Journal of Chromatography, 343 (1985) 77—84
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2656

GAS—LIQUID CHROMATOGRAPHIC EVALUATION OF BENCYCLANE IN BIOLOGICAL SAMPLES FOR PHARMACOKINETIC AND BIOAVAILABILITY INVESTIGATIONS: COMPARISON OF TWO ANALYTICAL METHODS*

A. MARZO and E. TREFFNER

B.T.B. Industria Chimica S.p.A., Life Science Division, Laboratory of Drug Metabolism and Pharmacokinetics, Via Paullo 9, 20067 Tribiano, Milan (Italy)

G. QUADRO*

Medea Research S.r.l., via C. Pisacane 34/A, 20129 Milan (Italy)

and

S. RAPELLI

Istituto di Fisiologia Generale e Chimica Biologica della Università di Milano, Facoltà di Farmacia, Via Saldini 50, Milan (Italy)

(First received January 14th, 1985; revised manuscript received March 22nd, 1985)

SUMMARY

Analytical conditions that allow bencyclane, a vasodilator, to be evaluated in biological samples for pharmacokinetic and bioavailability investigations are reported. Two gas chromatographic methods were developed, one employing a flame-ionization detector, reaching a sensitivity of 0.5—1 μg/ml, and the other employing a thermionic specific detector and reaching a sensitivity of 10 ng/ml. The extraction recovery, reproducibility and specificity were all satisfactory with both methods. The former method is suitable for chemical quality controls and the latter has a sufficient sensitivity and reproducibility for determination of the drug in biological samples as required in pharmacokinetic investigations.

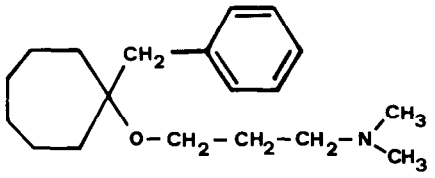
*This study was carried out within the framework of a National Research Council (CNR) programme (Progetto Finalizzato Chimica Fine e Secondaria), 1984. Some of the results reported were presented at the 2nd International Conference on Chromatography and Mass Spectrometry in Biochemical Sciences, Milan, 18—20 June, 1984.

0378-4347/85/$03.30

INTRODUCTION

Bencyclane, N,N-dimethyl-3-{[1-(phenylmethyl)cycloheptyl]oxy}-1-propanamine, $C_{19}H_{31}NO$, C.A.S. 2179-37-5, is a cycloalkane ether synthesized by Palos et al. [1] and has the molecular structure shown in Fig. 1. Its identity has been confirmed by mass spectrometry (Fig. 2).

Bencyclane is employed therapeutically as a spasmolytic agent, a muscle relaxant and a vasodilator in the treatment of peripheral and cerebral vascular disorders [2], its activity being attributed to the suppression of intracellular Ca^{2+} inflow with a consequent electromechanical decoupling at the smooth muscle. Other workers have already studied the pharmacokinetic and metabolic behaviour of bencyclane in animals and humans employing thin-layer chromatography with fluorimetric and densitometric detection, radioisotopic techniques [3–10] and, more recently, capillary gas chromatography combined with chemical-ionization mass spectrometry [11]. This paper reports two methods, one suitable for chemical quality control and the other for pharmacokinetic studies.



BENCYCLANE $C_{19}H_{31}NO$

M.W. = 289.45

Fig. 1. Molecular structure of bencyclane.

EXPERIMENTAL

Drugs, chemicals and instruments

Solvents and chemicals, all of analytical-reagent grade, were supplied by E. Merck (Darmstadt, F.R.G.). The internal standards (4-methoxy-4′-methylbenzophenone and mefenamic acid methyl ester) and bencyclane were synthesized in the Chemistry Department, B.T.B. Industria Chimica (Milan, Italy).

Supports and stationary phases for gas–liquid chromatography (GLC) were supplied by Supelchem (Milan, Italy). A Varian 3700 gas chromatograph and a VG Micromass MS 30/70 mass spectrometer were employed for analysis. The statistical evaluation was performed on a Hewlett-Packard HP 86 personal computer.

Evaluation by GLC with flame-ionization detection (GLC–FID)

A glass column (2 m × 6 mm O.D. × 2 mm I.D.) filled with 10% OV-11 on 100–120 mesh Supelcoport was used. The temperatures of the injection port,

oven and flame-ionization detector were maintained at 300, 295 and 300°C, respectively. Nitrogen was used as the carrier gas at a flow-rate of 90 ml/min. 4-Methoxy-4'-methylbenzophenone was used as the internal standard.

Evaluation by GLC with a thermionic specific detector

Extraction from biological fluids. A 0.5-ml volume of plasma (or blood) was placed in a glass-stoppered test-tube with 0.5 ml of sodium hydroxide solution (2 mol/l) and 4 ml of diethyl ether. The test-tube was vigorously stirred for 10 min and then centrifuged at 2400 *g* for 10 min. An aliquot (3.5 ml) of the ethereal phase was evaporated under a stream of nitrogen at 40°C and the residue was dissolved in about 50 µl of acetone. Mefenamic acid methyl ester was added as the internal standard. The solution was then ready for GLC analysis.

Analytical conditions. A Varian 3700 gas chromatograph was fitted with a glass column (2 m × 6 mm O.D. × 2 mm I.D.) packed with 10% OV-11 on 100—120 mesh Supelcoport. The temperatures of the injection port, oven and thermionic specific detector were maintained at 300, 290 and 350°C, respectively. Helium was used as the carrier gas at a flow-rate of 40 ml/min, as the thermionic specific detector showed a sensitivity almost twice as high as that observed with nitrogen. The thermionic specific detector was set at a hydrogen flow-rate of 4.5 ± 0.2 ml/min and an air flow-rate of 175 ± 5 ml/min. The chemical identity of bencyclane was confirmed by gas chromatography—mass spectrometry (Fig. 2).

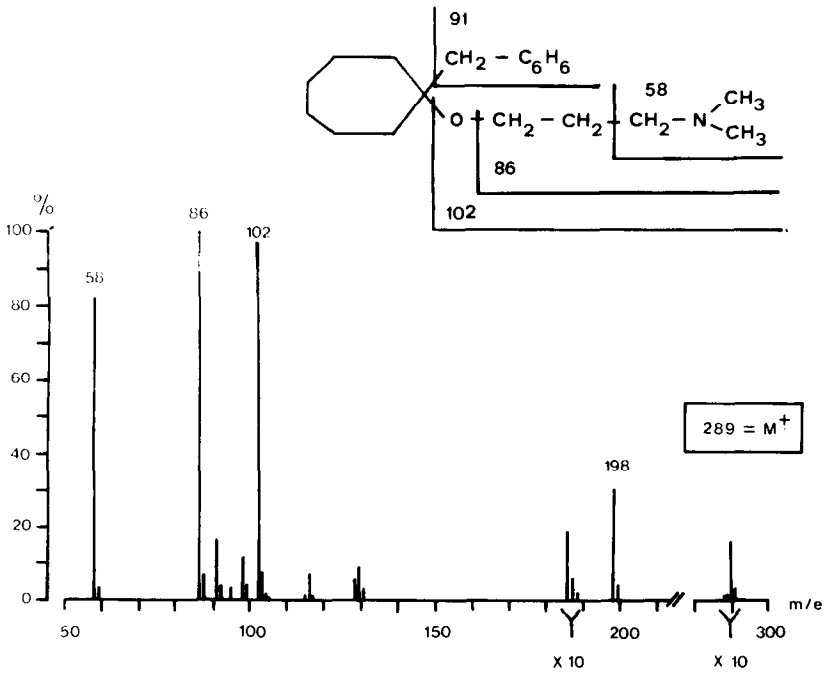


Fig. 2. Mass spectrum of bencyclane obtained in the electron-impact ionization mode (70 eV), the product being introduced through the gas chromatograph (gas chromatography—mass spectrometry).

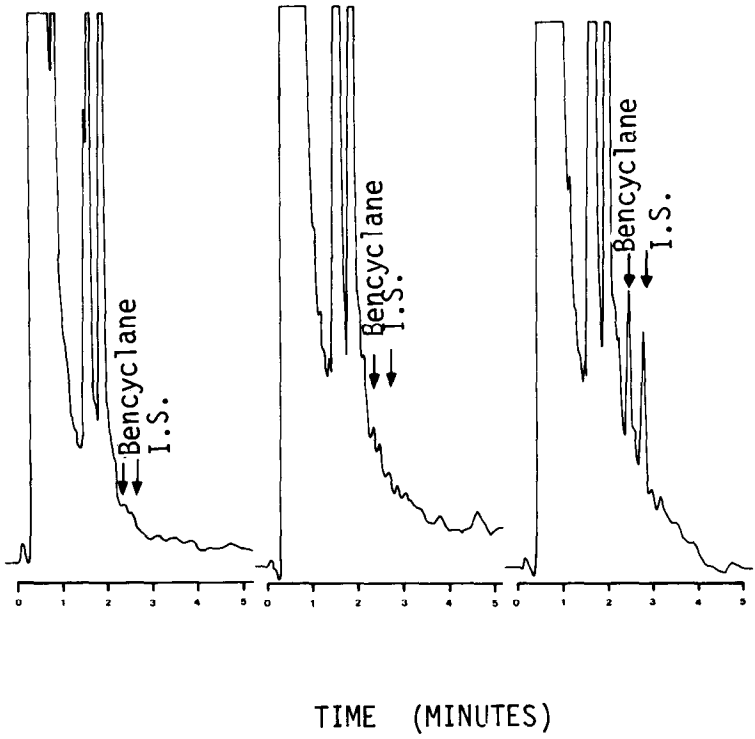


Fig. 3. Gas chromatograms of bencyclane and the internal standard (I.S.) after extraction from whole blood. Left, blank blood sample; middle, recovery test after adding 5 ng of bencyclane and 4 ng of I.S. to 0.5 ml of blood; right, recovery test after adding 50 ng of bencyclane and 40 ng of I.S. to 0.5 ml of blood.

Preliminary pharmacokinetic study in the rat

Twenty-eight Sprague—Dawley male rats, weighing 315 g on average, supplied by Charles River (Calco, Italy), were employed. A solution of bencyclane fumarate in water was prepared and administered orally to rats, starved overnight, at a dose of 50 mg/kg, expressed as base. The rats were divided into seven groups of four animals each and at each experimental time (0.5, 1, 2, 4, 6, 8 and 24 h) a group of four animals was killed by decapitation and heparinized blood samples were collected. Plasma was kept at −20° C until assayed according to the extractive and analytical methods described above. Figs. 3 and 4 show the original gas chromatograms obtained from blank samples of plasma and blood and four recovery tests obtained by adding 5 and 50 ng of bencyclane and 4 and 40 ng of internal standard to 0.5 ml of blood and plasma, respectively.

RESULTS

GLC—FID method

The retention times were 116 sec for bencyclane and 243 sec for the internal standard. The absolute sensitivity was 20 ng. The detector response proved to be linear over all the range explored: 20—20 000 ng.

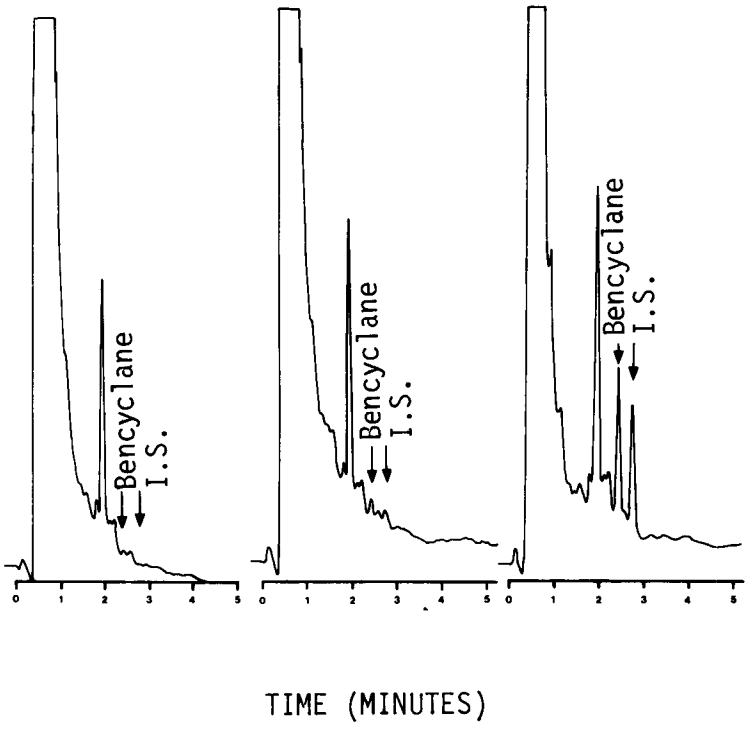


Fig. 4. Gas chromagograms of bencyclane and the internal standard after extraction from plasma. Left, blank plasma sample; middle, recovery test after having added 5 ng of bencyclane and 4 ng of I.S. to 0.5 ml of plasma; right, recovery test after adding 50 ng of bencyclane and 40 ng of I.S. to 0.5 ml of plasma.

GLC—thermionic specific detection method

The retention times were 114 sec for bencyclane and 159 sec for the internal standard (mefenamic acid methyl ester). Linearity was investigated over the range from 50 pg to 20 ng for each injection (Table I) by means of the detector response factor (d.r.f.), evaluated as follows:

$$\text{d.r.f.} = \frac{\text{weight of bencyclane}}{\text{weight of I.S.}} \cdot \frac{\text{I.S. peak area}}{\text{bencyclane peak area}}$$

This factor allows the different responses of the detector to the analytical substance and the internal standard to be counterbalanced. Table II shows the d.r.f. with a bencyclane to internal standard ratio ranging from 1:4 to 4:1. Linearity was verified at fixed and variable bencyclane-to-internal standard ratios. The intra-assay coefficient of variation was 8.2 when 100 pg were injected, and ranged from 1.4 to 5.8 when larger amounts were injected. The day-to-day variation in the determination of 2 ng of bencyclane proved to be virtually nil. The recovery of bencyclane from plasma and blood was investigated in the range from 10 ng/ml to 1 μg/ml, the analysis being performed in quadruplicate at each concentration. The mean recovery from blood over the whole range examined was 105%, and in the range between 20 ng/ml and 1 μg/ml 97.7%; the amount of bencyclane added (x) correlated well with that found (y)

TABLE I

LINEARITY OF RESPONSE WITH A FIXED BENCYCLANE-TO-INTERNAL STANDARD RATIO EVALUATED BY GLC—THERMIONIC SPECIFIC DETECTION

Linearity was confirmed by a constant detector response factor (d.r.f.). Bencyclane and the internal standard (mefenamic acid methyl ester) were injected at a 1:1 ratio.

Bencyclane injected (pg)	d.r.f. (mean ± S.D.) (n = 4)	R.S.D. (%)
50	N.D.*	
100	0.91 ± 0.075	8.2
200	0.98 ± 0.056	5.7
500	0.97 ± 0.056	5.8
1000	1.00 ± 0.014	1.4
2000**	1.03 ± 0.034	3.3
5000	0.99 ± 0.025	2.5
10000	0.99 ± 0.042	4.2
20000	0.98 ± 0.029	2.9

*N.D. = Not detectable.
**On repeating the assay with 2 ng on the following day, a response factor of 1.03 was obtained with an S.D. of 0.036 (3.5%).

TABLE II

DETECTOR RESPONSE FACTOR AT DIFFERENT BENCYCLANE TO INTERNAL STANDARD RATIOS (GLC—THERMIONIC SPECIFIC DETECTION METHOD) WITH AMOUNTS RANGING BETWEEN 1 ng AND 4 ng

Bencyclane-to-internal standard ratio	Detector response factor
1:4	0.969
2:4	1.005
4:4	1.008
4:2	1.016
4:1	0.993
Mean ± S.D.	0.998 ± 0.018

according to the correlation equation $y = -3.199 + 1.005x$, where the correlation factor $r^2 = 0.9990$.

The average recovery from plasma in the range 10—1000 ng/ml was 99.3%, with the relationship $y = 0.377 + 0.99x$ ($r^2 = 1.000$). The reproducibility, in terms of relative standard deviation at a bencyclane concentration of 10 ng/ml, was 13.3%, and at higher concentrations it ranged between 1.3 and 5.3%.

Figs. 3 and 4 show typical recordings from blank samples and from spiked samples containing 10 and 100 ng/ml bencyclane in blood and plasma, respectively.

At a concentration of 10 ng/ml bencyclane, the signal-to-noise ratio was 7:1 after extraction from plasma, and was 3:1 after extraction from blood.

The sensitivity for the evaluation of bencyclane can be considered to be around 10 ng/ml for plasma and 20 ng/ml for blood.

The mean plasma levels after oral administration of the drug to rats at a dose

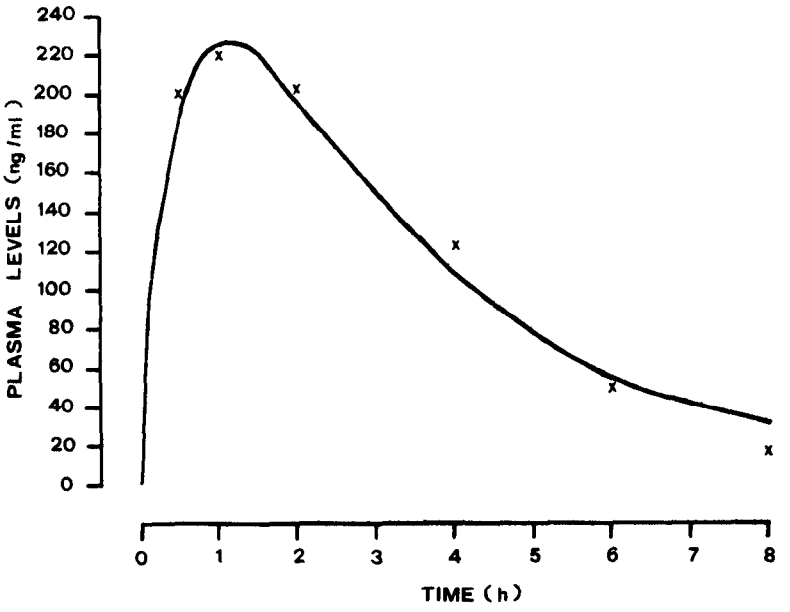


Fig. 5. Mean bencyclane plasma levels, each point obtained from four determinations of the drug after administration of 50 mg/kg per os to rats and the curve corresponding to the function calculated by means of the non-linear fitting method.

of 50 mg/kg are shown in Fig. 5. Maximal concentration was attained 1 h after administration, then the plasma levels decreased, being detectable in all four rats after 8 h but not after 24 h. A graph of the theoretical curve of plasma levels was drawn according to the one-compartment open model for the oral administration route, using the non-linear fitting method.

DISCUSSION

The difference between the plasma levels in humans obtained by Bock [9], who administered a single dose of bencyclane of 200 mg per os, and those obtained by Gielsdorf et al. [11], who found plasma levels ten to twenty times lower after administration of 100 mg per os, is remarkable. In the opinion of the latter workers this difference may be due to a higher selectivity of their method in comparison with the earlier ones, which may not have discriminated between bencyclane itself and its metabolites. Some workers have also suggested an enterohepatic reabsorption or a multi-compartmental behaviour of bencyclane, as a second peak of plasma levels after 7—8 h appeared in some subjects at higher doses of the drug [9, 11], and even a dose-dependent pharmacokinetic behaviour of the drug could be suggested.

The GLC—thermionic specific detection method reported here proved to be relatively inexpensive, highly specific and sensitive, as shown by the favourable analytical parameters reported, hence allowing pharmacokinetic investigations to be performed. The sensitivity was 10 ng/ml and the reproducibility was also very satisfactory for standards and after extraction from plasma, the relative standard deviation being lower than 6% in most cases. The high signal-to-

noise ratio (7:1) even at the lowest plasma concentrations confirmed the good specificity of the method. Hence the simple method described here may be very useful in clarifying the pharmacokinetic behaviour of bencyclane in man.

ACKNOWLEDGEMENTS

The authors express their gratitude to Mr. G. Meroni and Mr. M. Ripamonti of the B.T.B. Laboratory of Drug Metabolism and Pharmacokinetics for their invaluable technical assistance.

REFERENCES

1 L. Palos, Z. Budai and G. Zolyomi, Therapiewoche, 25 (1974) 2825.
2 A. Spagnoli and G. Tognoni, Drugs, 26 (1974) 44.
3 L. Gyarmati and L. Malè, Arzneim.-Forsch., 20 (1970) 1387.
4 K. Blasko, Arzneim.-Forsch., 20 (1970) 1374.
5 J. Nagy and K. Blasko, Arzneim.-Forsch., 20 (1970) 1376.
6 E. Faragò and J. Simon, Arzneim.-Forsch., 20 (1970) 1378.
7 E. Faragò and J. Simon, Arzneim.-Forsch., 20 (1970) 1380.
8 E. Faragò and J. Simon, Arzneim.-Forsch., 20 (1970) 1385.
9 P.R. Bock, Int. J. Clin. Pharmacol., 13 (1976) 246.
10 K. Kimura, A. Nagata and H. Miyawaki, Xenobiotica, 9 (1979) 119.
11 W. Gielsdorf, J.A. Settlage and H. Jaeger, Arzneim.-Forsch., 34 (1979) 290.