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Note

Thin-layer chromatography of 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine and other phenethylamine derivatives

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Many phenethylamine derivatives (amphetamine drugs) are used medicinally for therapeutic treatment. Illicit manufacture and use of many of these derivatives have increased greatly in recent years. The relative ease with which chemical groups can be manipulated on the basic phenethylamine nucleus (moiety) has resulted in the synthesis and use of many new hallucinogenic substances, including such drugs as 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MMDA). Substances such as these are a problem to the forensic analyst who must have the capability to screen for them. There are often not sufficient differences of physico-chemical properties to readily distinguish popular phenethylamine drugs by chromatographic means.

The following study was carried out to develop a method that could be used to screen for MDA and MMDA in thin-layer systems already in use in our laboratory. As with other current methods using thin-layer chromatography (TLC) as a screening technique, a sequential spraying pattern is carried out[1,2]. Indeed, the appearance of a particular color with a specific spray reagent, in conjunction with an accurate R_F value, can often serve to distinguish a particular substance from other chromatographically similar compounds. The use of a gallic acid spray, adapted from a procedure reported by DeMayo *et al.*[3], served to distinguish MDA from MMDA. The application of the fluorogenic reagent fluorescamine is described for the general detection of phenethylamine substances.

EXPERIMENTAL

Apparatus

The chromatography tanks used were glass, generally of the size 23 × 12 × 23 cm, with glass tops sealed with starch glycerin paste. The tanks were lined with filter paper. Analtech silica gel G plates (250 μm) were activated for 20 min at 115° before use (available from Mandel Scientific Co., Montreal, Canada).

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Reagents

Developing solvents. (I) Methanol–ammonia (100:1.5); (II) Benzene (distilled)–dioxane (distilled)–ethanol–ammonia (150:120:15:15); (III) Chloroform (distilled)–cyclohexane (distilled)–diethylamine (50:40:10).

Fluorescamine spray. Fluorescamine (Fluram ®, Roche Diagnostics, Vaudreuil, Canada), 5 mg/100 ml in acetone.

Ninhydrin spray. Ninhydrin 0.1 % (w/v) in acetone is prepared fresh.

Gallic acid spray. Gallic acid 1.0 % in sulphuric acid–ethanol (1 :1). Store in the dark.

Potassium iodoplatinate (acidified) spray. Platinic chloride (0.25 g) and potassium iodide (5 g) are mixed with water to produce 100 ml. Hydrochloric acid (2 ml) is added to this solution.

Drug standards. Solutions of either the free base or salt were prepared in a known concentration of approximately 5 mg/ml in ethanol or methanol.

Procedure

Approximately 50 μg of each drug were spotted and run. The running distance of each plate was 15 cm. Codeine was run on each plate as an internal standard. A reference mixture of amphetamine, benzphetamine and mescaline was included on most of the plates to monitor the tank condition. The plates obtained from using solvent III were sprayed with a 5 % ethanolic solution of hydrochloric acid prior to commencing the sequential spraying pattern to remove the effects of the residual diethylamine. After each run, the plate was air dried, sprayed with fluorescamine spray and viewed under ultraviolet light. All developed spots were noted. The plate was then oversprayed with ninhydrin spray, warmed at 70° for approximately 5 min and observed for any spot development. The plate was again oversprayed with gallic acid spray, warmed again for 10 min at 70° and observed. The plate was finally oversprayed with acidified potassium iodoplatinate spray for general development. After each spray sequence, the running distances of the spots were measured and the R_F and $R_{codeine}$ (R_F relative to codeine) values calculated. The values obtained for the phenethylamine substances and some other hallucinogens are given in Table I.

The sensitivity of detection of some of the drugs with ninhydrin spray, fluorescamine spray and gallic acid spray was determined by diluting the appropriate stock solutions and running the drug on the thin-layer plate in developing solvent I or II. These results are given in Table II.

The data in Table I show that it is often difficult to resolve MDA and MMDA using only R_F determinations. The inclusion of gallic acid spray in a sequential spraying pattern readily distinguishes these two compounds from each other with very good sensitivity. MDA appears as a green spot and MMDA appears as a blue spot. The other phenethylamine derivatives did not develop any interferences with this reagent.

A number of other TLC solvent systems had previously been investigated, but were discarded for reasons of poor resolution, poor sensitivity and difficult handling procedures. The three solvent systems used in this study are useful for many other drugs/poisons and generally give good reproducibility. A second spray reagent for MDA and MMDA was also considered —chromotropic acid, 1.0 % in sulphuric acid–ethanol (1 :1). A similar chromotropic reagent has been previously investigated by

TABLE I

TLC DATA

Substance	*Developing solvent*								
	I			*II*			*III*		
	R_F	$R_{codeine}$	$S.D._{codeine}$*	R_F	$R_{codeine}$	$S.D._{codeine}$*	R_F	$R_{codeine}$	$S.D._{codeine}$*
MDA	0.50	1.01	0.04	0.43	1.20	0.05	0.42	1.8	0.1
MMDA	0.55	0.98	0.04	0.41	1.10	0.05	0.40	1.7	0.1
Mescaline	0.46	0.81	0.02	0.19	0.57	0.05	0.26	1.10	0.09
Amphetamine	0.61	1.08	0.01	0.46	1.27	0.04	0.41	1.8	0.2
Methamphetamine	0.56	0.96	0.01	0.47	1.22	0.08	0.46	2.0	0.1
Phenethylamine	0.53	0.96	0.07	0.37	0.96	0.01	0.04	0.18	0.05
Ephedrine	0.48	0.85	0.04	0.26	0.71	0.05	0.13	0.60	0.04
Phenylephrine	0.46	1.81	0.03	0.03	0.09	0.02	0.05	0.20	0.03
Benzphetamine	0.78	1.37	0.03	0.76	2.3	0.3	0.71	3.0	0.2
Phenmetrazine	0.60	1.06	0.07	0.50	1.40	0.07	0.38	1.7	0.2
Phendimetrazine	0.02	0.02	0.01	0.01	0.04	0.01	0.18	0.9	0.2
Chlorphentermine	0.62	1.10	0.05	0.51	1.42	0.09	0.41	1.8	0.2
Dimethyltryptamine	0.59	1.04	0.01	0.48	1.34	0.04	0.21	0.89	0.04
Diethyltryptamine	0.65	1.15	0.03	0.61	1.70	0.08	0.31	1.33	0.03
PCP	0.71	1.27	0.05	0.82	2.30	0.20	0.72	3.1	0.3
LBJ	0.76	1.33	0.07	0.70	2.0	0.20	0.52	2.2	0.2
Codeine	0.57	1.00	—	0.36	1.00	—	0.24	1.00	—

* $S.D._{codeine}$ is the standard deviation of $R_{codeine}$.

DeMayo *et al.*[3]. The colors obtained with MDA and MMDA were pink and purple, respectively, and somewhat easier to differentiate than the gallic acid spray. However, the gallic acid spray was found to be more sensitive.

RESULTS AND DISCUSSION

The R_F values (and subsequent $R_{codeine}$ values) were obtained by measuring the running distances obtained from three separate tanks of the same solvent system, usually from three separate plates within that tank. Thus, most of the values recorded

TABLE II

DETECTION LIMITS (μg) OF SRAYS

Substance	*Fluorescamine*	*Ninhydrin*	*Gallic acid*
MDA	0.05	20	1
MMDA	0.05	10	1
Mescaline	0.05	5	
Amphetamine	0.05	20	
Methamphetamine	0.5	20	
Phenethylamine	0.05	5	
Ephedrine	3.0	10	
Phenylephrine	0.1	5	

in Table I are the result of nine values. In addition, similar data have been obtained separately in another laboratory.

The application of fluorescamine as a spray was investigated and found to be very useful. After spraying, the plate is viewed under ultraviolet light. Generally the phenethylamines are visible as green fluorescing spots or as dark-blue absorbing spots. The intensity of the latter may be increased by exposing the plate to a vapor of ammonia prior to spraying. Table II shows the detection limits obtained by the fluorescamine spray on some of the drugs.

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