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A SIMPLE, RAPID THIN-LAYER CHROMATOGRAPHIC DRUG SCREENING PROCEDURE

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SUMMARY

A rapid and sensitive thin-layer chromatography procedure for the screening of drugs of abuse is described. The procedure involves a single extraction of drugs from urine, chromatography on rapid "Toxi-Gram™" chromatography plates, and detection by consecutive spraying of appropriate reagents for the determination of amphetamines, barbiturates, and alkaloids on one chromatography plate. The procedure is ideal for routine screening of outpatients of a methadone clinic.

INTRODUCTION

With the advent of more and more methadone clinics for the treatment of drug addicts on an outpatient basis, simple and rapid screening procedures are needed for monitoring these patients. Attempts to use current kit methods proved unsatisfactory in our laboratory. Commercial drug screening kits have one or more of the following disadvantages: multiple extraction steps at different pH; multiple chromatography plates to be run for each patient sample; chromatography time in excess of 35 min; and, too small a migration distance for proper separation and accurate measurement of R_F values.

Stevenson[1] eliminated the need for multiple pH extractions of urine samples by showing that sufficient quantities of amphetamines, barbiturates, and alkaloids can be extracted from a 10-ml urine sample with a pH 9.5 buffer for detection of drugs of abuse on thin-layer chromatography plates. Weissman *et al.*[2] studied recovery and detection limits of pH 9.5 buffered urine extractions with consecutive spraying of detection reagents for amphetamines, barbiturates and alkaloids on one chromatography plate.

The drug screening procedure described in this paper employs rapid silica gel coated glass fiber chromatography plates, a convenient application system[3], as well as a one-step drug extraction from urine with a pH 9.5 buffer, combined with consecutive spraying with detection reagents for amphetamines, barbiturates, and alkaloid drugs and their metabolites on one chromatogram. Identification of drugs and/or their metabolites is carried out by comparison of R_F values as well as by colors formed on

reaction with the detection sprays. Such identification is used to detect the presence of methadone and its metabolite(s) in the urine of patients and to eliminate the possibility of there being other drugs present. The system described is sensitive, rapid and useful as a routine screening for monitoring outpatients of a methadone clinic, or as a routine screening for other purposes. It is easily set up at a minimal cost per test for both large and small hospital laboratories.

MATERIALS AND METHODS

All chemicals employed in this study were of reagent grade. Chromatography was carried out on "Toxi-Gram™" chromatography plates from ICL Scientific (Fountain Valley, Calif., U.S.A.). Evaporations were carried out at 65° in a "Vapo-Vent" water bath evaporator from Hycel (Houston, Texas, U.S.A.). Nickel evaporation crucibles, 70-ml capacity and 50 mm in top diameter, 47 mm high, were from Canadian Lab. Supplies (Toronto, Canada). Standards were measured and applied with 10-μl syringes from Hamilton (Reno, Nev., U.S.A.). Chromatogram sprayers, 50-ml capacity, were from Sargent-Welch Scientific Canada (Toronto, Canada). A "Black Ray UVL-22" detection lamp from Ultra-Violet Products (San Gabriel, Calif., U.S.A.), was used to detect spots by UV light.

Urine samples from the methadone clinic are generally run in batches once a week. Samples are stored in the freezer and thawed either at room temperature or in a 37° water-bath. All urines are centrifuged for 5 min at 4500 *g*. A 10-ml sample of urine is transferred by pipet to a 250-ml separatory funnel and 1 ml of an ammonium chloride–ammonium hydroxide buffer (pH 9.5) is added to the separatory funnel. The sample is swirled to mix the urine and buffer solution. Extracting solvent (50 ml) is added and any gas build up is allowed to escape via the stopcock. The separatory funnels are shaken for 5 min and the two phases are allowed to separate. The lower phase is drawn off into 70-ml metal cups which are placed in a Vapo-Vent evaporator until dry. A 0.1-ml methanol solution is used to rinse down the sides of the metal cups to concentrate the drugs. Two blank ITLC discs are placed into this concentrate and the metal cups are placed on an angle in the Vapo-Vent until dry. One of the blank discs is inserted into the ITLC plate for chromatography, while the second is filed as a spare. The plates are developed with ethyl acetate–methanol–ammonium hydroxide (85:10:5).

The plates are air dried in the fume hood for 10 min and then examined under UV. Further drug detection is done by consecutive spraying of the chromatogram with the following reagents: ninhydrin, diphenylcarbazone and mercuric sulfate, iodoplatinate, and Dragendorff's reagent. Reagent preparation and detailed instruction are reported in the procedure of Weissman *et al.*[2].

RESULTS

Identification of drugs using the rapid silica gel impregnated glass-fiber chromatograms is by R_F values and by the colors formed with the reagent sprays. Tabulated R_F values as well as colors formed are reported in Table I. Although R_F values differ with the procedure employed, the colors formed with the detection sprays correlate with those found by other researchers[2,4,5].

TABLE I

DRUG IDENTIFICATION

Drug	$R_F \times 100$	*UV light*	*Ninhydrin UV irradiation*	*Diphenyl-carbazone mercuric sulfate*	*Iodoplatinate*
Secobarbital	98			purple	
Butabarbital	97			purple	
Ethchlorvynol (Placidyl)	97			white, turns orange with heat	
Methadone	90		pink*		rose-purple
Methadone metabolite	86				rose-purple
Methaqualone	88				brown
Phenobarbital	81			purple	
Pentazocine (Talwin)	77				brownish purple
Cocaine	75				purple
Meperidine (Demerol)	72				brownish purple
Amitriptyline (Elavil)	70				maroon
Methedrine	68				purple
Caffeine	67				blue
Imipramine (Tofranil)	65	spot			maroon
Nicotine	63				blue
Promazine	61			orange, turns brown with heat	
Quinine	59	bluish			blue-purple
Perphenazine (Trilafon)	57	pale blue			blue-violet
Prochlorperazine (Stemetil)	56			tan, turns red with heat	
Nortriptyline (Aventyl)	55				purple
Codeine	54				purple
Desipramine (Pertofrane)	54				purple
Methamphetamine	48		rose*		rose-purple
Strychnine	41				
Morphine	33				blue
	5	greenish fluorescence			

* Visible at high concentrations.

Detection limits for morphine, methadone, and phenobarbital were determined to be 0.5, 1.0, and 2.0 μg, respectively.

DISCUSSION

The "Brinkman Drug-Skreen" system gives better resolution of drugs by R_F values than the "Kodak Chromat/O/Screen Analysis Kit" or the "Sepachrom™ Drug System", but it requires a minimum of 35 min for chromatography. From our experience with column extractions and liquid–liquid solvent extractions, we find that both work well. However, if one has to prepare chromatography columns, then one would save time by doing a liquid–liquid extraction.

The "Sepachrom™ Drug System" (Gelman Instrument, Ann Arbor, Mich., U.S.A.), a miniature chromatography system, proved to be very convenient and rapid, but the migration distance was too short for accurate R_F values. Under our laboratory conditions, the drugs did not separate properly. An added disadvantage is that for each patient three chambers must be run to determine amphetamines, barbiturates, and alkaloids. A large batch of drugs run once per week for a methadone clinic would require a multitude of steps and numerous chambers because of the limited number of samples that can be run per chamber.

The "Kodak Chromat/O/Screen Analysis Kit" is useful in routine screening, but because of the limited migration distance, R_F values are of little significance. Again, multiple handling and chromatography of samples is required to screen for amphetamines, barbiturates, and alkaloids.

The "Toxi-X™ Drug Kit" (containing the "Toxi-Gram™" chromatography plates) was examined for use as a routine screening procedure. Chromatography time was rapid and the replaceable disc application system proved very convenient, but drug separation using their solvent system was not satisfactory under our laboratory conditions. The replaceable disc application system, described in the literature by Popowicz[3], eliminated uneven spotting and gave a more uniform migration pattern. Drug standards can be stored on these discs. This eliminates standard spoilage due to evaporation, contamination or deterioration of standard drug solutions. Batches of drug standards can be prepared in advance or already prepared drug standards can be purchased (ICL Scientific). Drug standards purchased from ICL Scientific gave two bands for morphine by the procedure described in this paper. Both bands can be used for identification purposes. Carry-over effects often make identification of the first band, $R_F \times 100 = 5$, more difficult while the R_F of the second band, $R_F \times 100 = 33$, is less affected by carry-over. The cocaine spot was not found for the ICL Scientific drug standard. It appears that cocaine of the ICL Scientific drug standard is unstable. R_F values for cocaine standards prepared in this laboratory are reported in Table I.

If further identification is required, the second spare disc can be chromatographed, and detection with additional sprays such as potassium permanganate or diazotized *p*-nitrophenol can be carried out. In special cases, the chromatography solvent might be altered to obtain greatest separation prior to detection[6]. With the use of these additional techniques identification of most drugs is nearly specific. However, because of the similar R_F values and the nature of colors obtained with the iodoplatinate detection spray, it would be difficult to distinguish absolutely between Demerol, Elavil, methedrine and caffeine (See Table I). As an aid to the clinician it is important to know if the patient is a heavy coffee drinker and/or smoker. In our laboratory smokers were classified as: light, one pack of cigarettes per day or less; medium, two packs per day; heavy, three or more packs per day.

It is also important to note the nature of any medication that a patient may be taking when a drug screen is being performed. Codeine-containing analgesics would give a positive result for codeine during drug screening when using iodoplatinate as a detecting reagent. Since barbiturates migrate close to the solvent front, a glass plate can be overlaid covering the bottom 3/4 portion of the plate. A separate detection spray can be utilized for barbiturates, and the remainder of the plate can be sprayed with different detection sprays for other drugs.

The drug system which we describe uses a one-step pH 9.5 buffered drug ex-

traction, rapid chromatography plates, and consecutive spraying of reagents for the identification of amphetamines, barbiturates, and alkaloid drugs and their metabolites. The system is inexpensive and can easily be set up for routine screening of patients from a methadone clinic.

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