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3,4-Methylenedioxyamphetamine (MDA): Analytical and Forensic Aspects of Fatal Poisoning

3,4-Methylenedioxyamphetamine (MDA) (Fig. 1) is a synthetic amphetamine derivative with reported psychotropic properties [*1*]. In Canada, the drug is included in the "Restricted Drugs" schedule of the Federal Food and Drug Act, and, therefore, cannot be used legitimately. In spite of this tight control, MDA, known as the "love pill," has apparently become popular [*2*] in the drug oriented segment of our society. Tragic evidence of its popularity in Ontario was provided by five documented MDA fatalities examined in this laboratory within a 13 month period (1970–71).

Informative but brief mentions were found in the literature pertaining to the analytical toxicology of MDA [*3,4*], and only three references could be located that refer to the investigation of the drug in human subjects. Loman et al [*5*] found that MDA increased rigidity in a patient with Parkinson's disease. The subjective effects of the drug were evaluated and compared with amphetamine [*6*], and in 1967, Naranjo et al [*1*] evaluated MDA as an adjunct to psychotherapy.

The five MDA deaths, and the paucity of relevant information prompted the investigation of the analytical toxicology of MDA. The results of this work, a suggested procedure for the determination of MDA in body materials, and a discussion of the toxicological findings, are presented in this report.

Procedure

Blood (urine, bile, or fluid gastric contents)

1. Make a 10.0 ml of the sample distinctly alkaline with NaOH.
2. Extract twice with 100 ml chloroform. (5 min)
3. Wash combined chloroform layers with 10 ml 0.5*N* NaOH, and follow by a wash with 20 ml water. Filter (paper) the chloroform phase, and note the volume.
4. Extract the chloroform with 5.0 ml 0.1*N* sulfuric acid (5 min). Centrifuge the acidic extract if necessary.
5. Record the ultraviolet spectrum[2] of the clear acidic extract between 340 and 220 nm and measure the absorbance at 285 nm.

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[2] The second characteristic absorption peak of MDA at 234 nm may, particularly at low levels, be in part or altogether masked by coextracted impurities. Additional purification can be achieved by beginning the procedure with a wash of acidified sample with ether, followed by reextraction (steps 1 to 4).

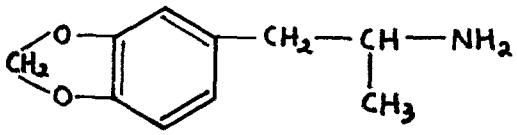

FIG. 1—*3,4,-Methylenedioxyamphetamine (MDA).*

6. Calculate the concentration of MDA from a previously prepared standard curve (concentration range from 0.5 to 2.0 mg MDA/100 ml).
7. Retain the acidic extract for confirmatory tests.

Liver (or other solid organs)

1. Using 100 g of tissue, proceed with the sodium tungstate precipitation method as outlined by Curry [7].
2. Extract the aqueous filtrate with ether. Retain the ether extract if required for analysis of acidic and neutral drugs.
3. Extract the alkalinized (NaOH) filtrate with 1000 ml chloroform (5 min). Filter (paper) the chloroform.
4. Proceed with steps 3, 4, 5, and 6 as outlined under *Blood*, but using suitable fractional volumes of the washing and extracting solutions.
5. Retain the sulfuric acid extract for confirmatory tests.

Confirmatory Tests

Make the sulfuric acid extracts from blood, liver, or other specimens alkaline (NaOH). Extract with a suitable quantity of chloroform and filter. Add 1 drop of concentrated HCl to the chloroform, and evaporate (without the use of heat) under a current of air to just near dryness. Dissolve the residue in a suitable aliquot of ethanol, and proceed as outlined under *Gas Liquid Chromatography* (qualitative or quantitative), and *Thin Layer Chromatography.*

Analysis

Ultraviolet Characteristics

The UV absorption spectrum of MDA hydrochloride is characterized by two absorption peaks of approximately equal heights:

a) 287 and 236 nm (ethanol)
b) 285 and 234 nm (0.1*N* H_2SO_4)
c) 285 and 234 nm (0.5*N* NaOH)

The optical density of a 1.0 mg/100 ml solution of MDA hydrochloride in 0.1*N* H_2SO_4 was 0.14 at 285 nm (Beckman DK-2A). Optical densities in ethanol and 0.5*N* NaOH were not significantly different.

Thin Layer Chromatography (TLC)[3]

Many developing solvents can be used. The solvent chloroform, ethanol, benzene, ammonium hydroxide (80:40:175:5) is satisfactory, avoiding tailing and yielding an Rf

[3] Silica gel GF (0.2 mm thickness), 20 by 20 cm glass plates prepared in the usual manner.

value of approximately 0.5. It can also be used for commonly encountered narcotics, such as morphine, codeine, and meperidine.

Visualization of MDA was first accomplished under a UV light (blue color), followed by a spray of a 1 percent aqueous solution of potassium permanganate. MDA was seen as a bright greenish spot which intensified upon standing for a few minutes. The color fades after about 1 h. 1 μg of MDA was distinctly observed.[4]

Elution of MDA by transferring the spot into 0.1*N* H_2SO_4, followed centrifugation and UV photometry was found to give quantitative recovery.

Gas Liquid Chromatography (GLC)

The conditions for gas chromatographic determination of MDA and its acetyl derivative for two instruments are as follows:

MDA

Instrument One	Varian Model 2100
Column	4 ft glass (OD ¼ in.)
Support	Aeropak 30 (80/100)
Coating	3 percent OV-17[5]
Carrier gas	N_2
Detector	FID
Temperatures	Column (135 C), detector (290 C), sampler (280 C)
Solvent	Ethanol
(Retention time = 10 min, approx.)	
Instrument Two	Beckman GC-4
Column	3 ft aluminum (OD ¼ in.)
Support	Aeropak 30 (80/100)
Coating	5 percent FFAP[6]
Carrier gas	N_2
Detector	FID
Temperatures	Column (160 C), detector (290 C), sampler (280 C)
Solvent	Ethanol
(Retention time = 2 min, approx.)	

Acetyl derivative of MDA[7]
OV-17—Column temperature 195 C (Varian Model 2100)
FFAP—Column temperature 240 C (Beckman GC-4)

Extraction (Isolation)

Recovery—Blood samples containing 1, 2, and 4 mg/100 ml of MDA hydrochloride were carried in duplicate through the procedure suggested for blood. An average of 86.2 percent (from 83–89 percent) of the drug added was recovered. The difference in absorbance at 285 nm was not greater than 0.02 for each duplicate analysis. The use of ether instead of chloroform resulted in a slightly lower recovery.

[4] Amphetamine, methamphetamine, and 4-methyl-2,5-dimethoxyamphetamine (STP) have similar Rf values in the solvent system used. However, with the exception of STP, the other amphetamines do not react significantly with the permanganate spray. MDA and STP can be differentiated by spraying with Marquis reagent [8] which gives a blue color with MDA and a yellow-green color with STP.

[5] The detection limit of MDA is considerably better on the OV-17 phase than on FFAP.

[6] Varian Aerograph Co., Cat. No. 82-001350-00.

[7] A few drops of acetic anydride are added to a chloroform solution of MDA prior to evaporation.

Liver tissue containing 2 mg/100 g of MDA hydrochloride was carried in quadruplet through the procedure suggested for liver. An average of 58.5 percent (from 53–67 percent) of the drug added was recovered.

Aqueous solutions of MDA hydrochloride were made basic and subjected to a routine steam distillation procedure. Pooling and extraction of 3 successive 10 ml portions of the distillate, resulted in a recovery of only 10 percent of the added drug. Pooling and extraction of the distillate collected over 1-h period (about 150 ml) resulted in a recovery of 45 percent of the drug added. The UV spectral characteristics of the steam distilled MDA did not change.

Effect of heating (on evaporation)—100 ml aliquots of a chloroform solution of MDA (approximately 2 mg/100 ml) were allowed to evaporate on a boiling waterbath. Absorption peak heights at 285 nm in ethanol were measured at the time when chloroform was just completely evaporated (0 time), as well as at successive hourly intervals. An average of 48 percent of the added MDA was lost at 0 time, and 80 percent was lost after 3 h of heating. Acetylation of the MDA in chloroform solutions prior to evaporation did not improve the recovery of the drug. Conversion of the drug to its hydrochloride salt in chloroform prior to evaporation improved the recovery of MDA considerably.

Case Histories and Toxicological Findings

The five cases illustrated in Table 1 involved four males and one female ranging in age from 17 to 30 years. Four of these were believed to have experimented with drugs previously. Three were dead on arrival (cases 1, 4, and 5), one died shortly after admission (case 3), and one died about 4 hours after admission (case 2). The symptoms observed or reported included agitation, hallucinations, delirium, and in two cases (2 and 5) convulsions. The drug was believed to have been taken orally in four cases and sniffed in case 2. In each case, the cause of death was officially attributed, directly or indirectly, to poisoning by MDA.

Little information was found in the literature as to the pharmacology and "therapeutic" dose range of MDA, and nothing as to the "therapeutic" concentrations in blood. Studies by Alles et al [*6*] of the subjective effects of MDA with oral doses between 60 and 120 mg, did not reveal, at the lower dose range, mood changes or sleeplessness, characteristic of even smaller doses of amphetamine. At the higher dose range there were observed distinct

TABLE 1—*Toxicological findings in five MDA deaths.*

Case No.	Sex	Age	Weight (kg)	Concentration in Biological Specimens: Blood mg/100 ml	Urine mg/100 ml	Bile mg/100 ml	Liver mg/100 g	Stomach (total content in mg)	Other Findings[a]
1	F	17	42	1.6	NA[b]	0.5	1.1	0.7	None
2	M	19	59	1.2	16	NA	NA	negative	Blood alcohol: negative Urine alcohol: 60 mg/100 ml
3	M	21	59	2.6	NA	NA	positive	positive	0.9 mg butabarbital per 100 ml blood
4	M	23	61	1.2	10	0.9	0.8	positive	Traces of amphetamine in urine, bile, and liver.
5	M	30	59	0.6	4.6	0.5	1.7	positive	None

[a] General screening examination of stomach contents and liver tissue. Specific examinations of blood for at least alcohol and barbiturates.

[b] Not available.

visual and related sensory changes, but no hallucinogenic effects. Naranjo et al [*1*] gave oral doses of 150 mg of MDA hydrochloride in a series of trials with eight volunteers and described subjective effects such as "intensification of feelings, a facilitation of self insight and a heightened empathy or aesthetic enjoyment at some point during the intoxication." These effects were noted between 40 and 60 min after ingestion, and lasted for approximately 8 h. Hallucinations or perceptual distortions were not reported by the subjects. Jackson and Reed [*2*] in their report of the effects of the drug as related to them by several adolescent patients, noted that the only visible change described was widely dilated pupils. Subjectively, the patients described effects such as "a mild sense of physical well being, some increase in taste sensation, a feeling of a decreased awareness of bodily sensations, and an almost overwhelming desire or need to be with and talk to other people." One of their patients reported several short visual hallucinations. The dose range of MDA was not given in this report

No references could be found on the human toxicity of MDA. The toxic symptoms noted in the cases described in Table 1, were predominantly suggestive of an excessive C.N.S. stimulation. The doses administered are not known. In case 3, however, the victim allegedly took about ¼ oz of MDA and a handful of butabarbital tablets. Because of the absence of any other cause of death, the blood and tissue concentrations of MDA given in Table 1 are believed to be indicative of fatal MDA poisoning. Highest concentrations were found in urine, followed by blood or liver tissue.

Summary

Some analytical aspects of MDA pertaining to routine toxicological analysis, have been investigated, and the results presented. A routine procedure has been suggested for analysis of MDA in body materials in cases of suspected overdose. Results of recovery and reproducibility studies have been given. Case histories and toxicological findings of five documented MDA fatailites have been given and discussed.

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