Journal of Analytical Toxicology, Vol. 6, May/June 1982

Differentiation of Amphetamine and Its Major Hallucinogenic Derivatives Using Thin-Layer Chromatography

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Abstract

Psychotropic, ring-substituted amphetamine derivatives can be differentiated from each other and from over-the-counter drugs using a sequential TLC detection technique. The improved detection is accomplished by distinct differences in color through four detection stages. Reported in the tables are R_f values in two solvent systems, the color characteristics through the four detection stages and in two confirmatory reagents, and the minimum detectible concentrations in urine of 19 amphetamine derivatives.

Introduction

Due to their psychotropic properties, various ring-substituted amphetamine derivatives have gained popularity as recreational drugs (1-3). Included in a list of illicitly marketed compounds compiled by Taylor *et al.* (4) are several of these psychotropic amphetamines, among them 2,5-dimethoxy-4-methylamphetamine (DOM or *STP*) (2,5), 3,4,5-trimethoxyamphetamine (TMA) (6), 3-methoxy-4,5-methylenedioxyamphetamine (MMDA) (7), and 3,4-methylenedioxyamphetamine (MDA) (1). Ratcliff indicated the occurrence of 4-methoxyamphetamine and 2,5-dimethoxyamphetamine in street drugs, alleged to be MDA (8). Other amphetamine derivatives appearing recently in street drugs are N-methyl-3,4-methylenedioxyamphetamine (MDM) (9,10) and N-ethyl-3,4-methylenedioxyamphetamine (MDE or *XTC*) (9,10,11).

Repeated mentions of this class of drugs in the literature indicate a need for a reliable differentiation technique to assist both clinicians and toxicologists.

Several workers have detected amphetamine derivatives by thin-layer chromatography (TLC); however, due to limitations of the methods used, results were considered presumptive until confirmed by other techniques (5,13-16). Usually TLC analysis of these substances is accompanied by gas chromatography (GC), and various researchers have reported methods to facilitate and improve the GC detection (13,15-18). Even with these improvements, GC is complicated, time-consuming, and expensive, requiring highly trained personnel to interpret the results and maintain proper instrument function. Alternative procedures such as immunoassay techniques do not provide a single, quick screen for many specific drugs, but instead, usually identify certain drug categories.

We report here an improved TLC technique for differentiating several amphetamines, which, by virtue of the detection methods used, greatly reduces the need for confirmation. When confirmation is necessary, the number of possible interfering substances is minimal. Because of the five-parameter specificity (color reactions with four reagents and R_f) the method of thin-layer chromatography for amphetamines has advantages over other analytical techniques in vogue.

Experimental

Applied Sciences (State College, Pennsylvania) supplied 4-methoxyamphetamine, 2,5-dimethoxyamphetamine, TMA, 2,4,5-trimethoxyamphetamine, 2,4,6-trimethoxyamphetamine, DOM, 2,5-dimethoxy-4-ethylamphetamine (DOE), and MMDA. Methylenedioxyamphetamine was supplied by U.S. Pharmacopeial Convention, Inc. (Rockville, Maryland). Mescaline (3,4,5-trimethoxyphenethylamine) was obtained from Sigma Chemical Co. (St. Louis, Missouri). Except for MDM and MDE, which were synthesized, all other drugs were purchased from the manufacturers.

Methanol and ethyl acetate were spectrophotometric quality. Other chemicals were reagent grade.

Mandelin's reagent was prepared by heating 200 mg of ammonium metavanadate with 250 mL of concentrated sulfuric acid until solution of the salt was complete.

To prepare modified Dragendorff's reagent (iodinated), 200 mg of bismuth subnitrate, 5 mL of water and 10 mL of glacial acetic acid were heated until the bismuth subnitrate dissolved (Solution A). In a separate container, 5 g of potassium iodide and 2 g of iodine were dissolved in 100 mL of distilled water (Solution B). Solution A was added to Solution B and the combination was diluted with water to 250 mL.

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Gallic acid and chromotropic acid reagents were prepared by heating 2.5 g gallic acid or 0.5 g chromotropic acid in 250 mL of concentrated sulfuric acid until solution was complete.

Syntheses for MDM and MDE have been included due to the difficulty in obtaining these standards from commercial sources.

The N-methyl derivative of MDA (MDM) was prepared by reacting 5 mg of free base MDA with 0.5 mL of methyl iodide. The solution was boiled for 10 minutes and allowed to cool to room temperature. The N-ethyl derivative (MDE) was prepared similarly using ethyl iodide (19). The products are the secondary amines (MDM or MDE), the tertiary amines (N,N-dimethyl-MDA or N,N-diethyl-MDA), and the quaternary ammonium salts. The amines were isolated from the salts by liquid/liquid extraction at pH 9.0 (Toxi-Tubes® , Analytical Systems, Laguna Hills, California), followed by separation on the TLC systems described below. The identities of MDM and MDE from these syntheses were verified by comparing their color reactions and R_f values with those of known standards (20).

Methanolic solutions of all drugs (1 μg/mL) were used to spike urine (drug free, except for caffeine). Aliquots of spiked urine were extracted at pH 9.0; 5 mL of urine were added to the liquid/liquid extraction tubes, which contain a mixture of 2.5 mL of organic solvents (methylene chloride and dichloroethane), pre-measured, buffered salts and a phase marking dye. The tubes were mixed by inversion for 1 minute, and then centrifuged. The organic extracts were removed and concentrated by heat and evaporation, onto small discs (3.5 mm) of glass microfiber media impregnated with silica gel (21). TLC plates (Toxi-Grams® , Analytical Systems) made of the same silica gel-impregnated material were inoculated by inserting the dried sample disc into one of the two center holes located near the lower edge of the chromatograms. Two solvent systems were used for separation: System I, ethyl acetate:methanol:water:NH_4OH (95:3.5:1.5 by volume plus 7.5 μL of concentrated ammonium hydroxide per mL of solvent); System II, acetone plus 5 μL of concentrated ammonium hydroxide per milliliter of solvent. Chromatograms were developed for 10 cm. The solvent was removed from the chromatograms by heating them on a hotplate at 70°C for about 30 seconds.

The main detection system for broad screening consisted of dipping the chromatograms into several reagents sequentially, and observing the results at four stages (21). At each stage, the chromatograms were removed from the reagents and viewed

Table I. R_f Values and Detection Characteristics of Amphetamine and the Major Substituted Derivatives

Drug	R_f I*	R_f II**	Detection Characteristics*** Stage I	Stage II	Stage III	Stage IV
Amphetamine	.37	.70	yellow→brown	pale olive	blue	brown
2,5-dimethoxyamphetamine (DMA)	.32	.69	yellowish green	bright green→ orange-yellow	deep orange	brown
2,5-dimethoxy-4-ethyl-amphetamine† (DOE)	.32	.65	greenish yellow	yellow	dull blue	brown
2,5-dimethoxy-4-methyl-amphetamine† (DOM, STP)	.32	.65	greenish yellow	yellow	dull blue	brown
N-ethyl-3,4-methylene-dioxyamphetamine (MDE)	.37	.35	blue-green	light olive	neg.	brown
Methamphetamine	.23	.20	yellow→brown	pale olive	blue	brown
4-methoxyamphetamine	.35	.68	blue-purple	fades	dull	brown
2-methoxy-4,5-methylene-dioxyamphetamine† (MMDA)	.32	.65	greenish yellow	greenish yellow	faint or neg.	brown
N-methyl-3,4-methylene-dioxyamphetamine (MDM)	.23	.20	blue-green	light olive	neg.	brown
3,4-methylenedioxy-amphetamine (MDA)	.37	.70	blue-green	grey-tan	bright blue	brown
Phendimetrazine‡	.66	.65	neg., slow yellow or yellow→green	neg., or pale green	neg., or blue	strong brown
Phenmetrazine‡	.39	.50	neg., slow yellow or yellow→green	neg., or pale green	neg., or blue	strong brown
Phentermine	.38	.40	yellow→brown	pale olive	blue	brown
Phenylpropanolamine (PPA)	.32	.90	yellow→green	pale green	blue	brown
Pseudoephedrine	.15	1.0	yellow→green	pale green	blue	brown
2,4,5-trimethoxyamphetamine	.20	.65	yellow	fades	neg.	brown
2,4,6-trimethoxyamphetamine	.20	.65	rose	pink-tan	faint or neg.	brown
3,4,5-trimethoxy-amphetamine (TMA)	.20	.65	yellow	fades	dull blue	brown
3,4,5-trimethoxyphen-ethylamine (mescaline)	.13	.60	orange	fades	dull green	brown

*System I, ethyl acetate:methanol:water (95:3.5:1.5) plus 7.5 μL of concentrated ammonium hydroxide per mL of solvent.

**System II, acetone plus 5 μL of concentrated ammonium hydroxide per mL of solvent.

***Please refer to text for description of Stages I-IV.

†No differentiation in the main detection system. Chromotropic acid or gallic acid reagents will differentiate MMDA from DOM and DOE.

‡Characteristics at Stages I-III are dependent on concentration and formaldehyde exposure.

while still wet. Following a two-minute exposure to formaldehyde vapors, the chromatograms were dipped slowly into Mandelin's reagent. The chromatograms were then viewed after about 20 seconds to one minute (Stage I). Next they were dipped quickly into and out of water (Stage II). At Stage II, the colors appeared immediately for some drugs; others appear within 10 seconds. The chromatograms were viewed subsequently under long wave (366 nm) ultraviolet light (Stage III). At this stage many of the drugs showed an immediate absorbance or fluorescence. At Stage IV, the chromatograms were dipped into modified Dragendorff's reagent. Various shades of brown were visible immediately.

For confirming the presence of a methylenedioxy group, we used either gallic acid or chromotropic acid reagents (22,23). Chromatograms were dipped into one or the other reagent, then were dipped once into water. A positive test is the immediate formation of a bright green chromophore with gallic acid, or a pale lavender chromophore with chromotropic acid.

Human urine specimens were analyzed for some amines: amphetamine, methamphetamine, phentermine, phenylpropanolamine, MDA, phenmetrazine and ephedrine. Urine specimens containing the more exotic amphetamine derivatives were unavailable for testing. In place of these, we tested spiked urine. At least five spiked specimens at each amphetamine concentration (0.5, 1.0, 1.5, 2.0 μg/mL) were extracted in order to determine the detection limits. The color patterns at the minimum detectible concentrations were then evaluated.

Results and Discussion

The characteristics of the amphetamines detected are listed in Table I, as are the R_f values with Systems I and II. Table II indicates the detection limits of each drug, which was defined as the lowest concentration of drug in urine that would give characteristic reactions in all stages of detection. Exceptions are stated in the table. Very little problem was encountered due to interference from other drugs. Table III indicates the color characteristics and R_f values in System I of some other drugs that are commonly used or abused.

The colors observed for amphetamine and the major ring-substituted derivatives, 4-methoxyamphetamine, MDA, DMA and TMA, were strikingly different. There was even a degree of differentiation among isomers of TMA. The N-methyl and N-ethyl derivatives of MDA (MDM and MDE) were distinguished from each other and from MDA by R_f in each solvent system, and by their lack of fluorescence in Stage III.

Derivatives that differ from amphetamine only in side-chain modifications (Figure 1), migrate to different positions in each solvent system. This distinction was reinforced by nuances of color in Stages I and II.

Though the distinction between MMDA, DOM and DOE is poor with the main detection system, MMDA can be differentiated from the others using gallic or chromotropic acid reagents. Both reagents form chromophores with formaldehyde, which is liberated when the methylenedioxy group decomposes in the sulfuric acid present in the two reagents (23). Water then causes a vivid color development, because the heat of solution of

Table II. Detection Limits in Urine of Amphetamine and the Major Substituted Derivatives

Drug	Detection Limit, μg/mL		
	Stages I-IV	Gallic Acid	Chromotropic Acid
Amphetamine	0.5	—	—
2,5-dimethoxyamphetamine	1.0	—	—
2,5-dimethoxy-4-ethylamphetamine	2.0	—	—
2,5-dimethoxy-4-methylamphetamine	2.0	—	—
Methamphetamine	0.5	—	—
4-methoxyamphetamine	0.5	—	—
Phendimetrazine	1.0*	—	—
Phenmetrazine	1.0*	—	—
Phentermine	3.0	—	—
Phenylpropanolamine	3.0	—	—
Pseudoephedrine	3.0	—	—
2,4,5-trimethoxyamphetamine	2.0	—	—
2,4,6-trimethoxyamphetamine	1.0	—	—
3,4,5-trimethoxyamphetamine	1.0	—	—
3,4,5-trimethoxyphenethylamine	1.0	—	—
N-ethyl-3,4-methylenedioxyamphetamine	1.5	0.25	0.25
2-methoxy-4,5-methylenedioxy-amphetamine	1.5	0.25	0.25
3,4-methylenedioxyamphetamine	1.0	0.25	0.25
N-methyl-3,4-methylenedioxy-amphetamine	1.5	0.25	0.25

* Stage IV only. 15 μg/mL is necessary to detect at Stage I.

Table III. R_f Values and Detection Characteristics of Other Commonly Used Drugs

Drug	R_f I*	Detection Characteristics**			
		Stage I	Stage II	Stage III	Stage IV
Acetaminophen	.73	blanch	honey	neg., or dull red	brown
Caffeine	.60	neg.	neg.	neg.	slate grey
Cocaine	.83	neg.	neg.	neg.	rose-brown
Codeine	.22	dark blue	blue→straw	absorbs or green	brown
Imipramine	.50	neg.	bright blue	blue-green	brown
Meprobamate	.82	neg.	neg.	neg.	yellow-tan
Methadone	.67	blue	fades	neg. or faint green	brown
Methaqualone	.89	neg.	neg.	neg.	rose-brown
Morphine	.13	grey-purple	straw	absorbs	brown
Nicotine	.43	neg.	neg.	neg.	rose-brown

* System I, ethyl acetate:methanol:water (95:3.5:1.5) plus 7.5 μL of concentrated ammonium hydroxide per mL of solvent.
** Please refer to text for a description of Stages I-IV.

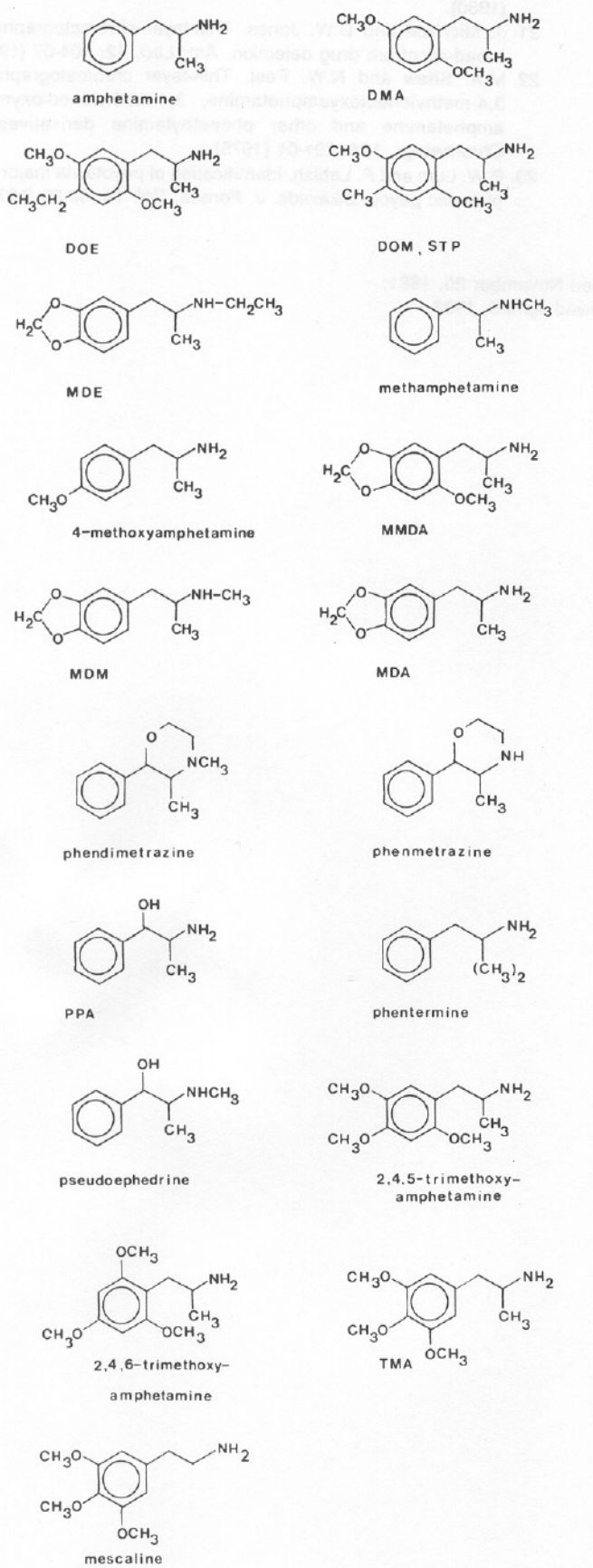

Figure 1. Structures of amphetamine and the major substituted derivatives.

sulfuric acid in water encourages further decomposition of the methylenedioxy group.

Thin-layer chromatography is a convenient screening method, but has not previously lent itself to more than presumptive identification of most of these substances. The unique method discussed here improves the level of confidence in differentiation of amphetamine derivatives; the improvement rests on distinct differences in color characteristics, which increase the specificity of the method and decrease the need for numerous solvent systems.

Acknowledgement

We thank Dr. Alexander T. Shulgin for gifts of MDM and MDE.

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Manuscript received November 25, 1981;
revision received April 9, 1982.