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# THIN-LAYER CHROMATOGRAPHIC SEPARATION OF METHADONE AND ITS PRIMARY METABOLITE IN THE PRESENCE OF OTHER DRUGS IN URINE SPECIMENS

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## SUMMARY

Three solvent systems for thin-layer chromatography have been developed for methadone and its primary metabolite (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine). These solvent systems separate methadone and its primary metabolite, and are not interfered by other drugs of abuse in urine specimens.

## INTRODUCTION

Most programs in the United States require laboratories to differentiate methadone from its primary metabolite (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)[1–5] in the urine of patients maintained on methadone. This distinction is necessary because a portion of methadone in urine is excreted as its primary metabolite[2]. In some instances, particularly when several days have passed since an individual last received a dose of methadone or when a low dose of methadone was administered, it may be the metabolite that is present in detectablea mounts. However, in our experience, we have detected both methadone and its primary metabolite for 7–10 days after a patient has stopped taking the drug. Currently available immunoassay methods, *viz.* radioimmunoassay (RIA), enzyme multiplied immunoassay technique (EMIT), and hemagglutination inhibition (HI), have antibodies for methadone which do not react with its primary metabolite. Several thin-layer chromatographic (TLC) solvent systems for methadone are described in the literature[3–9], but none of these is entirely satisfactory to separate methadone from its primary metabolite because those which do are interfered by other drugs of abuse.

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The three solvent systems reported here are: (I) ethyl acetate–methylene chloride–conc. ammonium hydroxide (90:10:0.9), (II) ethyl acetate–*n*-octanol–conc. ammonium hydroxide (93:7:1.0), and (III) ethyl acetate–diisopropyl ether–water–conc. ammonium hydroxide (90:10:1.1:1.0). These systems separate methadone and its primary metabolite and are not interfered by drugs such as morphine, codeine, nicotine, barbiturates, amphetamines, phenothiazines, propoxyphene, glutethimide, methaqualone, diazepam, dextromethorphan, methapyriline, caffeine, and chlordiazepoxide.

## EXPERIMENTAL

### *Apparatus*

Serological pipettes of 25 ml, 10 ml and 1 ml were used. Round-bottom centrifuge tubes, 50 ml (Cat. No. 8424) and conical centrifuge tubes, 40 ml (Cat. No. 8120) were supplied by Corning (Corning, N.Y., U.S.A.). The polypropylene funnels employed were 65 mm long. Whatman No. 1 PS 12.5-cm phase separating filter paper (W. & R. Balston, Maidstone, Great Britain) was used. The water-bath used, adjustable to 70° was from VWR Scientific (Brisbane, Calif., U.S.A.; Cat. No. 13472-523), the glass developing tank from Brinkmann, Westbury, N.Y., U.S.A. (Cat. No. 25 10 250-9). Kieselgel silica gel G TLC plates, 20 × 20 cm, were obtained from Camag, Muttenz, Switzerland (Cat. No. 30079), silica gel GF TLC plates with fluorescent indicator, 20 × 20 cm, from Analtech, Newark, Del., U.S.A. (Cat. No. 2011).

### *Reagents*

The following reagent-grade chemicals were applied: ammonium hydroxide (concentrated), chloroform, diisopropyl ether, ethyl acetate, methanol, methylene chloride, and *n*-octanol (obtained from J. T. Baker, Phillipsburg, N. J., U.S.A.).

Urine samples were spiked with concentrations ranging from 0.25 to 5 μg/ml of the following drugs: methadone, methadone metabolite (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine obtained from Eli Lilly, Indianapolis, Ind., U.S.A.), morphine, codeine, barbiturates, amphetamine, methamphetamine, cocaine, nicotine, phenothiazines, propoxyphene, methapyrilene, methaqualone, chlordiazepoxide, caffeine, diazepam, dextromethorphan and glutethimide.

Another set of urine samples was collected from patients taking methadone, heroin, codeine, chlordiazepoxide, methaqualone, propoxyphene, diazepam, barbiturates, amphetamine, methamphetamine, and from cigarette smokers and coffee drinkers.

The iodoplatinate spray was prepared as follows: 1 g chloroplatinic acid was dissolved in 10 ml distilled water then added to 30 g KI dissolved in 200 ml distilled water. The mixture was then diluted to 250 ml with distilled water.

### *Procedure*

The spiked urine samples and the urines from patients were extracted for spotting on TLC plates by a slightly modified method of Ho *et al.*[8]. Fifteen millilitres of urine, 0.2 ml conc. ammonium hydroxide, and 25 ml of chloroform–ethyl acetate–methanol (3:1:1) were placed in a 50-ml centrifuge tube and shaken vigorously for 5

min. After shaking, the top urine layer was aspirated and the organic layer was filtered with phase separating paper into a 40-ml conical centrifuge tube and evaporated to dryness under nitrogen in a 70° water-bath. The residue was reconstituted with 2–3 drops of methanol and spotted on a TLC plate. The plate was developed at room temperature, one plate per tank, to a height of 15 cm in one of the methadone TLC solvent systems (I, II, or III). The tanks were not equilibrated. After development, which took about 1 h, the plate was air dried and sprayed with iodoplatinate reagent.

## RESULTS

Table I gives the $R_F$ values for methadone, its primary metabolite, and other common drug standards in the three methadone solvent systems. The table illustrates that all three solvent systems give a good separation of methadone from its primary metabolite without interference by other drugs. All the drugs listed in Table I are vi-

TABLE I

$R_F$ VALUES OF DRUGS FROM URINE SPECIMENS OBTAINED IN THE THREE SOLVENT SYSTEMS

| *Drugs* | *Solvent system* | | | *Color with iodoplatinate* |
|---|---|---|---|---|
| | *I* | *II* | *III* | |
| Methadone | 0.66 | 0.75 | 0.63 | reddish brown |
| Methadone metabolite | 0.74 | 0.81 | 0.73 | reddish brown |
| Chlordiazepoxide | 0.20 | 0.31 | 0.26 | light brown |
| Chlorpromazine | 0.35 | 0.48 | 0.35 | brown |
| Cocaine | 0.77 | 0.84 | 0.78 | brown |
| Codeine | 0.04 | 0.03 | 0.04 | purple |
| Dextromethorphan | 0.07 | 0.11 | 0.09 | brown |
| Diazepam | 0.80 | 0.96 | 0.94 | gray |
| Diphenhydramine | 0.40 | 0.46 | 0.35 | brown |
| Ethinamate | 0.92 | 0.98 | 0.76 | yellow |
| Fluphenazine | 0.05 | 0.12 | 0.08 | brown |
| Glutethimide | 0.83 | 0.93 | 0.97 | yellow |
| Hydromorphinone | 0.01 | 0.02 | 0.01 | gray |
| Meperidine | 0.29 | 0.33 | 0.23 | brown |
| Mephentermine | 0.07 | 0.07 | 0.06 | gray |
| Methapyrilene | 0.28 | 0.40 | 0.30 | blue-gray |
| Methaqualone | 0.84 | 0.98 | 0.85 | brown |
| Morphine | 0.02 | 0.01 | 0.03 | blue |
| Nicotine | 0.19 | 0.29 | 0.28 | blue-gray |
| Oxazepam | 0.23 | 0.31 | 0.28 | yellow |
| Prochlorperazine | 0.06 | 0.11 | 0.09 | brown |
| Promazine | 0.19 | 0.26 | 0.14 | brown |
| Propoxyphene | 0.80 | 0.91 | 0.83 | red |
| Quinine | 0.03 | 0.02 | 0.04 | brown |
| Thiordazine | 0.21 | 0.32 | 0.25 | green-brown |
| Trifluoperazine | 0.08 | 0.15 | 0.17 | brown |
| Triflupromazine | 0.43 | 0.57 | 0.46 | brown |
| Tripelennamine | 0.32 | 0.42 | 0.48 | brown |

TABLE II

$R_F$ VALUES OF DRUGS FROM URINE SPECIMENS USING DIFFERENT VISUALIZING REAGENTS

Visualization reagents: (1) Reacts with iodoplatinate to give brownish red spot; (2) reacts with $H_2SO_4$ to produce white spots; (3) reacts with ninhydrin to produce pink spots; (4) shows a pink spot under UV light (254 nm) on a silica gel GF plate containing a fluorescent indicator; (5) reacts with furfural, HCl and heat to produce a black spot.

| *Drugs* | *Solvent system* | | | *Means of visualization* |
|---|---|---|---|---|
| | *I* | *II* | *III* | |
| Methadone | 0.66 | 0.75 | 0.63 | 1, 4 |
| Methadone metabolite | 0.74 | 0.80 | 0.73 | 1, 4 |
| Amobarbital | 0.88 | 0.87 | 0.94 | 2, 4 |
| Amphetamine | 0.16 | 0.19 | 0.16 | 3, 4 |
| Butabarbital | 0.88 | 0.85 | 0.91 | 2, 4 |
| Caffeine | 0.38 | 0.41 | 0.34 | 4 |
| Diphenylhydantoin | 0.53 | 0.87 | 0.82 | 2, 4 |
| Meprobromate | 0.66 | 0.76 | 0.55 | 5 |
| Methamphetamine | 0.10 | 0.13 | 0.11 | 3, 4 |
| Methaprylon | 0.51 | 0.65 | 0.45 | 2 |
| Pentobarbital | 0.84 | 0.90 | 0.95 | 2, 4 |
| Phenobarbital | 0.67 | 0.61 | 0.36 | 2, 4 |
| Salicylate | 0.01 | 0.02 | 0.01 | 4 |
| Secobarbital | 0.91 | 0.94 | 0.96 | 2, 4 |

sualized with the iodoplatinate reagent. Table II gives the $R_F$ values of drugs not visualizable by iodoplatinate, but obtained by other methods, as indicated in the table.

A sensitivity of 0.25 $\mu$g/ml for both methadone and its primary metabolite in urine is obtained by all three solvent systems with this procedure.

## DISCUSSION

We have tested nearly 300 different solvents in an attempt to find the best solvent to separate and detect drugs of abuse in urine specimens. While many of these systems separated methadone and its primary metabolite, nearly all of them were interfered by one or more common drugs of abuse. Some systems worked well for reference standards but not with those urines which were either spiked with the standards or collected from patients taking drugs. The three solvent systems presented here have been extensively tried, and they work well with actual urine specimens collected from patients taking drugs listed in Tables I and II.

Table I shows that methadone and its primary metabolite are separated and not interfered by any of the common drugs tested. We have analyzed (1) urines spiked with various concentrations of methadone and its primary metabolite, (2) urines spiked with other drugs, (3) urines spiked with various concentrations of methadone and its primary metabolite in combination with other drugs, (4) urines from patients taking methadone, (5) urines from patients taking other drugs, and (6) urines from heavy cigarette smokers and coffee drinkers. We have found that we can consistently detect 0.25-$\mu$g/ml levels of methadone and its primary metabolite without interference from any other drugs or substances found in the urine.

In testing various solvent systems, we have made a number of observations, which are illustrated in Table III. Ethyl acetate (solvent A) causes diazepam, glutethimide, and methaqualone to rise to the top of the plate, propoxyphene to rise slightly, and the remaining drugs to stay at the bottom. The addition of a small volume of ammonia or a small volume of water to a large volume of ethyl acetate (solvents B and C, respectively) increases the separation between methadone and its metabolite. The ammonia causes all the drugs to rise considerably higher whereas the water results in inhibiting the $R_F$ values of some of the drugs. In both cases, however, the separation of methadone and its metabolite is interfered by other drugs.

TABLE III

$R_F$ VALUES OF DRUGS OF ABUSE OBTAINED IN VARIOUS TLC SOLVENT SYSTEMS

Solvent systems: (A) ethyl acetate; (B) ethyl acetate–conc. ammonium hydroxide (99:1); (C) ethyl acetate–water (99:5); (D) methanol–cyclohexane–conc. ammonium hydroxide (90:10:0.8); (E) methanol–methyl ethyl ketone–conc. ammonium hydroxide (89:10:0.8); (F) ethyl acetate–*o*-dichlorobenzene–water–conc. ammonium hydroxide (90:10:1.5:1.0); (G) ethyl acetate–*n*-butanol–conc. ammonium hydroxide (93:7:0.9); (H) ethyl acetate–methanol–conc. ammonium hydroxide (89:10:0.75).

| *Drug* | *Solvent* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *D* | *E* | *F* | *G* | *H* |
| Methadone | 0.12 | 0.76 | 0.15 | 0.55 | 0.63 | 0.73 | 0.75 | 0.66 |
| Methadone metabolite | 0.10 | 0.91 | 0.05 | 0.25 | 0.26 | 0.78 | 0.80 | 0.58 |
| Chlordiazepoxide | 0.11 | 0.42 | 0.32 | 0.91 | 0.88 | 0.33 | 0.39 | 0.58 |
| Chlorpromazine | 0.07 | 0.49 | 0.10 | 0.56 | 0.58 | 0.51 | 0.51 | 0.90 |
| Diazepam | 0.79 | 0.80 | 0.81 | 0.94 | 0.90 | 0.81 | 0.87 | 0.91 |
| Fluphenazine | 0.02 | 0.18 | 0.04 | 0.75 | 0.72 | 0.14 | 0.14 | 0.24 |
| Glutethimide | 0.94 | 0.87 | 0.87 | 0.96 | 0.95 | 0.83 | 0.89 | 0.94 |
| Methaqualone | 0.83 | 0.88 | 0.81 | 0.95 | 0.91 | 0.83 | 0.88 | 0.91 |
| Morphine | 0.01 | 0.02 | 0.02 | 0.44 | 0.54 | 0.02 | 0.05 | 0.09 |
| Nicotine | 0.09 | 0.35 | 0.10 | 0.73 | 0.74 | 0.38 | 0.38 | 0.39 |
| Propoxyphene | 0.35 | 0.85 | 0.45 | 0.76 | 0.80 | 0.81 | 0.86 | 0.85 |
| Thioridazine | 0.03 | 0.33 | 0.06 | 0.61 | 0.56 | 0.40 | 0.36 | 0.35 |
| Trifluprom azine | 0.11 | 0.61 | 0.23 | 0.66 | 0.66 | 0.68 | 0.62 | 0.59 |

Another interesting phenomenon was that methanol-based systems (solvents D and E) gave higher $R_F$ values for methadone than its metabolite, whereas in ethyl acetate-based systems (solvents F and G) the order of $R_F$ values was reversed, except when small amounts of methanol were added to the ethyl acetate (solvent H).

The spots on the plate for methadone and its primary metabolite fade very rapidly after spraying with iodoplatinate, especially if the drugs are present in low concentrations. Thus, the plates must be read immediately after spraying. Occasionally, after several hours of sitting, the spots will come back into view as white spots on gray background instead of the normal brownish red.

We have found that the way the plate is placed in the tank is critical. Better results are obtained when only one plate per tank is developed and the tank is not equilibrated.

The three solvent systems described here give good results in separating and detecting various drugs of abuse from urine specimens, including methadone and its major metabolite.

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