Separation and Quantitation of Mixtures of the Most Commonly Abused Drugs

NICOLAS H. CHOULIS

Abstract □ TLC has been used for the separation and semiquantitative estimation of various mixtures of narcotics, barbiturates, amphetamines, and tranquilizers appearing in the illicit market. Of the solvent systems employed, chloroform–dioxane–ethyl acetate–concentrated ammonium hydroxide (25:60:10:5 v/v) gave complete separation for all 17 compounds examined. Potassium permanganate (0.1%) and iodine fumes were used as detection reagents. Twelve different mixtures of two, three, and four compounds, randomly selected, were separated and the spots were quantitated using a UV absorption–concentration relationship. The spots were eluted from the plates, and their absorption was measured and compared to standard curves. Recoveries were found to be between 93 and 95% for quantities as small as 0.1 mg./ml., while quantities as small as 1 mcg. could be detected.

Keyphrases □ Narcotic mixtures with barbiturates, amphetamines, tranquilizers—separation, analysis □ Barbiturate mixtures with narcotics, amphetamines, tranquilizers—separation, analysis □ Amphetamine mixtures with narcotics, barbiturates, tranquilizers—separation, analysis □ Tranquilizer mixtures with narcotics, barbiturates, amphetamines—separation, analysis □ Abuse drugs (narcotic, barbiturate, amphetamine, tranquilizer mixtures)—separation, analysis □ TLC—separation, mixtures of narcotics, barbiturates, amphetamines, tranquilizers □ UV spectrophotometry—analysis, narcotics, barbiturates, amphetamines, tranquilizers

Capsules, tablets, powders, solutions, and other forms in various sizes have appeared on the illicit market, identified under various names and consisting of mixtures of drugs. Some examples are: LSD–amphetamine (in various forms), heroin hydrochloride containing methapyrilene, marijuana cigarettes dipped in opium (better known as O. J., "opium joints"), phencyclidine hydrochloride, LSD (in capsules), amphetamine with cannabis (most commonly used), amphetamine with barbiturates (known as drimaryl, particularly in England), strychnine with LSD and methamphetamine (tablets, known as "blue cheer," particularly in Australia), LSD on marijuana, heroin with methamphetamine and cocaine, and barbital with heroin. Numerous methods have been used to investigate all of these compounds (1–32), but combinations of these drugs have not always been thoroughly examined.

The aim of the present investigation was to provide a simple, *in vitro*, routine TLC method for the separation and quantitative determination of various mixtures of the most commonly abused drugs.

EXPERIMENTAL

Materials—The following were used: sodium amobarbital[1], m.p. 288° [lit. (33) m.p. 287–289°]; dextroamphetamine, puriss.[2], b.p. 204° [lit. (33) b.p. 203–204°]; cocaine hydrochloride[3], m.p. 195° [lit. (33) m.p. 195°]; codeine[3], m.p. 154° [lit. (33) m.p. 154–156°]; ephedrine hydrochloride[4], m.p. 219° [lit. (33) m.p. 216–220°]; methadone hydrochloride[4], m.p. 235° [lit. (33) m.p. 235°]; methamphetamine[5], m.p. 173.5° [lit. (33) m.p. 170–175°]; morphine[4], m.p. 255° dec. [lit. (33) m.p. 254–256°]; sodium pentobarbital[1], m.p. 313°; phenobarbital[1], m.p. 175.5° [lit. (33) m.p. 174–178°]; and sodium secobarbital[1], m.p. 293° [lit. (34) m.p. 293°]. Cannabidiol, hashish, LSD, marijuana, and opium were supplied by the National Institute of Mental Health and were used without further testing.

Table I—λ_{max} of All Drugs Used for UV Studies

Drug	λ_{max}
Dextroamphetamine	258
Cannabidiol	280
Cocaine hydrochloride	231
Codeine	211
Ephedrine hydrochloride	250
LSD	237
Marijuana	280
Methamphetamine	257
Morphine	250
Opium	250
Phenobarbital	253
Sodium amobarbital	238
Sodium pentobarbital	239
Sodium secobarbital	240

Developing Systems—The following solvent systems were used: (1) chloroform–acetone (9:1 v/v), (2) ethanol–methanol–concentrated ammonium hydroxide (85:10:5 v/v), (3) methanol–concentrated ammonium hydroxide (100:2 v/v); and (4) chloroform–dioxane–ethyl acetate–concentrated ammonium hydroxide (25:60:10:5 v/v).

Detection of TLC Spots—The following detection reagents were used: (1) UV light (long wavelength), (2) iodine fumes, (3) Dragendorff, and (4) potassium permanganate, 0.1% solution. The Dragendorff reagent is prepared as follows. Dissolve 2.125 g. bismuth subnitrate in 100 ml. of distilled water (Solution A) and 50 g. of potassium iodide in 125 ml. of distilled water (Solution B). Combine 10 ml. of Solution A, 10 ml. of Solution B, 20 ml. of glacial acetic acid, and 100 ml. of distilled water. The minimum concentration of drug detectable by Reagents 2, 3, and 4 was 1 mcg., while 2–3 mcg. of compound was the sensitivity limit for UV light detections.

Preparation of Plates—The plates (20 × 20 cm.) were coated with silica gel G[6], 250 nm. thick, according to Stahl (35). Silica gel precoated plates[7] (250 nm. thick) were also used.

General Procedure—Solutions of the compounds were prepared by dissolving 3 mg. of the pure compounds in 1 ml. of the appropriate solvent, namely: (*a*) water for sodium amobarbital, cocaine hydrochloride, ephedrine hydrochloride, methadone hydrochloride, sodium pentobarbital, and sodium secobarbital; (*b*) ethanol for dextroamphetamine, codeine, methamphetamine, phenobarbital, and LSD; and (*c*) chloroform for opium, marijuana, hashish, cannabidiol, and morphine.

Using accurately calibrated micropipets, samples of the solutions were applied to the plates 1.5 cm. from the bottom edges, and ascending chromatograms were run at room temperature (about 23°). All chromatograms were carried out in chromatographic chambers

[1] Ruger Chemical Co.
[2] Aldrich.
[3] Merck & Co.
[4] Mallinckrodt.
[5] City Chemical Corp.
[6] Scientific Manufacturing Industries.
[7] Analtech, Inc.

Table II—R_f Values (in Increasing Order) of the Various Compounds Using Solvent System 4

Compounds	R_f Values	Detection Systems[a] 1	2	3	4
Marijuana	0.17[b], 0.95[c]	+	+	+	+
Hashish	0.17[b], 0.41[d], 0.95[c]	+	+	+	+
Opium	0.25[e], 0.44[f], 0.75[g]	−	+	+	+
Morphine	0.25[e]	−	+	+	+
Ephedrine hydrochloride	0.32	−	+	+	+
Methamphetamine	0.40	−	+	+	+
Codeine	0.44	−	+	+	+
Dextroamphetamine	0.50	−	+	−	+
LSD	0.70	+	+	+	+
Methadone hydrochloride	0.73	−	+	+	+
Amobarbital	0.76	+	+	−	+
Cocaine	0.78	−	+	+	+
Phenobarbital	0.80	−	+	−	+
Sodium pentobarbital	0.83	−	+	−	+
Sodium secobarbital	0.87	−	+	−	+
Cannabidiol	0.95	+	+	+	+

[a] 1 = UV light, 2 = iodine fumes, 3 = Dragendorff, and 4 = potassium permanganate, 0.1%. [b] Δ-9-Tetrahydrocannabinol. [c] Cannabidiol. [d] Cannabinol. [e] Morphine. [f] Codeine. [g] Papaverine.

saturated in solvent vapors. The solvent front was allowed to travel 10 cm.; the plates were removed, air dried, and sprayed with the appropriate reagent.

Mixtures of two, three, and four compounds, selected at random, were prepared for qualitative analysis. The same mixtures, formed by using accurately measured amounts, were prepared for separation and quantitative determination of the components.

For commercially available or counterfeit types of dosage forms, an extraction procedure was followed. The tablet (pulverized) or the capsule form was dissolved in water (10 ml.) and extracted with chloroform (3 × 10 ml.). The layers were then separated, and the chloroform layer was rendered alkaline with 0.5 *N* NaOH. The two formed layers were again separated, and the aqueous layer was treated with dilute hydrochloric acid and extracted with chloroform (3 × 10 ml.). The chloroform extract was washed twice with water (2 × 10 ml.), dried over anhydrous magnesium sulfate, filtered, concentrated, and chromatographed for the detection of acidic drugs.

For basic drugs the initial aqueous layer was treated with sodium hydroxide (0.5 *N*) and extracted with chloroform (3 × 10 ml.); the chloroform phase was extracted with dilute hydrochloric acid, and the aqueous acidic phase was rendered alkaline with sodium hydroxide (0.5 *N*), and extracted with chloroform (3 × 10 ml.). The chloroform extracts were combined, washed with water (2 × 10 ml.), dried, concentrated, and chromatographed.

Method—The relationship between UV absorption and concentration of the various compounds was established by preparing solutions of the compounds at various concentrations (0.5–2.5 mg./ml. at 0.5-mg. intervals) and measuring their absorption at λ_{max} (Table I) (33, 36), using a spectrophotometer[8] with 1-cm. cells. Standard curves of absorbance *versus* concentration of the various compounds were then constructed.

Accurately measured solutions of the various compounds were applied on thin layers; duplicates were run parallel. The chromatograms were developed and the parallel running spots were detected, using one of the spray reagents, and marked. The areas corresponding to the marked spots were carefully scraped off, and the layers were quantitatively transferred to 10-ml. conical flasks. The compounds were eluted from the coating material by adding about 7 ml. of the appropriate solvent; the flasks were shaken well for about 10 min., centrifuged, and filtered into 10-ml. volumetric flasks. Solvent was added to the volume. A similar elution process was followed for a blank sample of the coating material alone. Spectrophotometric measurements were carried out, at λ_{max}, using the spectrophotometer and the same cells. The absorbance values obtained (after reducing the absorbance due to the blank) were compared to the standard curves.

[8] Beckman DU.

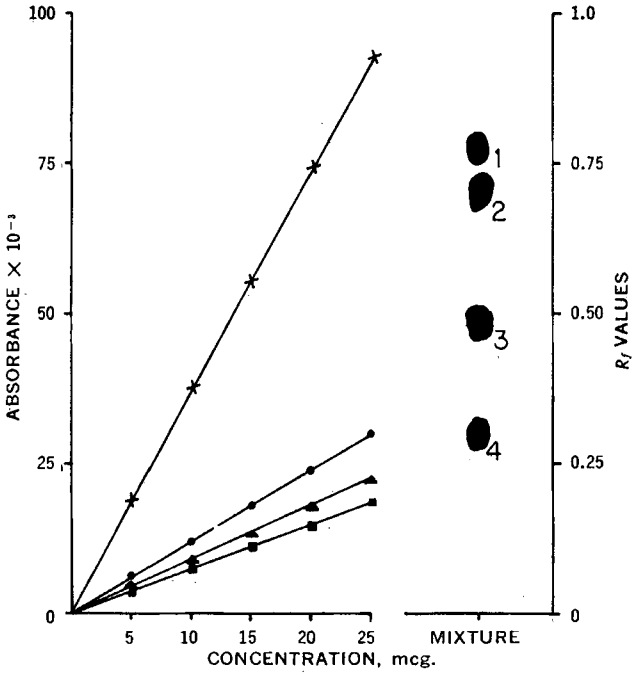

Figure 1—*UV absorption–concentration relationship (left) and* R_f *values (right) of the following compounds: 1,* ×, *phenobarbital; 2,* ●, *methadone hydrochloride; 3,* ▲, *dextroamphetamine; and 4,* ■, *ephedrine hydrochloride.*

RESULTS AND DISCUSSION

Only Solvent System 4 [chloroform–dioxane–ethyl acetate–concentrated ammonium hydroxide (25:60:10:5)] gave complete separation of all 17 compounds used; potassium permanganate, 0.1%, and iodine fumes detected all compounds. (See Table II, where the sensitivities of the detection reagents are also depicted.)

When a mixture of four compounds, namely ephedrine hydrochloride, dextroamphetamine, methadone hydrochloride, and phenobarbital was used, the chromatographic results (Fig. 1) indicated that the R_f values obtained agreed with the ones in Table II. Following the procedure described in the *Experimental* section (UV absorption–concentration relationship), quantitation of the four spots was carried out. The results obtained were compared to the standard curves of absorbance *versus* concentration of the four compounds (Fig. 1).

All experiments were performed at least in triplicate, and the mean values were recorded. No significant difference in these values was observed; 93–95% recoveries of the compounds from the spots were achieved. The results are tabulated in Table III, where the relationships of these compounds in concentration ranges between 0.5 and 2.5 mg./ml. were established.

The procedure was repeated using 12 different mixtures of two, three, and four compounds, randomly selected. The results were very similar to those already indicated.

Although the method is considered sufficiently accurate for the routine analysis of a wide variety of compounds commonly abused, some technical limitations may be imposed, particularly for compounds whose R_f values differ only between 2 and 3%, such as amobarbital, cocaine, and phenobarbital (R_f 0.76, 0.78, and 0.80, respectively), or for compounds for which more than one R_f value appears such as marijuana and opium (R_f 0.17, 0.95, and 0.25 and 0.44 and 0.75, respectively).

The R_f values are not absolute physical constants and may vary for many predictable and valid reasons. For example, the temperature of the experiments must be kept constant, and the purity of the solvents used must be examined so the R_f values will be reproducible. During the preparation of the plates, the variation of the thickness should be considered, since solvent vapor can be preadsorbed by the adsorbent easier on a thinner layer while a thick layer will not have time to reach a good equilibration. A lower proportion of adsorbed solvent will result with a thick layer and, therefore, a greater volume of solvent will be needed to wet a given

Table III—Absorbances of Amphetamine, Ephedrine Hydrochloride, Methadone Hydrochloride, and Phenobarbital at Various Concentrations

Concentrations, mg./ml.	Absorbances[a]							
	Amphetamine		Ephedrine Hydrochloride		Methadone Hydrochloride		Phenobarbital	
	1	2	1	2	1	2	1	2
0.5	0.004	0.003	0.003	0.002	0.006	0.004	0.018	0.012
1.0	0.009	0.008	0.007	0.006	0.012	0.011	0.037	0.035
1.5	0.014	0.014	0.011	0.0105	0.0175	0.017	0.056	0.052
2.0	0.0185	0.0175	0.015	0.014	0.024	0.023	0.075	0.072
2.5	0.0225	0.022	0.019	0.0185	0.030	0.029	0.092	0.089
Percent recovery		95		93		95		93.5

[a] 1 = pure compound, and 2 = TLC recovery.

area of the plate, resulting in higher R_f values on thicker plates. On the other hand, dry solvent vapor can displace some moisture from a partially activated plate, thus increasing its activity; and since it is likely that this will occur more rapidly and efficiently with a thin rather than a thick layer of silica gel, this effect could lead to an increase in R_f value with layer thickness. Readymade plates, such as the ones used in the present experiment, are often more reliable, provided that they all come from the same source.

The developing tank must be equilibrated with solvent prior to its use for at least 1 hr. at room temperature. The solvent system should not be reused more than five times, and freshly prepared solvents should not be kept for more than 48 hr.; otherwise, the R_f values obtained are not completely reproducible.

For quantitative determinations, if the compound is not detectable under UV light, parallel spots should be run simultaneously and should be detected first, separately, so that the positions of the spots to be quantitated can be determined without spraying over their area. Spray reagents interfere during spectrophotometric measurements, giving exaggerated results. Using this method, quantities as small as 1 mcg. can be detected, while quantities as small as 0.1 mg./ml. can be quantitated using UV spectrophotometry.

The described technique can be considered rapid and accurate for routine analysis. With certain modifications, it also can be used for the detection and quantitation of these compounds excreted from biological systems. For the latter, however, the presence of metabolites and urine contaminants should be taken into consideration.

REFERENCES

(1) A. Fiori and M. Marigo, *Nature (London)*, **182**, 943(1958).

(2) E. Stahl, "Thin Layer Chromatography," Springer Verlag, Berlin-Gottingen-Heidelberg, West Germany, 1965.

(3) U. Claussen and F. Korte, *Naturwissenschaften*, **53**, 541 (1966).

(4) F. Korte and H. Sieper, *Justus Liebigs Ann. Chem.*, **630**, 71 (1960).

(5) F. Korte and H. Sieper, *J. Chromatogr.*, **13**, 90(1964).

(6) *Ibid.*, **14**, 178(1964).

(7) M. Schantz, "Thin Layer Chromatography," Marini-Bettolo, Elsevier, 1964.

(8) K. Teichert, E. Mutshler, and H. Rochelmeyer, *Z. Anal. Chem.*, **181**, 326(1961).

(9) D. Waldi, K. Schnackerz, and F. Munter, *J. Chromatogr.*, **6**, 61(1961).

(10) M. Sali and M. Oesch, *ibid.*, **14**, 526(1964).

(11) D. Hale and C. Taylor, *Instrum. News*, **18**, 1(1967).

(12) N. H. Choulis, *J. Pharm. Sci.*, **56**, 1206(1967).

(13) N. H. Choulis, *J. Chromatogr.*, **30**, 618(1967).

(14) N. H. Choulis and C. Carey, *J. Pharm. Sci.*, **57**, 1048(1968).

(15) N. H. Choulis, *Can. J. Pharm. Sci.*, **3**, 13(1968).

(16) N. H. Choulis, *Biochem. Med.*, **5**, 311(1971).

(17) P. DeZan, R. F. Canaff, and R. Bianchi, *J. Ass. Offic. Anal. Chem.*, **54**, 925(1971).

(18) N. A. Rosenthal, M. M. Kaser, and K. N. Milewski, *Clin. Chim. Acta*, **33**, 51(1971).

(19) J. MacGee, *Clin. Chem.*, **17**, 587(1971).

(20) S. J. Mule, *J. Chromatogr.*, **55**, 255(1971).

(21) D. A. L. Bowen, D. M. Curr, and G. B. Oppenheim, *Clin. Toxicol.*, **3**, 89(1970).

(22) J. W. Steele, M. Bolan, and J. K. Eyolifson, *Can. J. Pharm. Sci.*, **5**, 107(1970).

(23) N. C. Law, V. Aandahl, H. M. Fales, and G. W. A. Milne, *Clin. Chim. Acta*, **32**, 221(1971).

(24) A. H. Beckett and A. C. Moffat, *J. Pharm. Pharmacol.*, **23**, 15(1971).

(25) V. P. Dole, W. K. Kim, and I. Eglitis, *J. Amer. Med. Ass.*, **198**, 115(1965).

(26) R. F. Skinner, *Proc. West. Pharmacol. Soc.*, **14**, 4(1971).

(27) A. M. Heaton and A. G. Blumberg, *J. Chromatogr.*, **41**, 367 (1969).

(28) A. Davidow, N. L. Petri, and B. Quame, *Amer. J. Clin. Pathol.*, **50**, 714(1968).

(29) I. Cochin and J. W. Daley, *J. Pharmacol. Exp. Ther.*, **139**, 104(1963).

(30) J. D. McChesney, D. K. Beal, and R. M. Fox, *J. Pharm. Sci.*, **61**, 310(1972).

(31) "Drug-Screen," Brinkmann Technical Bulletin No. 56, Brinkmann Instruments, Inc., Westburg, N. Y., 1970.

(32) E. G. C. Clarke, "Isolation and Identification of Drugs," The Pharmaceutical Press, London, England, 1969.

(33) "Handbook of Analytical Toxicology," I. Sunshine, Ed., The Chemical Rubber Co., 1969.

(34) "The Merck Index," 8th ed., Merck & Co., Rahway, N. J., 1968.

(35) E. Stahl, *Pharmazie*, **11**, 633(1956).

(36) "Handbook of Chemistry and Physics," 51st ed., R. C. Weast, Ed., The Chemical Rubber Co., Cleveland, Ohio, 1970–1971.

ACKNOWLEDGMENTS AND ADDRESSES

Received November 4, 1971, from the *School of Pharmacy, West Virginia University Medical Center, Morgantown, WV 26506*

Accepted for publication August 30, 1972.

Presented to the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, Houston meeting, April 1972.