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Note

Rapid detection of some basic drugs by thin-layer chromatography

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Rapid detection of basic drugs in illicit preparations may be limited by similarities in their spectral properties. Ultraviolet (UV) spectrophotometry is the most widely available screening method for illicit drugs but lacks specificity. A large number of basic drugs, notably phenethylamine derivatives show an UV absorption at approximately 257 nm with low absorptivities (low ε values). Thin-layer chromatography (TLC) of basic drugs allows a higher degree of specificity with increased detection sensitivity. Recently various workers have shown the advantages of derivative formation in TLC of phenethylamine derivatives[1–4]. The use of NBD-Cl (4-chloro-7-nitrobenzo-2,1,3-oxadiazole) has permitted the analyst to screen samples with increased speed and specificity[4]. Structures of reaction products have been elucidated by mass spectrometry[5].

This study was undertaken to provide additional TLC data in reported solvent systems[3,4] for phenethylamines and other basic drugs that exhibit a characteristic absorption at 257 nm in the UV region.

EXPERIMENTAL

Apparatus

The chromatography tanks used in this study were glass with approximate dimensions 23 × 12 × 23 cm. The tank tops were sealed with a starch–glycerin paste[6]; the tanks were lined with Whatman No. 3 chromatography paper. The developing tanks were allowed to equilibrate for 30 min after solvent introduction. Analtech prescored silica gel G (250 μm) plates (Mandel Scientific, Montreal, Canada) were used; the plates were not activated prior to use. The relative humidity throughout data collection was 23–29%.

Reagents

The following developing solvents were used: (I) ethyl acetate–cyclohexane (2:3); (II) ethyl acetate–cyclohexane (3:2); and (III) diethyl ether–benzene (1:1).

Derivative formation was accomplished with 0.1 *M* sodium bicarbonate solution and 1% (w/v) NBD-Cl (Aldrich, Milwaukee, Wisc., U.S.A.) in methyl isobutyl ketone.

Drug standards were prepared as follows: Solutions of 10 mg/ml of the salt of each drug were prepared in absolute ethanol or distilled water (depending upon the solubility of each drug).

TABLE I

TLC DATA OF NBD-Cl DERIVATIVES IN ETHYL ACETATE–CYCLOHEXANE (2:3)

Substance	R_F*	R_x**	R_x***	R_x§	*Visible colour*§§§
Alphaprodine	45	0.96	4.10	1.50	—
Amphetamine	47	1.00	4.60	1.69	Y
Anileridine	6	0.13	0.55	0.20	Or
Atropine	7	0.15	0.64	0.23	—
Benzphetamine	16	0.34	1.45	0.53	—
Chlorpheniramine	11	0.23	1.00	0.37	Y
Chlorphentermine	43	0.91	3.91	1.43	Y
3,4-Dimethoxy-amphetamine	10,21§§	0.21,0.45§§	0.91,1.91§§	0.33,0.70§§	Or,Y§§
Diphenhydramine	34	0.72	3.09	1.13	Y
Ephedrine	18	0.38	1.64	0.60	Y
Ethoheptazine	31	0.66	2.82	1.03	Y
N-Ethyl-3-piperidyl benzilate	12	0.26	1.09	0.40	—
Fenfluramine	30	0.64	2.72	1.00	Y
p-Hydroxyephedrine	4,9,3§§	0.30,0.19,0.06§§	1.27,0.82,0.27§§	0.47,0.30,0.09§§	Y,Y,Y§§
Hyoscyamine	7	0.15	0.64	0.23	—
3,4-Methylenedioxy-amphetamine	43	0.91	3.90	1.43	Y
Mescaline	16	0.34	1.45	0.53	Y
N-Methyl-phenethyl-amine	26	0.55	2.36	0.87	Y-Or
N-Methyl-3-piperidyl benzilate	11	0.23	1.00	0.37	—
Methylphenidate	37	0.79	3.36	1.23	—
Methamphetamine	30	0.66	2.82	1.03	Y-Or
3-Methoxy-4,5-methylenedioxy-amphetamine	34	0.72	3.09	1.13	Y
p-Methoxyamphet-amine	42	0.89	3.82	1.40	Y
Phenethylamine	45	0.96	4.09	1.50	Y
Phenmetrazine	31	0.66	2.64	1.03	Y
Phenylephrine	7,4§§	0.15,0.09§§	0.64,0.36§§	0.23,0.13§§	Y,Y§§
Phenylpropanol-amine	33,38§§	0.70,0.81§§	3.0,3.5§§	1.1,1.2§§	(F),Y§§
Piminodine	11	0.23	1.60	0.37	Y-Or
Piperidine	29	0.62	2.64	0.96	Or-Rd
Procyclidine	16	0.34	1.45	0.53	Or
Propoxyphene	11	0.23	1.00	0.37	Y
Pseudoephedrine	14	0.30	1.27	0.47	Y
2,5-Dimethoxy-4-methylamphet-amine	46	0.98	4.18	1.53	Or

* $R_F \times 100$ (average of 3 runs).
** R_x relative to amphetamine (standard).
*** R_x relative to chlorpheniramine (standard).
§ R_x relative to methamphetamine (standard).
§§ Multiple reaction products.
§§§ All spots fluorescent (yellow; 254 nm).

TABLE II

TLC DATA OF NBD-Cl DERIVATIVES IN ETHYL ACETATE–CYCLOHEXANE (3:2)

Substance	R_F*	R_x**	R_x***	R_x§	*Visible colour*§§§
Alphaprodine	60	0.88	2.35	1.16	—
Amphetamine	63	1.00	2.84	1.33	Y
Anileridine	19	0.29	0.84	0.39	Or
Atropine	25	0.39	1.10	0.51	—
Benzphetamine	36	0.54	1.47	0.71	—
Chlorpheniramine	23	0.35	1.00	0.47	Y
Chlorphentermine	59	0.95	2.77	1.27	Y
3,4-Dimethoxyamphetamine	24	0.37	1.05	0.49	Or/Y
Diphenhydramine	53	0.83	2.33	1.09	Y
Ephedrine	33	0.53	1.50	0.71	Y
Ethoheptazine	52	0.80	2.22	1.08	Y
N-Ethyl-3-piperidyl benzilate	28	0.41	0.98	0.54	—
Fenfluramine	50	0.78	2.20	1.03	Y
p-Hydroxyephedrine	30,25,12§§	0.47,0.39,0.19§§	1.34,1.1,0.54§§	0.62,0.51,0.25§§	Y,Y,Y§§
Hyoscyamine	26	0.40	1.13	0.53	—
3,4-Methylenedioxyamphetamine	58	0.91	2.59	1.20	Y
Mescaline	37	0.58	1.64	0.77	Y
N-Methyl-phenethylamine	42	0.66	1.71	0.88	Y-Or
N-Methyl-3-piperidyl benzilate	26	0.41	0.98	0.54	—
Methylphenidate	55	0.85	2.40	1.12	—
Methamphetamine	49	0.76	2.15	1.00	Y-Or
3-Methoxy-4,5-methylenedioxyamphetamine	53	0.82	2.33	1.08	Y
p-Methoxyamphetamine	57	0.89	2.53	1.17	Y
Phenethylamine	61	0.95	2.71	1.25	Y
Phenmetrazine	51	0.79	2.24	1.05	Y
Phenylephrine	23,16§§	0.35,0.25§§	1.05,0.75§§	0.47,0.34§§	Y Y§§
Phenylpropanolamine	53	0.82	2.33	1.09	—,Y
Piminodine	25	0.39	1.10	0.51	Y-Or
Piperidine	43	0.66	1.71	0.88	Or-Rd
Procyclidine	31	0.49	1.21	0.64	Or
Propoxyphene	22	0.35	1.01	0.46	Y
Pseudoephedrine	32	0.49	1.40	0.65	Y
2,5-Dimethoxy-4-methylamphetamine	60	0.93	2.65	1.23	Or

* $R_F \times 100$ (average of 3 runs).
** R_x relative to amphetamine (standard).
*** R_x relative to chlorpheniramine (standard).
§ R_x relative to methamphetamine (standard).
§§ Multiple reaction products.
§§§ All spots fluorescent (yellow; 254 nm).

Procedure

Approximately 100 μg of each drug were added to a 10 × 75 mm test-tube, followed by 0.2 ml of the sodium bicarbonate solution. Each tube was shaken and 0.2 ml of the NBD-Cl solution were added. The tubes were stoppered and placed in an oven for 30 min at 80°. Aliquots of 5 μl of the upper (methyl isobutyl ketone) layer were spotted and each TLC plate was eluted to a distance of 15 cm. Spots were observed under visible light and UV light (254 nm). Each R_F value was recorded.

The R_X values (relative to a standard mixture, 10 mg/ml of chlorpheniramine, methamphetamine, and amphetamine) were then calculated. The standard mixture allowed monitoring chromatographic conditions and the collection of R_X values.

RESULTS AND DISCUSSION

TLC data for some basic drugs with UV maxima at approximately 257 nm are tabulated in Tables I–III. Standard deviations in all R_F values are less than 0.05.

TABLE III

TLC DATA OF NBD-Cl DERIVATIVES IN DIETHYL ETHER–BENZENE (1:1)

Compound	R_F*	*Visible colour*§
Amphetamine	69	Y
Anileridine	18	Or**
Atropine	15	—
Benzphetamine	27	—
Chlorpheniramine	36	Or
Diphenhydramine	63	Or
Ephedrine	42	Or
N-Ethyl-3-piperidyl benzilate	26	—
Fenfluramine	57	Or-Rd
p-Hydroxyephedrine	13,28,36***	Or,Or,Y***
3,4-Methylenedioxyamphetamine	63	Or
Mescaline	37	Y
N-Methyl-phenethylamine	52	Or
N-Methyl-3-piperidyl benzilate	27	—
Methylphenidate	64	—
Methamphetamine	59	Or
3-Methoxy-4,5-methylenedioxyamphetamine	57	Or
p-Methoxyamphetamine	63	Y
Phenethylamine	66	Y
Phenmetrazine	57	Or
Phenylephrine	17,23***	Or,Or***
Phenylpropanolamine	49	Y
Piperidine	55	Or-Rd
Procyclidine	62	Rd
Propoxyphene	35	Y
Pseudoephedrine	35	Or
2,5-Dimethoxy-4-methylamphetamine	61	Or

* R_F × 100 (average of 3 runs; 1 run per freshly prepared tank).

** Streak.

*** Multiple reaction products.

§ All spots fluorescent (yellow; 254 nm).

The developing solvents I and II were found to be the most suitable for NBD-Cl derivatives.

Table III gives data for the first run in each tank only. The second and third runs gave data with standard deviations greater than 0.10 and were not included.

Comparative R_F values for basic drugs in the three solvent systems permitted tentative identification (see Table IV). The inclusion of UV spectra provided a rapid method for identification of the basic drugs tabulated. For example, chlorpheniramine

TABLE IV

COMPARATIVE R_F VALUES OF NBD-Cl DERIVATIVES

Compound	$R_F \times 100$			*Visible colour***
	Ethyl acetate–cyclohexane (2:3)	*Ethyl acetate–cyclohexane (3:2)*	*Diethyl ether–benzene (1:1)*	
Anileridine	6	19	18	Or
Atropine	7	25	15	—
Hyoscyamine	7	26	—	—
Phenylephrine	7,4*	23,16*	17,23*	Y-Or,Y*
3,4-Dimethoxyamphetamine	10,20*	24	—	Y
Chlorpheniramine	11	23	36	Y
Propoxyphene	11	22	35	Y
Piminodine	11	25	—	Y-Or
N-Methyl-3-piperidyl benzilate	11	26	27	—
N-Ethyl-3-piperidyl benzilate	12	26	26	—
Pseudoephedrine	14	32	35	Y
p-Hydroxyephedrine	14,9,3*	30,25,12*	13,28,36*	Y
Benzphetamine	16	36	27	—
Mescaline	16	37	37	Y
Procyclidine	16	31	62	Or
Ephedrine	18	33	42	Y
N-Methylphenethylamine	26	42	52	Y-Or
Piperidine	29	43	55	Or-Rd
Fenfluramine	30	50	57	Or
Ethoheptazine	31	52	—	Y-Or
Methamphetamine	30	49	59	Y-Or
Phenmetrazine	31	51	57	Y
Phenylpropanolamine	33,38*	53	49	—,Y*
Diphenhydramine	34	53	63	Y
3-Methoxy-4,5-methylene-dioxyamphetamine	34	53	57	Y
Methylphenidate	37	55	64	—
p-Methoxyamphetamine	42	57	63	Y
Chlorphentermine	43	59	—	Y
3,4-Methylenedioxyamphet-amine	43	58	63	Y
Alphaprodine	45	60	—	—
Phenethylamine	45	61	66	Y
2,5-Dimethoxy-4-methyl-amphetamine	46	60	61	Or
Amphetamine	47	64	69	Y

* Multiple reaction products.

** All spots fluorescent (yellow; 254 nm).

and propoxyphene have identical R_F values by the NBD-Cl method; their UV spectra, however, are markedly different. Chlorpheniramine in acid shows an absorption at 265 nm, while propoxyphene in acid has an absorption at 257 nm.

Slight differences in colours of reaction products and the presence or absence of a fluorescent reaction product permitted differentiation between most of the drugs tabulated. Multiple reaction products, where detected, are indicated by more than one R_F value. The nature of these products has not been investigated.

Bethanidine, eucatropine, homatropine, hyoscine, mephentermine, methadone, phendimetrazine and propylhexedrine did not react with NBD-Cl, and pethidive gave a weak reaction only. Some of these compounds also show an absorption at approximately 257 nm. This fact should be taken into consideration when interpreting analytical data.

Typical sensitivities for selected drugs after reaction with NBD-Cl and TLC elution, are listed in Table V. These sensitivities indicate the application of NBD-Cl on small samples, for example, syringe washings. At present the NBD-Cl procedure is being used satisfactorily in forensic drug analysis and drug screens on basic extracts of toxicological material.

TABLE V
SENSITIVITIES OF SELECTED COMPOUNDS WITH NBD-Cl
Sensitivities were determined after TLC elution.

Compound	*Sensitivity (μg)*
Amphetamine	0.01
Anileridine	0.03
Chlorpheniramine	0.01
Chlorphentermine	0.50
Diphenhydramine	0.01
Methylphenidate	0.50
Methamphetamine	0.01
Phenethylamine	0.01
Phenmetrazine	0.05
3,4-Methylenedioxyamphetamine	0.35
3-Methoxy-4,5-methylenedioxyamphetamine	0.35
p-Hydroxyephedrine	0.50

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