% and 8.1%, respectively (Table I).

The concentrations of cibenzoline in plasma samples from four subjects each given a 50-mg oral dose of cibenzoline are given in Table II. These samples were also analyzed using the published HPLC method [4]. Plasma concentration—time curves based on the mean concentration data reported in Table II for both methods are shown in Fig. 4. Data from the two methods are linearly related (correlation coefficient = 0.96, HPLC concentration = 1.105 · GC—MS concentration − 1.44 ng/ml). A two-tailed paired Student's t-test of the two sets of concentration data suggests that they are not statistically different at the $p = 0.05$ level of significance [8]. The same result is also obtained if natural logarithms of the concentrations are calculated prior to the paired Student's t-test in order to normalize the relatively wide range of concentrations compared [8].

In summary, a GC—MS assay for cibenzoline was developed which is approximately 2.5 times more sensitive than a published HPLC assay [4] for the compound. Plasma concentration data determined by the GC—MS and HPLC assays [4] are not statistically different.

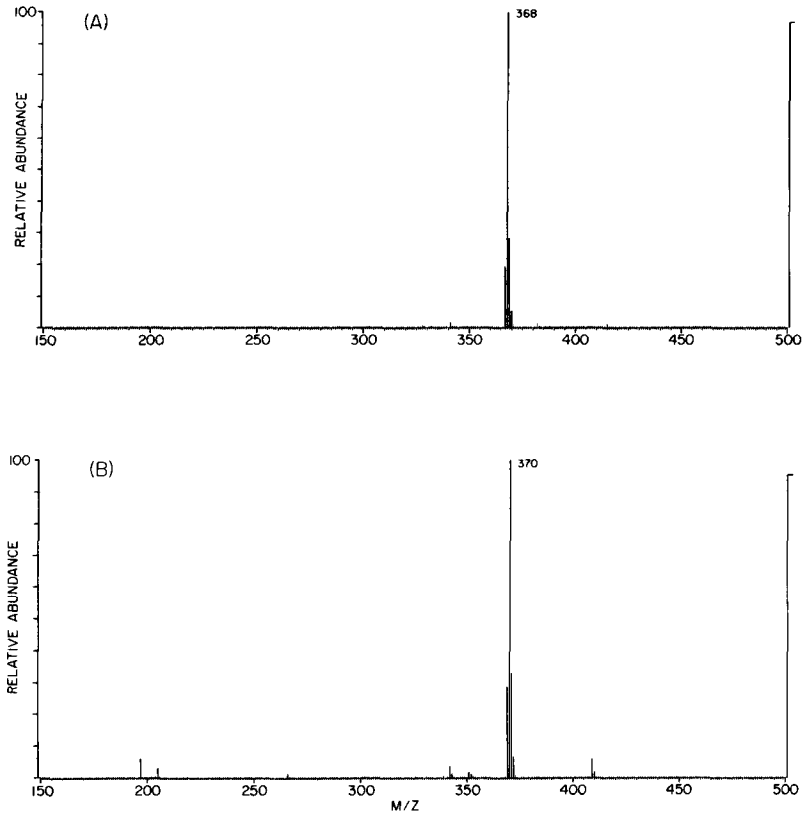

Fig. 2. NICI mass spectra of the PFP derivatives of (A) cibenzoline (MW = 408) and (B) [$^{15}N_2$]-cibenzoline (MW = 410). Methane was the CI reagent gas.

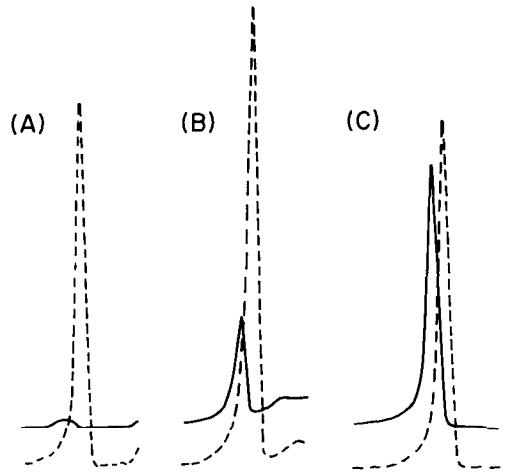

Fig. 3. Selected-ion current profiles from the analysis of 1-ml plasma samples each containing 10 ng of [$^{15}N_2$]-cibenzoline (- - -). Profiles A and B are from plasma samples also fortified with 0 and 1 ng of cibenzoline, respectively. Profile C is from plasma from a subject given cibenzoline. The concentration of cibenzoline in this sample is 5.0 ng/ml.

TABLE I

STATISTICAL VALIDATION OF THE GC—NICI-MS ASSAY FOR CIBENZOLINE IN PLASMA

Amount added (ng/ml)	Intra-assay*		Inter-assay**	
	Amount found ± S.D. (ng/ml)	R.S.D.*** (%)	Amount found ± S.D. (ng/ml)	R.S.D. (%)
1.00	1.06 ± 0.04	3.4	0.81 ± 0.15	18.6
5.00	4.59 ± 0.08	1.7	5.25 ± 0.48	9.1
10.00	10.17 ± 0.01	0.1	10.64 ± 0.72	6.7
25.00	26.20 ± 1.06	4.0	26.60 ± 0.94	3.5
50.00	49.12 ± 0.72	1.5	48.05 ± 1.21	2.5
	Average = 2.1%		Average = 8.1%	

*Intra-assay precision from the analysis of the data from three separate calibration curves run on the same day.

**Inter-assay precision from the analysis of the data from four separate calibration curves run over a 12-day period.

***Relative standard deviation.

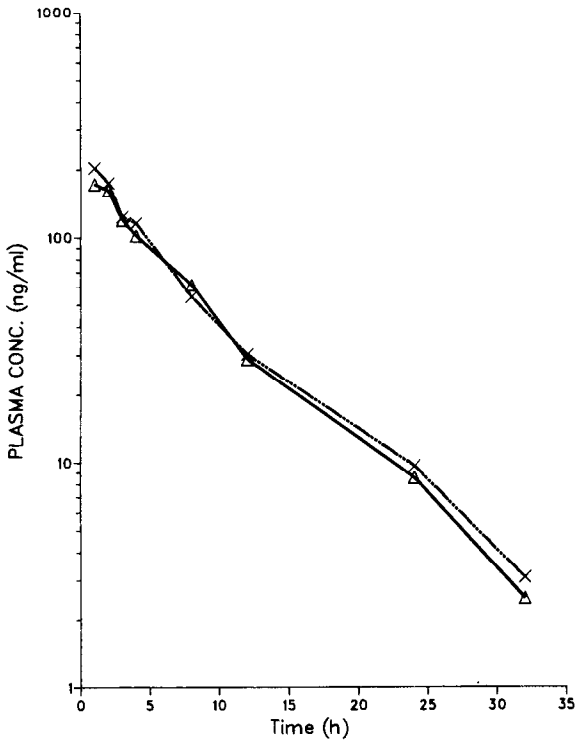

Fig. 4. Plasma concentration—time curves based on the mean concentration data reported in Table II for both the GC—MS (△) and HPLC (×) methods.

TABLE II

CONCENTRATIONS (ng/ml) OF CIBENZOLINE IN PLASMA FOLLOWING A SINGLE 50-mg ORAL ADMINISTRATION OF CIBENZOLINE TO FOUR MALE VOLUNTEERS

Time (h)	Concentration (ng/ml)									
	Subject 1		Subject 2		Subject 3		Subject 4		Mean ± S.D.	
	GC—MS*	HPLC**	GC—MS	HPLC	GC—MS	HPLC	GC—MS	HPLC	GC—MS	HPLC
0.00	NM***	NM	NM	NM	NM	NM	NM	NM	NM	NM
1.00	183.00	200.00	195.00	299.00	104.00	109.00	209.00	206.00	172.8 ± 47.0	203.5 ± 77.6
2.00	152.00	170.00	198.00	230.00	101.00	111.00	198.00	191.00	162.3 ± 96.2	175.5 ± 49.7
3.00	95.60	124.00	154.00	140.00	86.40	84.70	144.00	147.00	120.0 ± 33.9	123.9 ± 27.9
4.00	76.50	118.00	147.00	148.00	62.10	72.80	122.00	125.00	101.9 ± 39.4	116.0 ± 31.5
8.00	54.80	51.70	99.90	74.90	32.20	36.70	59.10	53.90	61.5 ± 28.1	54.3 ± 15.7
12.00	29.70	32.60	34.70	45.30	22.30	14.60	28.50	28.70	28.8 ± 5.1	30.3 ± 12.6
24.00	8.12	9.75	14.50	16.20	3.20	4.66	8.47	8.25	8.6 ± 4.6	9.7 ± 4.8
32.00	4.23	3.60	3.18	5.84	NM	NM	2.66	2.84	2.5 ± 1.8	3.1 ± 2.4
48.00	1.68	NM	NM	NM	NM	NM	NM	NM	NM	NM
72.00	NM	NM	NM	NM	NM	NM	NM	NM	NM	NM

*Concentrations obtained using the assay reported in this paper.
**Concentrations obtained using the assay reported in ref. 4.
***NM = non-measurable.

REFERENCES

1 D. Herpin, P. Gaudeau, A. Boutand, A. Amiel, B. Tourdias and T. Demange, J. Curr. Ther. Res., 30 (1981) 742.
2 R. Brazzell, K. Aogaichi, J. Heger, J. Somberg, N. Carliner and J. Morganroth, Clin. Pharmacol. Ther., 35 (1984) 307.
3 M. Canal, B. Flouvat, D. Tremblay and A. Dufour, Eur. J. Clin. Pharmacol., 24 (1983) 509.
4 M.R. Hackman, T.L. Lee and M.A. Brooks, J. Chromatogr., 273 (1983) 347.
5 W.A. Garland and B.H. Min, J. Chromatogr., 172 (1979) 279.
6 P.H. Wood, Biomed. Mass Spectrom., 9 (1982) 302.
7 A.J. Levy and S.P. Markey, Science, 201 (1978) 743.
8 ALSTAT, A Statistical Computing Program for Conversational Data Analysis, Computer Section, Alcoa Laboratories, Alcoa Co., Alcoa Center, PA 15069.