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THIN-LAYER CHROMATOGRAPHIC SCREENING AND CONFIRMATION OF BASIC DRUGS OF ABUSE IN URINE

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SUMMARY

Thin-layer chromatographic procedures are presented for the positive identification of methadone, primary metabolite of methadone (2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine), propoxyphene, norpropoxyphene, cocaine, benzoylecgonine, methaqualone, and phencyclidine from urine specimens. Initial screening of specimens is done by developing plates in ethyl acetate–methanol–diethylamine (90:10:1.6). Samples screened positive are confirmed in methylene chloride–methyl ethyl ketone–concentrated ammonium hydroxide (74:25:0.8), depending on the drug(s) indicated by the screening procedure. The method is quite sensitive, detecting most of the listed drugs at levels of 1.0 μg/ml or less.

INTRODUCTION

The widespread use and abuse of drugs necessitate simple and rapid methods that can be used to detect drugs in urine specimens. In this study we present a thin-layer chromatographic (TLC) procedure to detect a group of basic drugs including methaqualone, propoxyphene, cocaine, phencyclidine and methadone along with some of their primary metabolites such as norpropoxyphene, benzoylecgonine, and 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine.

In our screening procedure, urine specimens are made alkaline and extracted with an organic solvent. The organic layer, containing the basic drugs, is evaporated to dryness and reconstituted in a small volume of methanol. Half of the concentrate is spotted on a TLC plate while the remaining concentrate is saved for possible confirmation analysis. The plate is developed in the screening solvent system ethyl

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acetate–methanol–diethylamine (90:10:1.6). The drug spots are then visualized by iodoplatinate reagent. Extracts of specimens which are found positive in the initial screening are spotted on a second TLC plate, which is developed in the confirmation solvent system methylene chloride–methyl ethyl ketone–concentrated ammonium hydroxide (90:10:0.8). However, if norpropoxyphene, benzoylecgonine, methadone and/or its metabolite 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine are suspected in the initial screen, improved results are obtained by confirming on a plate developed in methanol–chloroform–concentrated ammonium hydroxide (74:25:0.8).

Six other solvent systems are presented as alternate screening and/or confirmation methods for the basic drugs.

EXPERIMENTAL

Materials

Merck silica gel G TLC plates, 20 × 20 cm, obtained from EM Laboratories (Elmsford, N.Y., U.S.A.), were used. The iodoplatinate spray was prepared as follows: 1 g of chloroplatinic acid was dissolved in 10 ml of distilled water, then added to 30 g of potassium iodide dissolved in 200 ml of distilled water. Five milliliters of conc. hydrochloric acid were added and the mixture was then diluted to 250 ml with distilled water.

Solvent systems

The solvent systems used are listed in Table I.

Procedure

Sample preparation. Twenty milliliters of urine, 0.2 ml of conc. ammonium hydroxide and 25 ml of 5% isopropanol–chloroform are placed in a 50-ml centrifuge tube and shaken horizontally at 70 cycles/min for 15 min. After shaking, the top urine layer is aspirated off and the organic layer filtered through phase-separating paper into a 40-ml conical centrifuge tube. One tenth milliliter of 1% hydrochloric acid in methanol is added and the sample is then evaporated to dryness under nitrogen in a 70° water-bath.

Screening. The residue is reconstituted in 100 μl of methanol. Fifty microliters are spotted on a TLC plate (the remainder is saved for confirmation analysis). The plate is developed in the screening solvent system (solvent system I). After development, which takes a little over 1 h, the plate is air dried and sprayed with iodoplatinate reagent to visualize the spots for methadone, methadone metabolite, cocaine, benzoylecgonine, phencyclidine, propoxyphene, norpropoxyphene, and methaqualone.

Confirmation. (i) Unless norpropoxyphene, benzoylecgonine, methadone and/or methadone metabolite are suspected to be present in the initial screen, the remainder of the extract is spotted on a second TLC plate, which is then developed in the confirmation solvent system (solvent system II). It may be necessary to add another drop or two of methanol if the extract is completely dry. After development, the plate is air dried and then sprayed with iodoplatinate reagent to visualize the drug spots.

(ii) If norpropoxyphene, benzoylecgonine, methadone and/or methadone metabolite are observed in the initial screen, the remainder of the extract is spotted on a second TLC plate. This plate is developed in methadone confirmation solvent

TABLE I

R_F VALUES OF COMMON DRUGS ON VARIOUS SOLVENT SYSTEMS

Solvent systems: (I) ethyl acetate–methanol–diethylamine (90:10:1.6); (II) methylene chloride–methyl ethyl ketone–conc. NH_4OH (90:10:0.8); (III) methanol–chloroform–conc. NH_4OH (74:25:0.8); (IV) ethyl acetate–methylene chloride–conc. NH_4OH (90:10:0.7); (V) ethyl acetate–benzene–chloroform (40:40:20); (VI) methylene chloride–butanol–conc. NH_4OH (85:15:0.2); (VII) acetone–methanol–diethylamine (90:10:0.7); (VIII) acetone–methanol (60:40); (IX) acetone–chloroform (70:30).

Drug	*Solvent system*								
	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*
Benzoylecgonine	0.04	0.01	0.37	0.01	0	0.01	0.03	0.18	0
Chlordiazepoxide	0.63	0.04	0.87	0.20	0.09	0.61	0.82	0.82	0.64
Cocaine	0.55	0.56	0.79	0.77	0.12	0.65	0.64	0.52	0.30
Codeine	0.20	0.01	0.46	0.04	0	0.09	0.21	0.23	0.03
Diazepam	0.86	0.57	0.87	0.80	0.50	0.96	0.90	0.85	0.87
Meperidine	0.27	0.13	0.68	0.21	0.03	0.27	0.30	0.36	0.15
Methadone	0.35	0.23	0.55	0.66	0.05	0.21	0.41	0.25	0.13
Methadone metabolite	0.30	0.15	0.15	0.74	0.05	0.04	0.27	0.05	0.07
Methaqualone	0.88	0.74	0.89	0.84	0.55	0.97	0.91	0.87	0.91
Morphine	0.16	0	0.43	0.02	0	0.04	0.18	0.23	0.02
Nicotine	0.26	0.10	0.73	0.10	0.05	0.12	0.37	0.44	0.21
Norpropoxyphene	0.23	0.03	streak	0.11	0	streak	0.19	streak	0.01
Oxazepam	0.84	0.04	0.83	0.23	0.32	0.79	0.89	0.88	0.84
Phencyclidine	0.58	0.45	0.67	0.89	0.07	0.38	0.59	0.38	0.26
Propoxyphene	0.59	0.50	0.84	0.80	0.18	0.68	0.66	0.57	0.43

system (solvent system III). After development, the plate is air dried and sprayed with iodoplatinate to visualize the drug spots.

Interpretation

Positive specimen. We consider that a positive specimen must contain spots for the drug(s) of interest on both solvent systems. The spots must have the same R_F values and colors as known standards.

Negative specimen. Specimens which do not meet the above criteria are considered to be negative.

RESULTS AND DISCUSSION

Table I gives the R_F values of the drugs of interest as well as other drugs that may be found in the urine on nine different solvent systems. Table II gives the colors produced with iodoplatinate reagent and the minimum detection level of each drug of interest. Table III gives the preferred solvent systems for screening and confirmation of individual drugs.

We have developed this procedure to screen and confirm urine specimens for the presence of methadone, primary metabolite of methadone, propoxyphene, norpropoxyphene, cocaine, benzoylecgonine, methaqualone, and phencyclidine. The method is suitable for large-scale testing of these basic drugs. The procedure requires only one extraction, but, by splitting the sample, still allows the dual analysis required

TABLE II

DRUG DETECTION LEVELS AND COLORS WITH IODOPLATINATE

Drug	*Color*	*Minimum detectable level (μg/ml)*
Benzoylecgonine	purple	3.0***
Cocaine	purple*	1.0
Methadone	red-brown	0.5
Methadone metabolite	red-brown	0.5
Methaqualone	red-brown	1.0
Norpropoxyphene	blue**	1.0
Phencyclidine	purple-brown	1.0
Propoxyphene	red-brown	1.0
Chlordiazepoxide	purple	1.0
Codeine	purple	0.5
Diazepam	brown	1.0
Meperidine	brown	1.0
Morphine	blue	0.5
Nicotine	blue-green	1.0
Oxazepam	yellow	2.0

* With an orange halo if the plate is sprayed before being completely dry.

** Blue on solvent systems III, VI, and VIII; red-brown with a blue halo on other solvent systems.

*** Obtained using a modified extraction procedure by adding K_2CO_3 instead of ammonia as described in Results and discussion.

for a positive identification. No effort is made to detect amphetamine, methamphetamine, barbiturates or opiates by this method because our laboratory screens these drugs by immunochemical procedures[1–4].

Nine solvent systems have been developed to screen and confirm various members of the group of basic drugs and their metabolites listed above. Other possible drugs of interest, such as nicotine, morphine, codeine, chlordiazepoxide, meperidine,

TABLE III

PREFERRED SOLVENT SYSTEM FOR THE IDENTIFICATION OF EACH DRUG

For solvent systems I–IX, see Table I.

Drug	*Preferred screening system*	*Alternative screening system(s)*	*Preferred confirmation system*	*Alternative confirmation system(s)*
Benzoylecgonine	I	VII	III	VIII
Chlordiazepoxide	I	IV, VII	IX	VI
Cocaine	I	VII	II	VIII, IX
Diazepam	I	VII	V	II
Meperidine	VII	IV, I	VI	II
Methadone	IV	I, VII	III	II, VI
Methadone metabolite	IV	I, VII	III	II, VI
Methaqualone	I	VII	V	II
Norpropoxyphene	I	VII	III	II, VI, VIII
Oxazepam	I	IV, VII	V	IX, VI
Phencyclidine	I	VII	VI	II, IX
Propoxyphene	I	VII	II	IX, VIII

diazepam, and oxazepam, which are also extractable into organic solvent at a basic pH, can be identified with these solvent systems. The preferred solvent system for screening and confirmation depends upon the drug(s) to be analyzed, as illustrated in Table III.

Solvent system I is a good all-purpose screening solvent system. It roughly divides the basic drugs into four groups. In the first group, at the top of the plate, are methaqualone, diazepam, and oxazepam. There is virtually no separation of these three, but some idea as to which drug is present can be obtained from the coloration of the spot. In the second group, in the middle of the plate, are chlordiazepoxide, propoxyphene, phencyclidine, and cocaine. There is a slight separation of these four as well as some color differentiation to aid in their identification. In the third group, about one quarter of the way up the plate, are methadone, primary metabolite of methadone, meperidine, nicotine, norpropoxyphene, morphine, and codeine. Methadone and its primary metabolite are separated at the top of this group of drugs, easily differentiated from other drugs and urinary substances. Nicotine and meperidine remain unseparated at the center of the group. Norpropoxyphene, codeine and morphine are separated from one another, remain below the other members of the group, and are subject to interference by normal urinary substances. Color differences will aid greatly in presumptively identifying "unknown" spots in this group of drugs. In the fourth group is benzoylecgonine, just slightly above the origin of the plate. It is subject to interference, but can be identified on the basis of its purple color.

Solvent system II is an excellent confirmation solvent system for most drugs of interest. It gives clear separation without interference from other drugs or urinary substances, of methaqualone, cocaine, phencyclidine, propoxyphene, methadone, its primary metabolite, meperidine, and norpropoxyphene. It can therefore confirm all the drugs of interest screened by solvent system I, although methadone, its primary metabolite, and norpropoxyphene can be better confirmed by solvent system III. Generally, only one spot for both methadone and its metabolite will be seen if the total concentration of the two methadone drugs is greater than 4–5 μg/ml. Because of very close R_F values, the two spots appear united in the event of such high concentrations of the two drugs. Norpropoxyphene does not migrate very far from the origin, and is likely to be interfered with by the urinary components present in this area of the TLC plate. Benzoylecgonine does not migrate from the origin and cannot be confirmed confidently by this solvent system.

Solvent system III is a good confirmation solvent system for methadone, primary metabolite of methadone, benzoylecgonine, and norpropoxyphene. Methadone and its primary metabolite are widely separated without interference from other drugs or urinary substances. Confusion of one for the other is virtually impossible. Benzoylecgonine is found near the center of the plate without interference from other drugs. Norpropoxyphene produces a large blue streak with an R_F value from 0–0.6, indicating propoxyphene use. It should be noted that a very strong norpropoxyphene streak could obscure a weak benzoylecgonine, methadone and/or methadone metabolite spot. Under such circumstances, a confirmation is best made by using solvent system II, IV or VII. Morphine and codeine can also be confirmed using solvent system II. However, this system is not good for other drugs since many drugs migrate toward the top of the plate, where they are unseparated and subject to interference by normal urinary constituents.

Solvent system IV is a good screening solvent system for methadone and its primary metabolite[5]. It gives R_F values for methadone and its primary metabolite in reverse order from those produced by solvent systems I, II, III, VI, and VIII. Chlordiazepoxide and meperidine, although not separated from one another, are separated from other drugs and urinary substances. Nearly all other drugs of possible interest migrate, as in solvent system III, to the top of the plate, where there is little separation and much interference by urinary substances.

Solvent system V is the most effective system for the confirmation of methaqualone. Methaqualone, diazepam and oxazepam are clearly separated from one another and from all other drugs. This solvent system, however, is not useful for the confirmation of other drugs, since most other drugs remain near the origin of the plate.

Solvent system VI is the best solvent system for the confirmation of phencyclidine, clearly separating it from all the other drugs of interest and common urinary substances. Chlordiazepoxide, meperidine, methadone, norpropoxyphene, cocaine, oxazepam, and propoxyphene can also be confirmed by this solvent system, although better separations are obtained on other systems.

Solvent system VII is an alternate screening solvent system. Most of the drugs of interest are well separated from each other with minimal interference from common urinary substances. The separations are similar to those obtained with solvent system I. Solvent system VII is superior to solvent system I in that (i) methadone and its primary metabolite are further separated, (ii) there is better separation between nicotine and meperidine, and (iii) phencyclidine is better separated from cocaine and propoxyphene. It is, however, inferior to solvent system I in that (i) there is less separation between methaqualone and urinary substances rising to the top of the plate, (ii) there is no separation between cocaine and propoxyphene, and (iii) there is less separation between methaqualone and oxazepam.

Solvent system VIII is the best solvent system for the confirmation of a specimen containing both cocaine and its metabolite, benzoylecgonine. This is the only solvent system presented which does not cause these two drugs to be interfered by other compounds or urinary substances. Methadone, methadone metabolite, propoxyphene and norpropoxyphene can also be confirmed by this solvent system, although better separations are obtained on other systems.

Solvent system IX is a good confirmation solvent system for chlordiazepoxide. Chlordiazepoxide is well separated from the other drugs of interest and is not interfered with by other urinary substances. This solvent system can be used on the basis of spot coloration for the further confirmation of cocaine, propoxyphene, and phencyclidine, although the separation of the three drugs is only slight and propoxyphene and cocaine tail at large concentrations. Solvent system II is much better for the confirmation of these three drugs.

In the procedure described for the analysis of methaqualone, cocaine, propoxyphene, phencyclidine, and methadone, we recommend screening all specimens using solvent system I and confirming all specimens on solvent system II with the exception of those specimens screened positive for norpropoxyphene, benzoylecgonine, methadone, and/or methadone metabolite. Such specimens are confirmed on solvent system III.

We have successfully run over 19,000 urine samples on our "two-plate" drug analysis system. The only problem encountered has been with methaqualone. Both

screening solvent systems I and VII yield a large number of false positives for methaqualone owing to the proximity of the methaqualone R_F value to the R_F values of common urinary substances carried by the solvent systems to the top of the plate. This problem is eliminated by chromatographing suspected methaqualone specimens with two confirmation solvent systems. We obtained best results by first screening urine specimens for methaqualone on solvent system I or VII, and confirming them on solvent systems II and V.

The identification of several drugs is further confirmed by the presence of their metabolites on the plate. We have found that methadone is considerably metabolized into 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine. In our experience, the two compounds have always been present in roughly equal concentrations[6–9] in the urine of individuals on methadone therapy. We have found that propoxyphene is largely metabolized into norpropoxyphene[8–10]. In most cases of propoxyphene use, only the norpropoxyphene is present in the urine in levels detectable by TLC. Propoxyphene itself is found only if the concentration of norpropoxyphene is very large. Cocaine is extensively metabolized into benzoylecgonine[8,9,11,12]. As with propoxyphene, in most instances of cocaine use only benzoylecgonine may be present in the urine in detectable levels; cocaine itself is observed only when the concentration of benzoylecgonine is very high.

Our standard analysis procedure is somewhat insensitive to benzoylecgonine since it is not readily extracted. We have found that by adding approximately 7 g of potassium carbonate to the urine instead of ammonia, benzoylecgonine is extracted much better. A sensitivity of 3 μg/ml can be achieved.

In contrast to norpropoxyphene and benzoylecgonine, the metabolites of methaqualone and phencyclidine, although present in much larger concentrations than the parent drugs[13–15], are not detectable using this TLC method. These metabolites are either unextractable or cannot be visualized with iodoplatinate reagent.

In our laboratory we have successfully detected 678 persons taking methadone, 584 persons taking propoxyphene, 7 taking methaqualone, 47 taking cocaine, and 24 taking phencyclidine in over 19,000 urine specimens submitted for analysis. We have not seen detectable levels of chlordiazepoxide, diazepam, or oxazepam. This is due to the extensive metabolism of these drugs[10,11]. Spiked urine samples containing 1 μg/ml of these drugs are easily detected by this method, thus confirming earlier work[10] suggesting that the benzodiazepine drugs are not excreted unchanged in urine in sufficient quantity to be readily detected.

NOTE BY THE EDITOR

The use of several solvent systems is certainly an improvement over the schemes which claim that one solvent system is enough for identification. However, it should be borne in mind that R_F values can vary considerably on silica gel plates (even from one batch to another) and that one R_F value can never and several R_F values can hardly be enough for "court evidence". The recent literature seems to show that at least one other kind of chemical evidence is needed.

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