# Laboratory Methods

# *A Gas Chromatographic Assay for Ketamine in Human Plasma*

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A highly sensitive, quantitative assay procedure for ketamine and two of its metabolites, based upon gas–liquid chromatography of a heptafluoro-butyryl derivative, has been developed. No appreciable interference occurred with procaine, atropine, meperidine or promethazine. Anesthetic doses in man (iv) produced ketamine plasma levels above 1 $\mu$g/ml, with a biological half-life of 17 min. The presence of the N-dealkylated metabolite of ketamine in human plasma was clearly demonstrated. (Key words: Ketamine; Assay; Gas chromatography; Metabolites.)

AN ASSAY PROCEDURE in which a fluorescent dye (xylene red B) was extracted into an organic solvent in direct proportion to the amount of ketamine present was described recently.[1] However, the assay was not highly specific, and considerable interference from the N-dealkylated amine metabolite of ketamine (I) and the cyclohexanone oxidation product (II) was encountered.‡ As a result, we resorted to quantitative gas–liquid chromatography, based in part upon our earlier experience with this technique.[2] Gas–liquid chromatography has been found to possess adequate sensitivity for measurement of plasma levels of ketamine and metabolites I and II in human subjects receiving the usual anesthetic doses of ketamine.

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‡ Ketamine (Ketalar, Parke-Davis) is 2-(o-chlorophenyl)-2-(methylamino) cyclohexanone. Metabolite I is 2-amino-2-(o-chlorophenyl)-cyclohexanone; metabolite II is 2-amino-2-(o-chlorophenyl)-3-cyclohexen-1-one. The drug was administered in the form of the hydrochloride salt. All assays are expressed in terms of ketamine base equivalents.

## Materials

### APPARATUS

All analyses were carried out with a Hewlett-Packard Model 402 Biomedical Gas Chromatograph equipped with a $^{63}$Ni electron-capture detector and a 1-mv Honeywell recorder. The column used was a 6 foot (3 mm ID) U-shaped glass tube packed with OV-17 (3
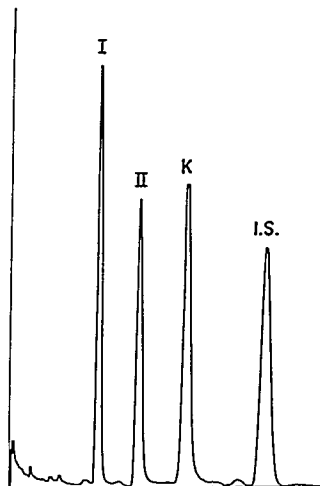


FIG. 1. Gas–liquid chromatogram of heptafluorobutyryl derivatives of ketamine (K), metabolites I and II, and internal marker (I.S.). Column: 6 foot OV-17 (3 per cent) on GasChrom Q at 180 C; electron-capture detector. Five ng of each component present in the injection mixture.
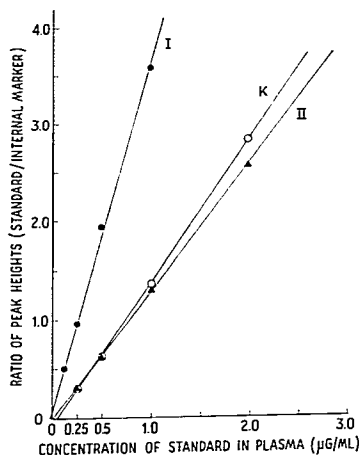
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Fig. 2. Relation of peak height ratio (X/I.S.) to drug concentration in plasma. Known amounts of internal marked (1 μg) and ketamine (0.25, 0.50, 1.00, 2.00 μg), or metabolite I (0.125, 0.25, 0.50, 1.00 μg) or metabolite II (0.25, 0.50, 1.00, 2.00 μg) were added to 1 ml plasma and run through the entire gas–liquid chromatography procedure. Mean of two assays per point; regression lines calculated by the method of least squares. Column temperature 195 C.

per cent) on GasChrom Q (100/120 mesh). The temperature was maintained at 195 C for routine assays, with the flash heater at 220 and the detector at 250 C. The carrier gas (45 ml/min) consisted of 5 per cent methane in argon; the same gas mixture was also used to purge the detector (130 ml/min). The detector voltage pulse control was set at 150 microsec. Electrometer attenuation was held at 32 × 10⁻¹¹ A.

CHEMICALS

Ketamine hydrochloride, metabolite I, metabolite II, and the internal marker, 2-amino-2-(o-bromophenyl)-2 methylamino-cyclohexanone (Parke, Davis & Co., Detroit, Mich.); benzene, nanograde; sodium sulfate, anhydrous (Mallinckrodt, St. Louis, Mo.); heptafluorobutyric anhydride in sealed glass ampules (Pierce Chemical Co., Rockford, Ill.); pyridine (J. T.

Baker Chemical Co., Phillipsburg, N. J.); pretested 3 per cent OV-17 on GasChrom Q, 100/120 mesh (Applied Science Laboratories, College Station, Pa.).

REAGENTS

Borax–alkali: A solution of 10 per cent borax (Na₂B₄O₇·10 H₂O) is made up in 5 N sodium hydroxide.

Pyridine: Dry pyridine over KOH pellets.

Procedure

The internal marker (I.S.) is made up in 0.1 N HCl to contain 1 μg/ml. One ml of plasma, 0.1 ml borax buffer, 1 ml of I.S. (1 μg/ml) and 2.5 ml of benzene are measured by pipette into a 15 × 135-mm glass-stoppered conical-tip centrifuge tube, then shaken for 10 min in a mechanical shaker. Following centrifugation, 2 ml of the benzene layer are transferred to a 10 × 75-mm disposable culture tube containing about 1.5 g anhydrous sodium sulfate. The mixture is agitated in a Vortex mixer at intervals over a 15–30-min period to insure complete removal of water. The dried benzene solution is then decanted directly into a 10 × 100-mm glass-stoppered test tube, and 0.1 ml of heptafluorobutyric anhydride and 0.1 ml of pyridine are added. The contents are thoroughly mixed and the lower half of the stoppered tube is heated for an hour at 75 C in a metal heating block. The tube is then removed, cooled to room temperature, and the contents are shaken for 5 min with 2 ml of 0.5 N NaOH to remove excess reagent. Approximately 1.5 ml of the benzene layer is transferred into a second glass-stoppered test tube and shaken for 5 min with 2 ml of 0.25 N HCl. After centrifugation, 1 ml of the benzene layer is transferred to a cork-stoppered 10 × 75-mm disposable culture tube containing 0.5 g of anhydrous sodium sulfate. From 1 to 3 μl of this solution are injected directly into the column. The retention times are short enough to permit repeated injections of samples at 10-minute intervals.

Standard curves for ketamine, I, and II are set up by relating the ratio of peak heights (ketamine/I.S., I/I.S., II/I.S.) to known amounts of ketamine, I, or II added to 1 ml of control plasma. Stock solutions of ketamine and II are made up in 0.1 N HCl to contain

FIG. 3. Plasma levels of ketamine and metabolite I in a human subject. A 24-year-old woman weighing 55.4 kg was given a single iv injection of 122 mg ketamine (2.2 mg/kg). Duration of anesthesia = 20 min. Gas–liquid chromatography assays of heparinized plasma for ketamine are indicated by circles; regression line calculated by the method of least squares. Metabolite I assays are indicated by triangles. Metabolite II was not observed in the chromatograms.
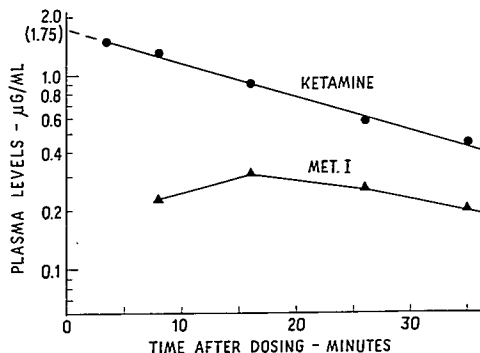
200 μg base per ml; I and I.S. are made up to contain 100 μg base per ml. For use, a mixture of the four stock solutions is prepared by pipetting 1 ml of each into a 100-ml volumetric flask and diluting to mark with 0.1 N HCl. Further dilutions of 1:2, 1:4, and 1:8 are made in 0.1 N HCl. One milliliter of each dilution is then added to 1 ml of normal plasma, and the assay procedure is carried out as described above. The ratios of the peak heights of ketamine, I, and II to that of the internal marker provide a direct index of actual plasma concentrations. Since column conditions and detector responses may show some change, standard curves are prepared with control plasma for each series of analyses.

## Results and Discussion

The heptafluorobutyryl derivatives was chosen for this work because of the high sensitivity of the electron-capture detector for halogenated compounds. The o-bromophenyl analogy of ketamine served as an excellent internal standard because its extraction properties were similar to those of ketamine and metabolites I and II, but its retention time on the OV-17 column was sufficiently different to permit a clean separation. A typical chromatogram, representing injection of 5 ng of each compound on the column, is shown in figure 1. No peaks were observed when the heptafluoro-

butyryl reagent was omitted. The reaction with this reagent was essentially complete within 30 min, but the samples were routinely heated for an hour to insure completeness of reaction. Extraction of the mixture with aqueous alkali and acid was necessary to remove excess reagent, which would otherwise interfere with chromatography. The heptafluorobutyryl derivatives appeared to be stable in the benzene solution for several days.

The essential linearity of response of the electron-capture detector to known amounts of ketamine, I, II, and internal marker is shown in figure 2. All samples (0–2 μg/ml plasma) were run in duplicate, and the ratios of peak heights to the I.S. peaks were calculated for each of four concentrations of drug or metabolite added to plasma. The ratios were directly proportional to drug concentrations over the range tested, and the lowest concentrations employed (0.125–0.25 μg/ml) were readily detectable. Reproducibility of the peak height ratios was established in another trial by running six replicate assays of each of four concentrations of drug; the standard errors were within 2 per cent of the mean in each series.

The recovery of ketamine, I, and II added in known amounts to control plasma was nearly the same as the recovery of each component from protein-free aqueous solutions. For ketamine, the mean recovery from plasma

at four different concentrations was $98 \pm 1.3$ per cent (SE); the mean recovery of metabolite I was $97 \pm 1.4$ per cent and that of metabolite II, $90 \pm 0.7$ per cent. These slight differences are compensated for by running recovery standards from plasma with each set of assays. Other known metabolites of ketamine are not extractable with benzene, and did not interfere with this assay procedure.

Application of the gas–liquid chromatography procedure to control plasma specimens revealed no interfering peaks due to normally-occurring constituents. Procaine, atropine, meperidine, and promethazine in concentrations of 20 $\mu$g/ml did not interfere with the gas–liquid chromatography assay, although these drugs produced serious interference with the fluorimetric procedure.[1] Similarly, the use of polyethylene tubing in collection of blood specimens had no effect on the assay.

Typical assays for plasma levels of ketamine and metabolite I in an adult human subject are shown in figure 3. The ketamine levels fell exponentially from 1.49 $\mu$g/ml 4 min after dosing to 0.44 $\mu$g/ml in 35 min; the plasma half-life was estimated to be 17 min. The apparent volume of distribution, estimated by back-extrapolation of plasma level data to zero time, was about 70 liters (distribution coefficient = 1.26 ml/g). The initial plasma levels of metabolite I were considerably lower than those of ketamine, reaching values a third to half as great in 15–30 min. No metabolite II was detected in the plasma of this subject, although small amounts (0.1–0.2 $\mu$g/ml) were observed in other subjects. In most of the specimens of plasma examined to date, the levels of ketamine and its metabolites fell within the concentration ranges covered by the standard curves. In a few cases involving drug infusion, values as high as 3 $\mu$g/ml were attained. The sensitivity of the procedure can be increased by using larger samples of plasma or by concentrating the final reaction mixtures to smaller volumes.

## References

1. Dill WA, Chucot L, Chang T, et al: Determination of ketamine in blood plasma. ANESTHESIOLOGY 34:73–76, 1971
2. Chang T, Dill WA, Glazko AJ: Metabolic disposition of 2-(o-chlorophenyl)-2-(methylamino)cyclohexanone-HCl (CI-581) in laboratory animals and in man. Fed Proc 24: 268, 1965

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## Transfusion

**GAMMA-GLOBULIN AND POSTTRANSFUSION HEPATITIS** A hundred patients were followed for at least six months after transfusion with whole blood or plasma. All patients were examined every two weeks for the first two months and every month for the last four months, venous blood being obtained for serum glutamic oxalacetic transaminase (SGOT) and serum pyruvic oxalacetic transaminase (SPOT) determinations. When these were found to be abnormal, further liver function tests were performed. Gamma-globulin administration was randomized; patients who fell into the group that received gamma-globulin were given 10 ml within seven days of the first blood transfusion, and this dose was repeated 30 days thereafter. Of 100 patients, 21 per cent developed hepatitis; of 53 who received gamma-