Determination of Therapeutic Blood Levels of Methamphetamine and Pentobarbital by GC

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Abstract □ A GC method is presented for the assay of methamphetamine and pentobarbital in blood following oral administration of the combination. The trichloroacetamide derivative of methamphetamine has higher electron-capture sensitivity and is more easily chromatographed than previously reported derivatives. Details concerning the extraction procedure, derivative formation, percent recoveries of both drugs, and blood level data are given.

Keyphrases □ Methamphetamine—GLC analysis, blood □ Pentobarbital—GLC analysis, blood □ GLC—analysis, methamphetamine and pentobarbital in blood

Various methods have been used for the determination of amphetamines (1–5) and barbiturates (6–10) from body fluids. Most of the more sensitive methods utilized GLC equipped with flame-ionization detectors. These methods have proved useful for therapeutic doses of barbiturates (11–15) but lack the necessary sensitivity required to monitor blood levels over a 24-hr. period from a small single dose of methamphetamine (15–18). Inquiries concerning the bioavailability of certain products led to the development of an analytical technique for the determination of either methamphetamine alone or in combination with a barbiturate.

The advent of the electron-capture detector (ECD), with its high sensitivity toward halogenated compounds, led to the formation of suitable derivatives by some workers (19–22). Bruce and Maynard (23) described a method based on the formation of heptafluorobutyramide from some secondary (*i.e.*, methamphetamine) and primary amines. Noonan *et al.* (24) used the trichloroacetamide derivative for the determination of amphetamine. Wilkinson (25) showed the pentafluorobenzamide of amphetamine to be highly sensitive, with excellent possibilities for monitoring blood levels.

Results obtained from different derivatives in this laboratory indicate that the trichloroacetamide is the derivative of choice for methamphetamine. The high order of sensitivity: trichloroacetamide > heptafluorobutyramide > pentaflurobenzamide, indicates its desirability from this standpoint. In addition, its lower volatility compared to heptafluorobutyramide negates

Table I—Percent Recovery of Methamphetamine[a]

Total Nanograms Added	Trial 1, %	Trial 2, %	Mean Recovery, %
	Urine, 1 ml.		
0	0	0	0
25	91.6	92.5	92.1
50	93.5	93.1	93.2
125	90.7	91.2	91.0
250	92.3	93.8	93.1
500	96.7	95.1	95.9
750	90.9	96.2	93.6
1000	95.2	95.7	95.5
1500	98.1	97.8	98.0
2000	94.4	93.2	93.8
	Blood, 15 ml.[b]		
0	0	0	0
25	67.3	65.9	66.1
50	66.1	68.2	67.2
100	65.2	68.7	67.0
200	68.1	72.1	70.1
400	71.4	71.3	71.4
800	66.0	72.4	69.3
1200	69.7	67.5	68.6
1600	66.0	72.3	69.2
2000	73.2	68.8	70.1

[a] Trial values were determined by: peak height spiked sample/peak height pure derivative. [b] The lower recoveries from blood could be due to erythrocyte binding. However, preliminary studies indicated no significant difference in percent recoveries when plasma or whole blood was used.

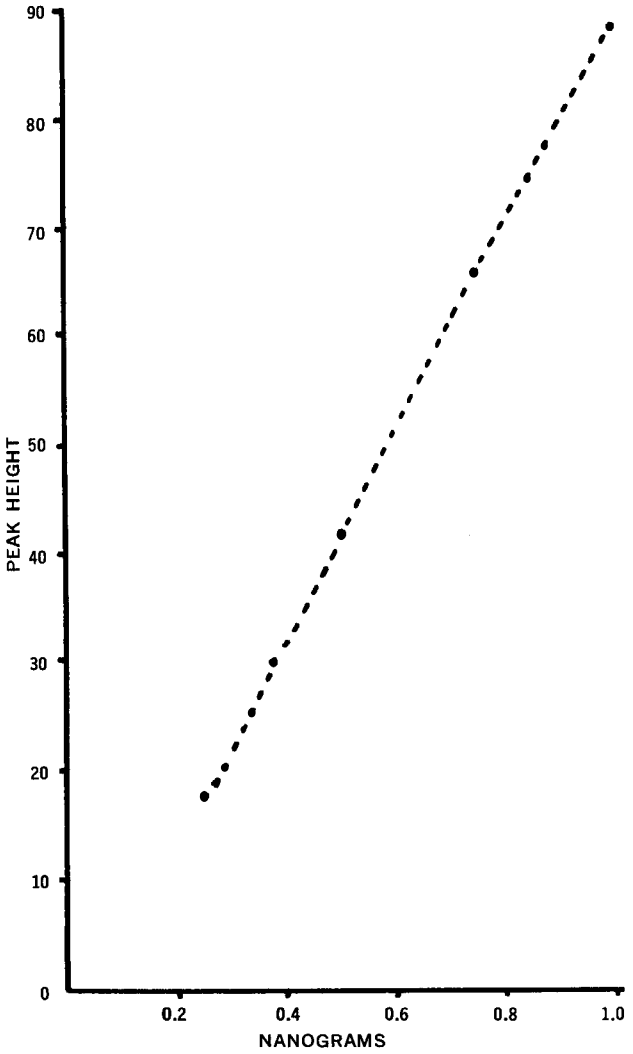

Figure 1—*Standard curve of methamphetamine trichloroacetamide, 3×10^{-9} amp.*

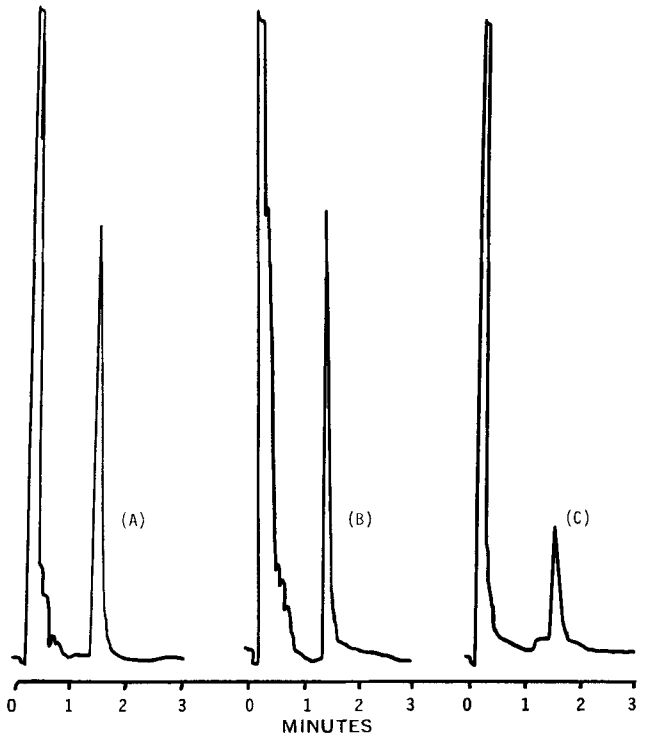

Figure 2—*Chromatographic responses of different methamphetamine derivatives. Key: (A), trichloroacetamide, 0.5 ng.; (B), heptafluorobutyramide, 2.0 ng.; and (C), pentafluorobenzamide, 2.0 ng.*

the need for temperature programming during successive blood determinations.

Samples containing barbiturates in addition to methamphetamine were selectively extracted; the barbiturate was determined by GLC, using a flame-ionization detector, with a method similar to that of Kazyak and Knoblock (15).

EXPERIMENTAL

Reagents—The following were used: trichloroacetyl chloride[1], redistilled, 17.8-cm. (7-in.) column; pyridine[2], distilled over CaH_2, 50.8-cm. (20-in.) column; and hexane[3], 1000 ml. washed with concentrated H_2SO_4 (6 × 50 ml.), 7 *N* NaOH (1 × 50 ml.), and H_2O (2 × 50 ml.), and distilled over CaH_2, 50.8-cm. (20-in.) column.

Apparatus—The following were used: gas chromatograph[4], chart speed 2 min./in.; electrometer, sensitivity range 3 × 10^{-6} to 10^{-12} amp.; flame-ionization detector, with air flow 600–700 ml./min. and hydrogen 60–70 ml./min.; and ECD, with 150 mc. tritium on titanium foil.

Procedures—*Preparation of Blood Sample*—The blood samples were collected in tubes containing a balanced mixture of potassium oxalate and ammonium oxalate[5]. The plasma layer of centrifuged whole blood (10 ml.) was removed and placed in a screw-cap tube (25 × 150 mm.) fitted with a Teflon liner. To this fraction was added distilled H_2O (10 ml.), 10% sodium tungstate (5 ml.), and 1.3 *N* H_2SO_4 (6 ml.). The mixture was allowed to stand for 10 min., followed by the addition of ether (25 ml.), and then briskly shaken and centrifuged. The ether extract was removed and placed in another tube, and the aqueous acid extract was placed in a 50-ml. centrifuge tube. The residue in the screw-cap tube was again treated with 1.3 *N* H_2SO_4 (10 ml.) and ether (25 ml.) and shaken. The ether and acid washings were combined with their respective extracts.

[1] Eastman.
[2] Purified grade, Matheson, Coleman and Bell.
[3] Nanograde, Mallinckrodt.
[4] Packard model 7301, equipped with Honeywell Class 19 recorders.
[5] Vacutainer specimen tube (7 ml.), containing 6 mg. ammonium and 4 mg. potassium oxalate, Becton Dickinson 4761.

Table II—Percent Recovery of Pentobarbital from Blood[a]

Micrograms per Milliliter	Trial 1, %	Trial 2, %	Mean Recovery, %
0	0	0	0
0.10	88.6	89.9	89.3
0.20	90.1	92.7	91.4
0.30	93.6	97.5	95.6
0.50	94.0	92.9	93.5
1.00	96.7	98.4	97.6

[a] Trial values were determined by: peak height spiked sample/standard $CHCl_3$ solution of pentobarbital.

Barbiturate Assay—The ether extract was dried (anhydrous Na_2SO_4, about 1 g.) and filtered through a pledget of glass wool, with the Na_2SO_4 being rinsed with ether (2 × 5 ml.). The combined rinsings and extract were evaporated to dryness under a stream of nitrogen. The evaporated extract was redissolved in chloroform (0.10 ml.), and 5 μl. was injected into a GC fitted with a flame-ionization detector. The column was glass [1.8 m. (6 ft.) long × 0.63 cm. (0.25 in.) o.d.], packed with acid-washed Chromosorb W[6], silylated with dimethylchlorosilane, and having a 5% SE-30 liquid load.

The instrument's operating conditions were as follows: inlet port, 220°; column, 195°; detector, 275°[7]; and a gas flow (nitrogen), 90 ml./min. Retention time for pentobarbital was 4.0 min. Since some high boiling compounds are extracted by the procedure, the system was temperature programmed after each injection. The

Figure 3—*Chromatogram of methamphetamine trichloroacetamide, 25 pg.*

[6] Hewlett-Packard.
[7] Detector temperature was maintained at 275° to avoid condensation of high boiling materials during temperature programming.

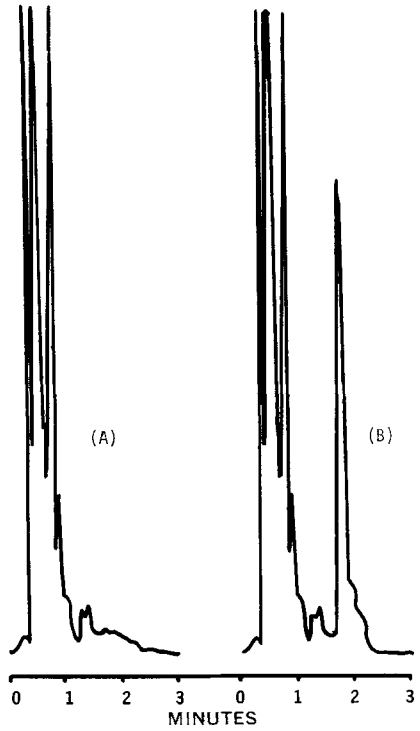

Figure 4—*Chromatograms of trichloroacetylated human plasma extracts. Key: (A), before administration; and (B), 2 hr. after administration of methamphetamine hydrochloride, 12.5 mg.*

initial hold of 195° was 5 min., with a programmed rate of 5°/min. to 275° and a final hold of 5 min.

Methamphetamine—The combined aqueous layers removed in the extraction procedure were made alkaline (about pH 14)[8] by the addition of 7 *N* NaOH (5 ml.) and extracted with hexane (3 × 25 ml.). The hexane extracts were combined and dried over NaOH pellets and decanted, and the pellets were rinsed with hexane (2 × 10 ml.). The combined rinsings and extract were evaporated under a stream of nitrogen to 1–2 ml. To this evaporated extract was added the derivatizing mixture[9] (0.1 ml.). The reaction was gently agitated

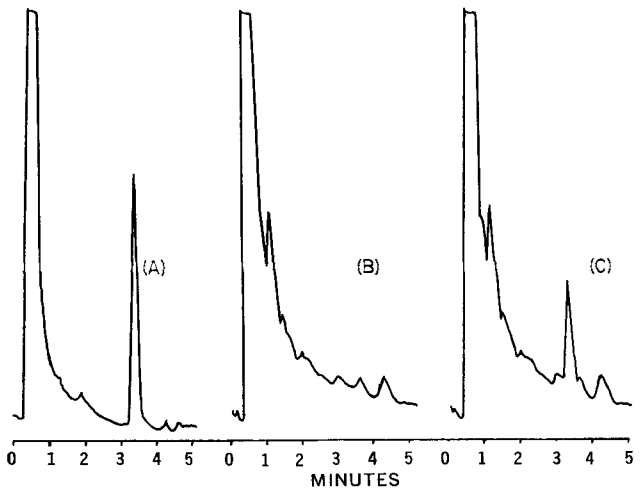

Figure 5—*Chromatograms of: (A), pentobarbital, 0.5 mcg. standard; (B), blank plasma; and (C), 6-hr. blood sample.*

[8] This pH gave cleaner chromatograms than those resulting from extractions at lower pH values. This may be due to the elimination of biogenic phenolic amines by sodium salt formations.

[9] Derivatizing mixture: To a solution of redistilled pyridine (1.580 g., 0.020 mole) in hexane (15 ml.) was added a solution of redistilled trichloroacetyl chloride (1.818 g., 0.010 mole) in hexane (30 ml.). The mixture was gently agitated and stoppered.

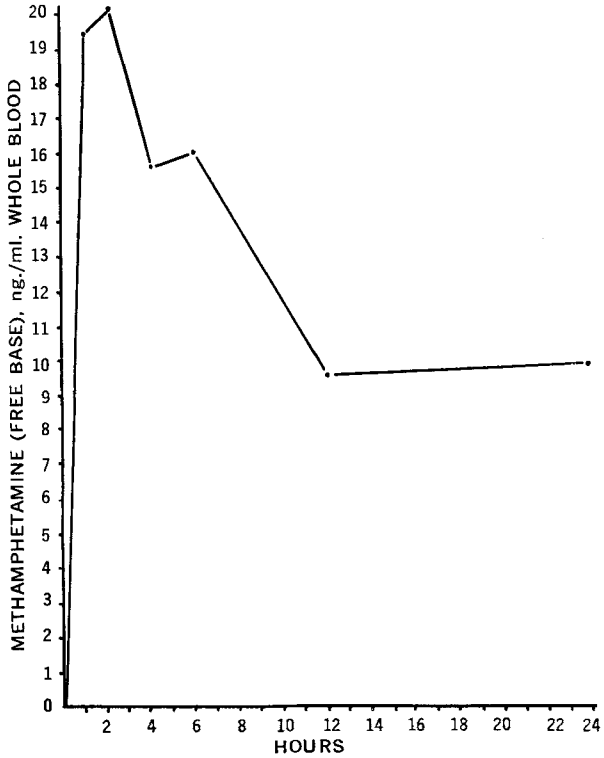

Figure 6—*Average blood level of 10 patients, each of whom received an oral liquid dose of 12.5 mg. methamphetamine hydrochloride.*

and allowed to stand at room temperature for 10 min.; distilled H_2O (1.0 ml.) was added, and the mixture was vigorously shaken. The sample was centrifuged, and the water layer was removed with a Pasteur pipet fitted with a 5-ml. syringe. In a similar manner, the following washings were completed: 10% HCl (1 ml.), 0.7 *N* NaOH (1 ml.), and H_2O (2 × 1 ml.).

The hexane extract was dried (anhydrous Na_2SO_4, about 0.25 g.) and transferred to a test tube (10 ml. graduated in 0.1 ml.). The Na_2SO_4 was rinsed with hexane (2 × 0.5 ml.), and the combined rinsings and extract were evaporated to 0.4 ml. under a stream of nitrogen. Quantities of 3–4 μl. of this solution were injected into a GC fitted with an ECD. The column was glass [1.2 m. (4 ft.) long by 0.63 cm. (0.25 in.) o.d.], packed with a 3% load of OV-1[10] on 80–100 mesh, Gas Chrom Q[10]. Retention time for the methamphetamine derivative was 1.5 min., with column, inlet port, and detector temperatures of 185° and a gas flow (nitrogen) of 105 ml./min. Confirmation of the trichloroacetamide derivative was accomplished by synthesizing a larger quantity under similar conditions and characterization by IR and elemental analysis. Gas chromatograms of the reference standard were identical under similar conditions to those of the submicro samples.

Methamphetamine Trichloroacetamide[11]—To a stirred solution of pyridine (7.91 g., 0.110 mole) and desoxyephedrine (7.46 g., 0.050 mole) in hexane (75 ml.) was added a solution of trichloroacetyl chloride (9.98 g., 0.055 mole) in hexane (25 ml.) over 10 min. A mild exothermic reaction occurred, accompanied by the precipitation of pyridine hydrochloride. The mixture was stirred for 3.5 hr. at room temperature and filtered, and the filtrate was washed with H_2O (1 × 25 ml.), 10% HCl (1 × 25 ml.), 0.7 *N* NaOH (1 × 25 ml.), and H_2O (2 × 25 ml.). The organic layer was dried (anhydrous Na_2SO_4), filtered, and evaporated under vacuum. The clear, yellow residue was distilled to give 12.6 g. (86%) of a colorless oil, b.p. 131–132°/0.09 mm.

Anal.—Calc. for $C_{12}H_{14}Cl_3NO$: C, 49.19; H, 4.91; N, 4.79. Found: C, 48.92; H, 4.79; N, 4.75.

[10] Applied Science Labs.

[11] Analyses were performed by Baron Consulting Co., Orange, CT 06477

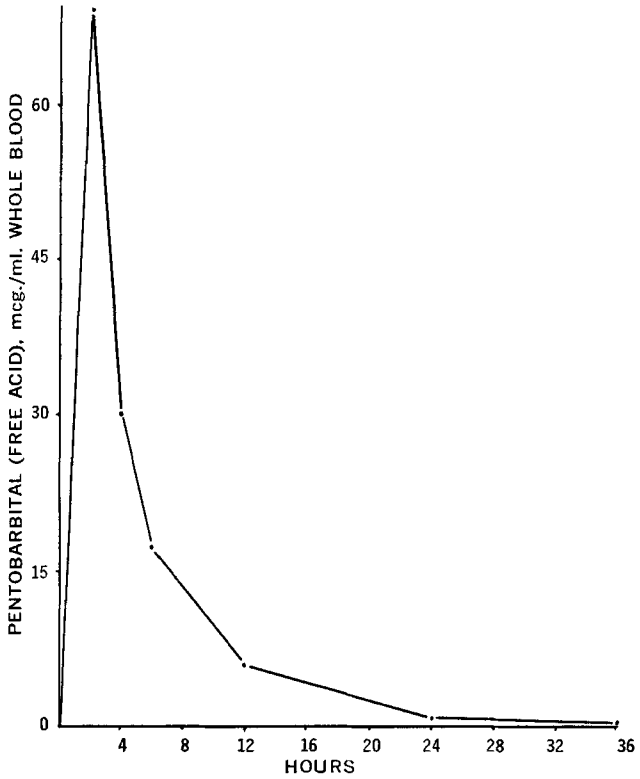

Figure 7—*Average blood level of 10 patients, each of whom received an oral liquid dose of 50 mg. pentobarbital (free acid).*

RESULTS AND DISCUSSION

Recoveries of methamphetamine from samples spiked with methamphetamine hydrochloride are given in Table I. The average percent recoveries for urine and blood, 18 trials each, were 94.0 ± 4.1% (range) and 68.4 ± 4.4%, respectively. Noonan *et al.* (24) reported approximately 50% recovery of amphetamine from both water and plasma based on the trichloroacetamide. Differences in recoveries between the two methods may be due in part to the use of a proton acceptor (pyridine). One might expect lower extraction recoveries from plasma (protein binding) than water. However, amines extracted from plasma could act as proton acceptors and facilitate higher reaction yields. The combination of both factors may explain the similar percent recoveries of amphetamine by Noonan *et al.* (24).

Electron-capture responses to the pure methamphetamine trichloroacetamide (Fig. 1) were linear over a 0–1-ng. range, as were responses from the derivatized urine and plasma extracts. Chromatograms obtained from different derivatives of methamphetamine (Fig. 2) illustrate the trichloroacetamide's greater sensitivity in relation to heptafluorobutyramide and pentafluorobenzamide, with 25 pg. of the trichloroacetamide easily discernible (Fig. 3). Efforts to reconcile these results, which deviate from some existing theories concerning the electron-capturing ability of various derivatives, will not be attempted in this paper. However, preliminary results indicate that electron-capture response is more difficult to predict than was previously thought.

Pentobarbital recoveries (Table II), 93.4 ± 4.8%, were in agreement with Ander's Method B (14), 92.0 ± 2.4%. Biological materials interfering with pentobarbital were not encountered. However, high boiling compounds were extracted, and temperature programming was necessary. Before the initiation of the procedure and after programming, it was essential to saturate the column by injecting relatively high concentrations of pentobarbital until constant peak heights were observed (usually, after programming, two or three injections of 0.5 mcg. were satisfactory).

Quantification of plasma levels for both methamphetamine and pentobarbital were based on peak height determinations. Blank blood samples were spiked with known amounts of each drug and carried through the entire procedure. Standard curves were run at the beginning and end of each day, with the mean values used for calculations. Chromatograms of methamphetamine from blood (Fig. 4) are practically free from extraneous peaks. Those of pentobarbital (Fig. 5) are of lesser quantity but quite acceptable and readily quantitated.

Ten female subjects were used to determine blood levels of both drugs. Each subject received a single oral liquid dose, containing 12.5 mg. methamphetamine hydrochloride and 50 mg. pentobarbital (free acid). Blood samples were taken at 1, 2, 4, 6, 12, and 24 hr. Average blood levels for methamphetamine and pentobarbital are given in Figs. 6 and 7, respectively. Methamphetamine levels were considerably lower than those reported by Bruce and Maynard (23) but in general agreement with Lebish's *et al.* (18) 1-and 2-hr. results[12].

The method described in the article was extremely sensitive for methamphetamine. Shortened work-up, usually accompanied by partial loss of chromatographic definition, can be accomplished by decreasing the number of washings after derivatization to a base and water wash. Failure to obtain consistently clean chromatograms was traceable to impure solvents, reagents, nitrogen (used for evaporating), and non-Teflon-lined caps. This method has been adapted for urinary methamphetamine metabolism and excretion studies and will be presented in a future publication.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 9, 1971, from *Beecham-Massengill Pharmaceuticals, Division of Beecham Inc., Bristol, TN 37620*

Accepted for publication June 18, 1971.

[12] One- and two-hour results were the only levels reported.