Journal of Chromatography, 146 (1978) 344—349
Biomedical Applications
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CHROMBIO. 191

Note

Rapid gas chromatographic analysis of plasma levels of ketamine and major metabolites employing either nitrogen selective or mass spectroscopic detection

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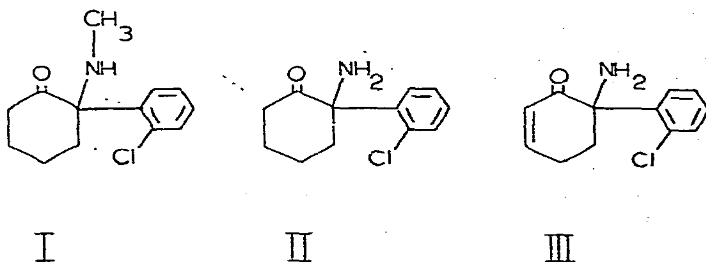
(Received January 2nd, 1978)

Ketamine [2-(*o*-chlorophenyl)-2-(methylamino)cyclohexanone] (I), a parenteral anesthetic with unusual pharmacological properties [1], has been studied by numerous investigators. Many groups [2—4] have sought to clarify therapeutic relationships between this compound and its major metabolites. Two biotransformation products that have received much attention include 2-(*o*-chlorophenyl)-2-aminocyclohexanone (II) and 6-(*o*-chlorophenyl)-6-amino-



cyclohex-2,3-en-1-one (III). Most investigations of this type have focused upon biodisposition and metabolism. Thus suitable chemical analyses of these compounds remain essential to these experiments.

Most researchers employ modifications of the gas chromatographic (GC) assays developed by Chang and Glazko [4, 5] and Lo and Cumming [6]. Additionally, a high-pressure liquid chromatographic (HPLC) method has been described [7]. All of these analytical processes require derivatization and/or extensive extraction and concentration steps.

This report offers new analytical methods for I—III based upon either the convenience and sensitivity of computer assisted integrated gas chromatography—mass spectroscopy (GC—MS) or the advantages of GC separation

coupled with selective nitrogen detection. Additionally, these procedures simplify methodology and reduce assay time. Finally, qualitative identification of compounds is greatly facilitated by examination of MS data.

EXPERIMENTAL

Supplies

Compounds I, II, III and diphenhydramine were supplied courtesy of Parke Davis & Co. (Detroit, Mich., U.S.A.). All solvents and additional chemicals were standard reagent-grade materials.

Gas chromatography

GC separations were made with a Perkin-Elmer 3920 B gas chromatograph equipped with a phosphorous—nitrogen (P—N) detector. The column consisted of a 6 ft. × 2 mm I.D. glass tube packed with 3% SP-2300 on Supelcoport (100—120 mesh; Supelco, Bellefonte, Pa., U.S.A.). Nitrogen carrier gas flow-rate was maintained at 40 ml/min, hydrogen flow-rate at 5 ml/min and air flow-rate at 100 ml/min. The rubidium bead current control was set at 500. Injection port and interface temperatures were 270°, and the column oven operated isothermally at 220°.

A Hewlett-Packard 5720 gas chromatograph equipped with a Ni^{63} detector was employed to compare the results of this study with the electron capture method [5].

Mass spectroscopy

Mass spectra were recorded with a Dupont Instruments Dimaspec 321 gas chromatograph—mass spectrometer interfaced with a DuPont Instruments 320 data system. Source temperature was 225° with ionization at 75 eV, and the accelerating voltage maintained over the range of 12,300—600 eV. The jet separator was operated at 225°. Other GC parameters were identical to those noted above except that helium was employed as the carrier gas.

Procedure

Male Swiss—Webster mice (20—30 g) were injected intraperitoneally with 125 mg/kg ketamine hydrochloride dissolved in normal saline. After 15 min, animals were sacrificed by decapitation and blood was collected in a small beaker. Plasma was obtained by standard centrifugation methods and saved for analysis. Routinely 0.4—0.5 ml of plasma per animal was obtained by this process.

For a normal assay procedure 0.1—1.0 ml of plasma was transferred to a glass stoppered centrifuge tube. To this was added 0.10 ml of 4 *N* NaOH solution, 0.1 ml of diphenhydramine hydrochloride solution (equivalent to 25 μg/ml free base) and sufficient distilled water to produce a final volume of 1.2 ml. Methylene chloride (2.0 ml) was added and the tube was shaken by hand for approximately 30 sec. Separation of the layers was facilitated by centrifugation, and the organic (lower) phase was transferred to a conical centrifuge tube. After evaporation of the methylene chloride under a stream of nitrogen at 20°, 0.05 ml of acetone was added and the tube vortexed for a few seconds.

A 1—2 μl volume of this solution was injected into the gas chromatograph.

Standard curves were obtained as described above except that known quantities of compounds I—III (0.4—10 μg) were added to plasma samples obtained from animals that had been injected with normal saline.

RESULTS AND DISCUSSION

Under the conditions employed for GC analysis of various samples, excellent separation of all four compounds (I, II, III, and diphenhydramine, IV) was achieved (Fig. 1). Relative retention times for compounds I—III were 2.5, 3.5 and 5.5 min respectively. The average GC analysis time of approximately 10

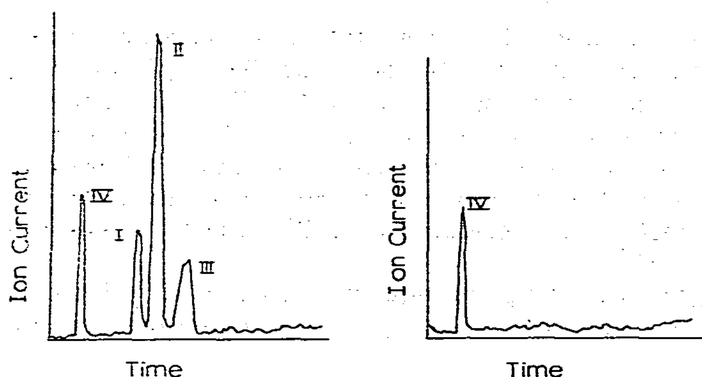


Fig. 1. Reconstructed gas chromatograms of compounds I, II, III and IV (diphenhydramine). Column: 6 ft. × 2 mm I.D., 3% SP 2300 on Supelcoport (100—120 mesh) at 240°. The injection mixture (left chromatogram) was obtained initially from 0.2 ml of plasma containing 4.0 μg I and III, 10 μg II and 2.5 μg IV, while the other chromatogram resulted from a plasma sample containing only the internal standard (IV).

min, permitted rapid processing of several samples. As can be seen no significant interfering peaks were observed. Similar separations were obtained using an instrument equipped with a P—N detector.

Qualitative identification was facilitated by examination of mass spectral data. All compounds showed extensive fragmentation which was consistent with known chemical structures. Compounds I and II displayed very weak molecular ions while the M^+ ion of III was highly significant in the mass spectrum. Base peaks for all compounds contained chlorine as evidenced from the relative intensities of ions due to isotope distribution (Table I).

Quantitation of compounds I—III was accomplished by two methods, the most expeditious of which utilized the available software contained in the GC—MS data-acquisition system. This essentially involved integrating total ion currents produced by compounds I—III and comparing each of these to the analogous response shown by the internal marker, diphenhydramine.

Standard peak-height ratio methods were used to quantitate data obtained in experiments employing the P—N detector equipped instrument. Results were essentially the same as those obtained through the computer analyses described above. Since the P—N detector is available to more laboratories, the

TABLE I

RELATIVE INTENSITIES OF MAJOR IONS IN THE MASS SPECTRA OF COMPOUNDS I—III

See Experimental for conditions

Compound	*m/e*	Relative intensity
I	180	100
	182	35.3
	209	26.9
	211	9.4
	237 M	0.9
	239 M + 2	0.3
II	166	100
	168	34.8
	195	26.9
	197	8.9
	223 M	0.2
	225 M+ 2	0.07
III	153	100
	155	32.2
	221 M	40.4
	223 M + 2	13.2

data illustrated (Fig. 2) was compiled from experiments using this type of instrumentation. As can be noted, standard curves for compounds I—III were linear in the range 0.8—10.0 μg/ml. Other experiments showed that this range of linearity could be extended to at least 40 μg/ml.

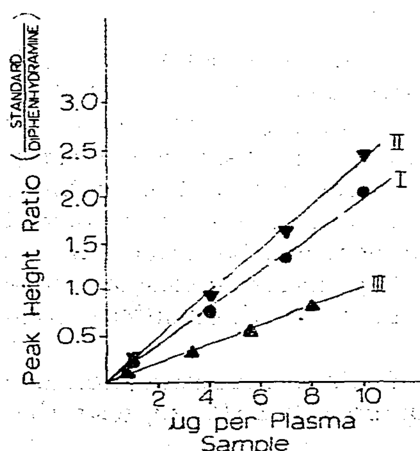


Figure 2: Standard curves for ketamine and major metabolites. • = Ketamine (I); ▾ = N-demethylated product (II); ▴ = N-demethylated cyclohexenone analog (III). Known quantities of I—III were added to 0.2 ml plasma. After addition of 2.5 μg diphenhydramine (IV) to each sample, GC analysis was carried out using a P—N detector equipped instrument. Ratios of standards to internal marker (IV) were calculated. Points represent mean of three determinations. Regression lines were calculated by least-squares method.

Percentage recovery of I—III was determined by extracting 1.0 μg of each from plasma samples. Peak-height ratios of compounds I—III versus internal marker were compared with those obtained from acetone solutions of similar concentrations. Four determinations resulted in mean per cent recoveries for I, II, and III of 97±2.4, 98±1.9 and 93±3.2, respectively.

To establish the utility of this method for application to in vivo studies, mice were injected with ketamine (see Experimental) and plasma samples analyzed for the presence of compounds I—III. The results (Table II) indicated that all compounds could be readily identified and quantitated, using these procedures. In addition to plasma samples, liver and brain specimens were also subjected to analysis. Again this method proved quite satisfactory for monitoring compounds I—III in these tissues. Similar findings resulted when laboratory rats were used as test animals.

TABLE II

PLASMA CONCENTRATIONS OF COMPOUNDS I—III IN ANIMALS

Measured for male, Swiss-Webster mice (n = 8), 25 min after receiving ketamine (125 mg/kg) intraperitoneally.
Values determined by peak-height ratio method from data obtained using P—N detection. No significant differences were noted when computer analysis of peak areas of reconstructed gas chromatograms were quantitated.

Compound	Concentration (μg/ml)
I	8.5 ± 1.16
II	13.9 ± 1.28
III	2.58 ± 0.21

Analytical methods for ketamine and its major metabolites described in this paper offer rapid and accurate procedures for the separation, identification and quantitation of these compounds. Several advantages over published methods can be cited, a major difference is that no expensive and lengthy derivatizations, such as those required for electron capture detection methods [5], are necessary. Additionally, extensive extraction, back extraction, and concentration steps are eliminated. Similar advantages can be noted for this method when compared with HPLC analysis [7]. In general one plasma sample can be processed and assayed in approximately 15 min with sensitivity comparable to other methods.

Computer assisted GC—MS processing greatly facilitates identification and quantitation. The extensive fragmentation of compounds I—III suggests that chemical ionization would probably be valuable.

Finally, it should be noted that use of a nitrogen-selective GC detector provides similar results to GC—MS computer methods. The latter allows for more rapid analyses but the former may be more attractive for economic reasons. However, both offer advantages over previously published methods.

REFERENCES

1 C.F. Lanning and M.H. Harmel, Annu. Rev. Med., 26 (1975) 137.
2 M.L. Cohen and A.J. Trevor, J. Pharmacol. Exp. Ther., 189 (1974) 351.
3 M.P. Marietta, W.L. Way, N. Castagnoli, Jr. and A.J. Trevor, J. Pharmacol. Exp. Ther., 202 (1977) 157.
4 T. Chang and A.J. Glazko, in G. Dal Santo (Editor), Biotransformation of General Anesthetics, International Anesthesiology Clinics, Little Brown and Company, Boston, Mass., 1974, pp. 157—177.
5 T. Chang and A.J. Glazko, Anesthesiology, 36 (1972) 401.
6 J.N. Lo and J.F. Cumming, Anesthesiology, 43 (1975) 307.
7 L.L. Needham and M.M. Kochhar, J. Chromatogr., 114 (1975) 220.