CLINICAL TOXICOLOGY 11(2), pp. 265-275 (1977)

The Identification of Ketamine and Its Metabolites in Biologic Fluids by Gas-Chromatography-Mass Spectrometry

MAN M. KOCHHAR, Ph.D.

Drug Abuse Screening Laboratory
Department of Toxicology
School of Pharmacy
Auburn University
Auburn, Alabama

In recent months there have been reports concerning the abuse of ketamine as an hallucinogenic agent (side effects of ketamine). Ketamine, a member of arylcycloalkylamine, is a dissociative anesthesic agent [1]. It causes psychotomimetic effects similar to phencyclidine but of shorter duration [1, 2]. The psychological manifestations vary in severity between pleasant dream-like states, vivid imagery, hallucinations, and emergence delirium. It is metabolized very rapidly in man and animals (Fig. 1) with the appearance of several metabolites [1, 3-5]. Ketamine levels in biologic specimens have been determined by gas chromatography [6, 7], thin-layer chromatography [7], colorimetric method [8], and by high pressure liquid chromatography [9]. With the exception of the gas chromatographic method, the methods lack either sensitivity or accuracy. A gas chromatography-mass spectrometric method was therefore developed for the accurate and sensitive determination of ketamine and its metabolites in biologic fluids.

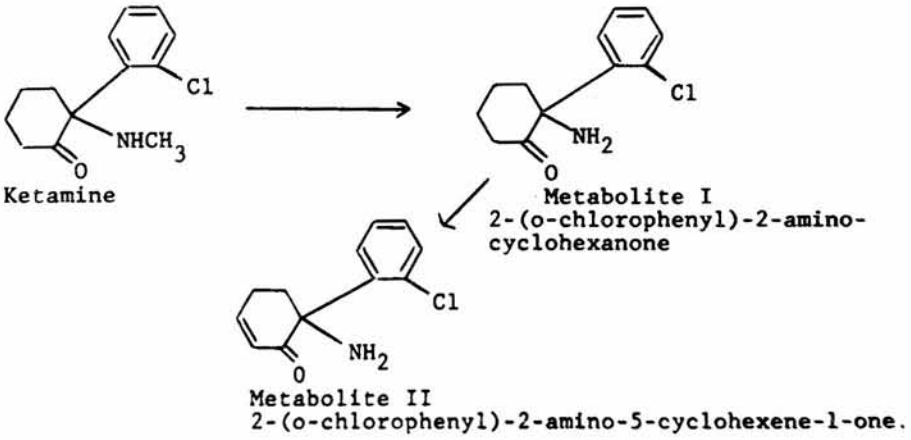


FIG. 1. Metabolism of ketamine.

MATERIALS AND METHODS

Analysis of Urine, Blood, Plasma, and Serum

Male Sprague-Dawley rats (185-215 gm) were injected intraperitoneally with 100 mg ketamine (Parke-Davis & Co., Chicago, Illinois) per kg. Urine was collected over two successive 6-hr periods, and blood samples were collected after half an hour by cardiac puncture. The blood was divided into three groups; (i) whole blood, which was collected in sodium-heparin tubes (143 U.S.P. units) to avoid clotting; (ii) plasma was obtained from these heparinized tubes by centrifugation at 30,000g for 25 min; (iii) serum was obtained by centrifuging clotting blood for 25 min at 30,000g.

Fifteen-milliliter aliquots of urine and 10-ml of blood, serum, and plasma samples were made alkaline (pH 10.0) with ammonium hydroxide:ammonium chloride buffer. The mixture was extracted three times with 15 ml chloroform:isopropanol (3:1) mixture. The organic phases were combined, dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness at 60°C. The residue was dissolved in 1.0 ml of ethanol:acetone (1:1) mixture.

Gas Chromatography-Mass Spectrometry

1 μl solution of each extract, prepared as described above, was injected into a Finnigan Model 3200 gas chromatograph-mass spec-

trometer, which was coupled to a Finnigan Model 6100 data system. The samples were chromatographed through a 6 ft × 2 mm glass column packed with 1% Carbowax 20M on Gas Chrom G, AW-DMCS 100-120 mesh. A column temperature of 210°C and a flow rate of 50 ml/min of helium was used in this study. The electron impact (EI) mass spectra were obtained at 70 eV with the ion source set at 70°C.

For the chemical-ionization (CI) mass spectra, methane was used as the reactant gas with a source pressure of 1 Torr. The emission was 1 mA, power voltage 17 kV, and the source temperature 120°C.

RESULTS AND DISCUSSION

The gas chromatogram and gas chromatogram-mass spectra were similar both for samples and for authentic standards supplied by Parke-Davis and Company. The mass spectra of ketamine, metabolite I, and metabolite II are shown in Figs. 2-4, respectively. The retention times for ketamine, metabolite I, and metabolite II were 6.0, 7.4, and 10.0 min, respectively.

The fragmentation patterns of ketamine and its metabolites are summarized in Tables 1 and 2. These compounds did not show prominent parent ions by EI. The major route of decomposition was the loss of the alkyl portion of the molecule. The CI spectra of ketamine showed M + 1 ion as the base peak, whereas loss of ammonia from the M + 1 ion was the base peak in metabolites I and II.

No significant quantities of metabolite II were found in serum, plasma, and blood although it represents the principle excretion product in urine. The presence of unchanged ketamine in urine samples indicated that only two thirds of the ketamine is biotransformed. Urine seems to be a better fluid for the detection of ketamine and its metabolites because of the higher concentration present up to 12 hr. The toxicity of ketamine may be due to its storage in body depots [1].

The method developed, however, should be adaptable to tissue samples. The method was sensitive in detecting 0.1 μg/ml. A technique for rapid detection of ketamine and its metabolites in blood and urine has been described.

TABLE 1. Fragmentation Patterns of Ketamine, Metabolite I, and Metabolite II by EI

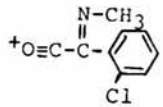
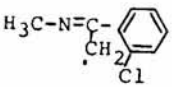
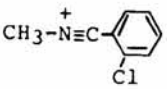
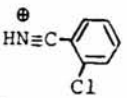
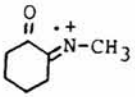
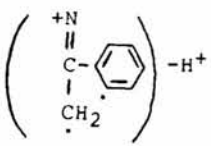
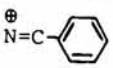
Compound	Fragment or fragment cleared from parent	Peak, m/e	Intensity relative to base peak
Ketamine	-CO	209	03
		180	100
		166	12
		152	30
	-Cl;CO, and $-C_2H_4$	146	15
		138	28
		125	13
		115	31
		102	38
	HCl	36	50
Metabolite I	-CO	195	5

TABLE 1 (continued)

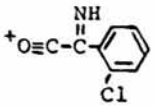
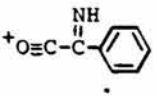
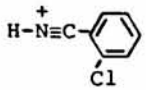
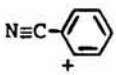
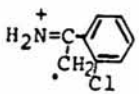
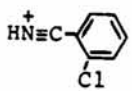
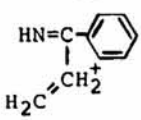
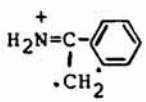
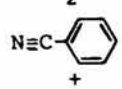
Compound	Fragment or fragment cleared from parent	Peak, m/e	Intensity relative to base peak
Metabolite I		166	100
		131	28
		138	19
		102	21
		91	8
		77	18
Metabolite II		153	100
		138	44
		130	8
		118	50
		102	32

TABLE 2. Fragmentation Patterns of Ketamine, Metabolite I and Metabolite II by CI

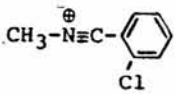
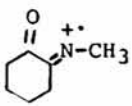
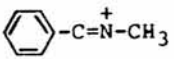
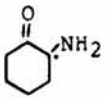
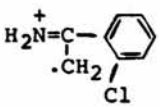
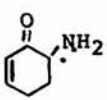
Compound	M or (M + 1) fragment	Peak	Intensity relative to base peak
Ketamine	(M+1)	238	100
	$(M+1)-H_2O$	220	22
	$(M+1)-CH_3\overset{\cdot+}{N}H_2$	207	30
	(M+1)-HCl	202	11
	M-CO and $-NH-CH_3$	179	19
		152	6
		125	22
		117	4
	$H_2O + C_3H_6$	60	48
Metabolite I	(M+1)	224	62
	$(M+1)-NH_3$	207	100

TABLE 2 (continued)

Compound	M or (M + 1) fragment	Peak	Intensity relative to base peak
Metabolite I	M - CO	195	14
	(M+1)-CO and NH_3	179	55
		125	70
		112	24
Metabolite II	M+1	222	45
	(M+1)-NH_3	205	100
	(M+1)-CO and NH_3	177	31
		153	15
		110	18

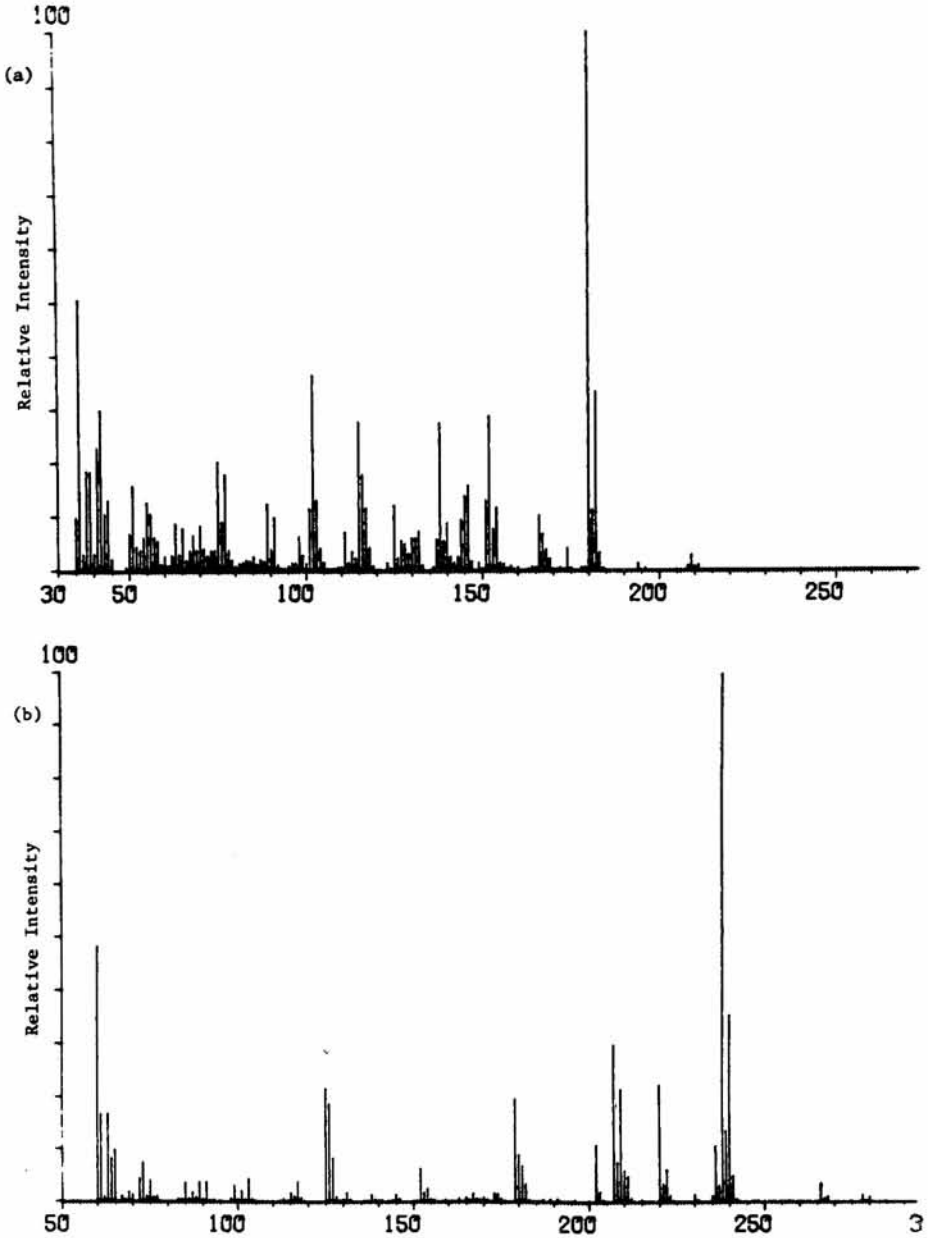


FIG. 2. Mass spectra of ketamine by (a) EI and (b) CI.

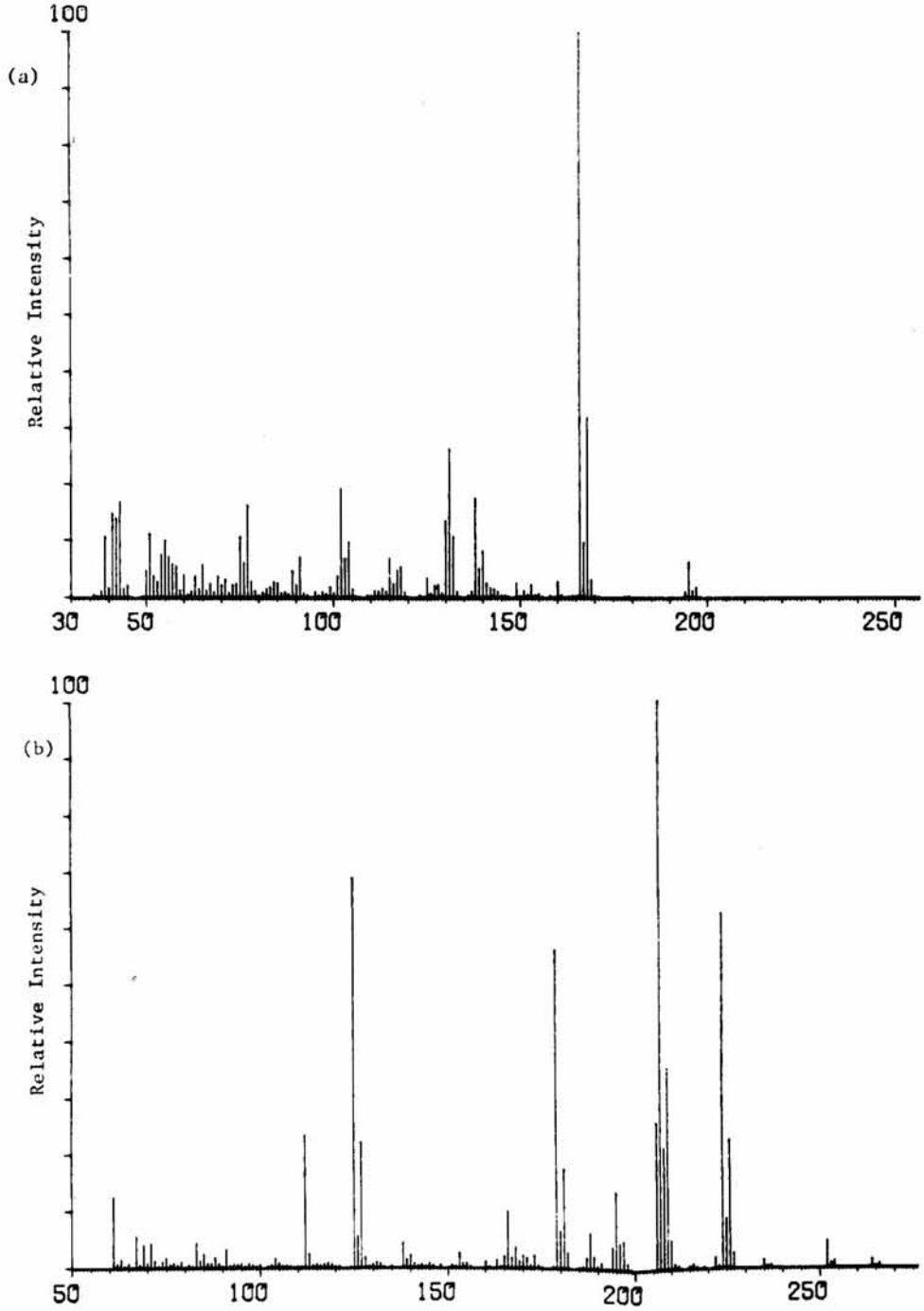


FIG. 3. Mass spectra of metabolite I by (a) EI and (b) CI.

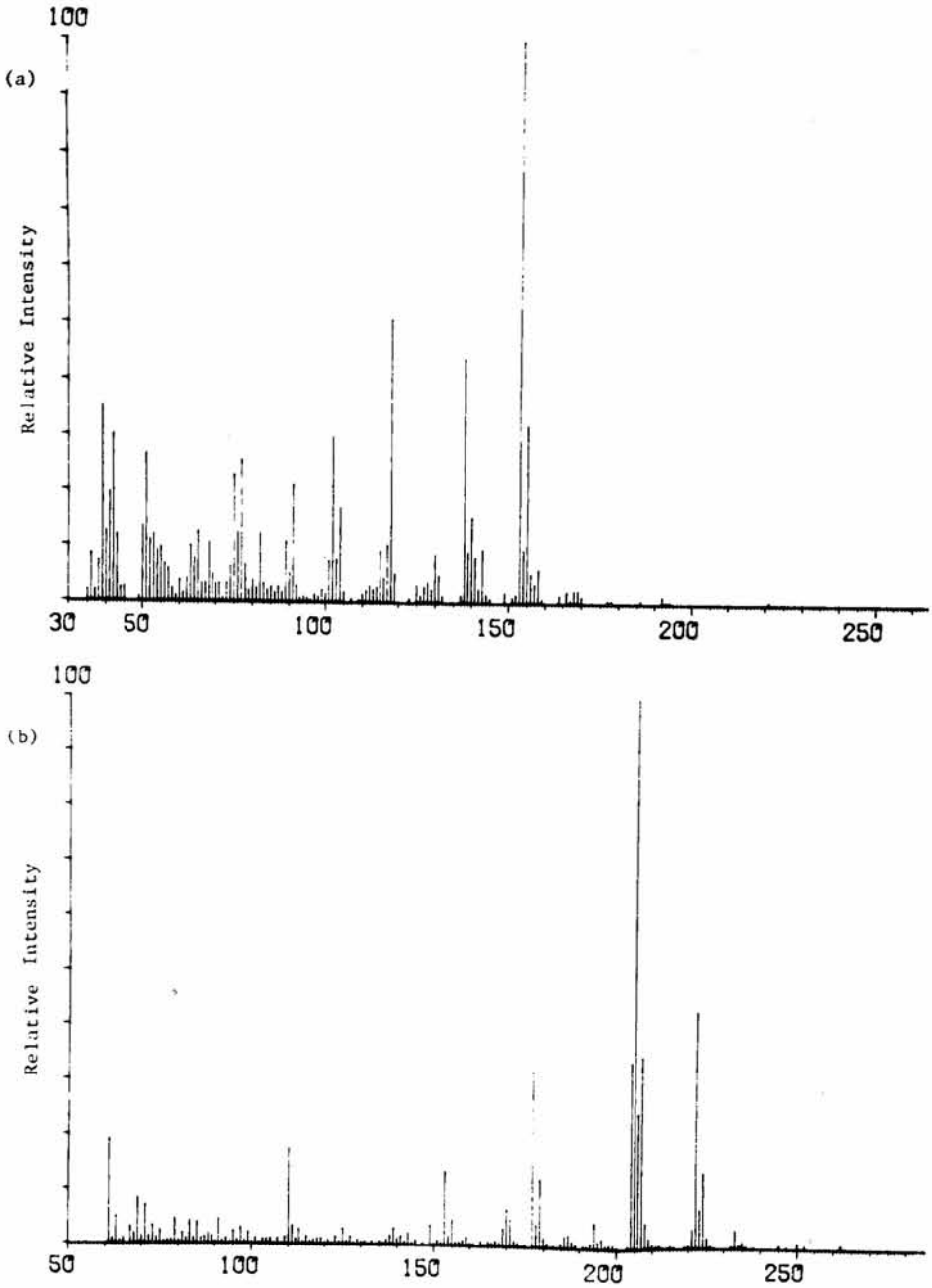


FIG. 4. Mass spectra of metabolite II by (a) EI and (b) CI.

SUMMARY

A simple gas chromatography-mass spectrometric method for the identification of ketamine and its metabolites in urine, blood, serum, and plasma has been developed in order to investigate a coma due to an overdose of the drug.

REFERENCES

[1] H. Kreuscher, Ketamine, Springer-Verlag, New York, 1969, pp. 1-7.

[2] G. Chen, The neuropharmacology of 2-(o-chlorophenyl)-2-methylaminocyclohexanone hydrochloride, J. Pharmacol., 152, 332 (1966).

[3] D. A. McCarthy, G. Chen, D. H. Kaump, and C. R. Ensor, General anesthetic and other pharmacological properties of 2-(o-chlorophenyl)-2-methylaminocyclohexanone hydrochloride, J. New Drugs, 5, 21 (1965).

[4] Y. Kayama, Central action of ketamine, dissociation of excitation, Japan. J. Anesth., 22, 1223 (1973).

[5] M. L. Cohen, S. L. Chan, W. L. Way, and A. J. Trevor, Distribution in the brain and metabolism of ketamine in the rat after intravenous administration, Anesth., 39, 370 (1973).

[6] T. Chang and A. J. Glazko, A gas chromatographic assay for ketamine in human plasma, Anesthesiology, 36, 401 (1972).

[7] M. M. Kochhar, L. T. Bavda, and S. Bhushan, Thin-layer and gas chromatographic determination of ketamine and its biotransformed products in biological fluids, Res. Commun. Chem. Pathol. Pharmacol., 14, 367 (1976).

[8] T. Chang, W. A. Dill, and A. J. Glazko, Metabolic disposition of 2-(o-chlorophenyl)-2-(methylamino)cyclohexanone, Federation Proc., 24, 268 (1965).

[9] L. L. Needham and M. M. Kochhar, Determination of ketamine and some in vivo metabolites using high pressure liquid chromatography, J. Chromatogr., 114, 220 (1975).