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## Simplified multiple sample urinalysis in support of a methadone clinic

Analytical procedures for the detection of acidic, neutral and basic drugs in urine are nearly always based on organic extraction. Several procedures are currently available[1-4]. We have borrowed from these procedures and have modified the techniques to fit our needs. As a result we have developed a procedure utilizing two technicians and one chemist, capable of handling 400–500 urine samples a week.

*Materials and methods*

*Reagents.* Silica Gel GF with 10% $CaSO_4 \cdot 1/2H_2O$ was used as sorbent.

Extracting solvents used were: (a) Chloroform, (b) chloroform–isopropanol (9:1).

Developing solvents used were: (a) chloroform–methanol–ammonium hydroxide (85:10:1), (b) ethyl acetate–methanol–ammonium hydroxide (85:10:1.5), (c) chloroform–acetone (9:1).

The following spray reagents were used: (a) Ninhydrin–acetone. 0.4 g of ninhydrin is dissolved in acetone and diluted to 100 ml with acetone. This is stored in a sealed bottle. (b) Ninhydrin–acid. 0.3 g of ninhydrin is dissolved in 100 ml of isopropanol and 1 ml of glacial acetic acid. (c) Potassium iodoplatinate. 1 g of platinum chloride is dissolved in 100 ml of water containing 10 g of potassium iodide. This mixture is diluted to 500 ml with water and stored in dark bottles. (d) Concentrated hydrochloric acid. (e) Mercuric sulfate. 5.0 g of mercuric oxide are suspended in 100 ml of distilled water. 20 ml of concentrated sulfuric acid are slowly added with stirring. This is then cooled to room temperature and diluted to 250 ml with water.

The reference drugs were: (a) Barbiturate reference. 30 mg each of amobarbital, phenobarbital and seconal are dissolved in ethanol. This is filtered and concentrated to 5 ml. (b) Amphetamine and narcotic reference. 30 mg each of morphine, codeine, benzadrine, methamphetamine, methadone and cocaine are dissolved in ethanol. This is filtered and concentrated to 5 ml.

*Preparation of drying apparatus.* A 12-in. electric frying pan is filled about three-fourths full of cement. Eighty holes of the appropriate size are made using evenly arranged test tubes.

The test tubes are held in an even upright position by a piece of ½-in. pine with eighty holes corresponding to the tube holes in the frying pan. The board is supported on a 2-in. frame that rests on the concrete.

*Preparation of air distributor.* To speed solvent evaporation, air is directed to each sample in the dryer through an air distributor. The air distributor is made from two sheets of clear acrylic plastic and glass medicine droppers. The medicine droppers are set into place with epoxy glue. The distributor is made to fit only half the tubes in the frying pan.

*Preparation of plates.* A large number of plates can be easily made at one time. They are easily stored and activated for use when needed. Five glass plates are coated with a 300-$\mu$ layer of Silica Gel GF prepared from a slurry consisting of 30 g Silica Gel GF, 55 ml demineralized water, and 5 ml methanol. The silica gel should contain

*Separation of drugs from urine.* 10 ml of urine are poured into each of two screw cap test tubes. Barbiturates are extracted from one tube while opiates and amphetamines are extracted from the other tube.

*Barbiturate extraction.* To one of the screw cap tubes containing urine, three drops of 4 *N* sulfuric acid are added. This should adjust the pH to about 2. 4 ml of chloroform are added, the screw cap is put on and the sample mixed well. The tubes are then centrifuged for 3 min. The urine is removed with a water aspirator, and the chloroform is transferred to a test tube. A glass bead is added to prevent bumping and the tube is placed in the drying apparatus set at 60°. The use of a medium flow of air through the air distributor will greatly speed evaporation. The chloroform will evaporate in about 30 min.

When the chloroform has evaporated, 50 $\mu$l of chloroform is added to redissolve the residue. The sample is spotted on a chromatogram plate with a disposable pasteur pipet along with the appropriate reference. Fourteen samples and four references can be spotted on each plate.

The plate is then developed in the chloroform–acetone (9:1) solvent. When the solvent has traveled 10–12 cm from the spot, the plate is removed and air dried. The plate is then sprayed heavily with mercuric sulfate. The barbiturates will show as white spots on a grey background. These spots fade as the plate dries so the results must be recorded immediately.

*Alkaloid and amphetamine separation.* To the other 10 ml urine sample, 1.0 ml of concentrated hydrochloric acid is added. The tubes are then put in a No. 2 ½ "tin can" and placed in the pressure cooker. The cooker is allowed to come up to pressure and remain for exactly 5 min. It is then slowly cooled until atmospheric pressure is reached (about 15 min). Force-cooling of the pressure cooker should be avoided since some of the acidified urine will be lost. It is lost because the pressure cooker can be cooled faster than the samples inside. This causes them to "bump" and boil over.

No significant morphine losses due to acid hydrolysis have been observed under these conditions. At higher concentrations of hydrochloric acid, morphine losses become apparent and significant. However, this is not the case with the amount of acid used here.

The test tubes are then removed from the pressure cooker and cooled to room temperature. The acidified urine is then neutralized by adding 1.5 ml concentrated ammonium hydroxide. This adjusts the pH to about 9. 7.5 ml of chloroform–isopropanol (9:1) are then added to each tube. The tubes are capped, mixed for about 5 sec and the layers allowed to separate for at least 5 min. It may be necessary to centrifuge some samples to complete the separation. The urine is then removed by a water aspirator and the solvent poured into a 16 × 125 mm test tube. A glass bead, 0.3–0.5 ml methanol–hydrochloric acid (100:1) are added and the sample dried in the electric frying pan at 60° as previously described for the barbiturate extraction. It is critical that the right amount of methanol–hydrochloric acid is added. Too little or too much decreases the sensitivity of methadone, morphine and codeine significantly. The other drugs are affected to a lesser degree.

50 $\mu$l of chloroform–isopropanol (9:1) are added to each tube to redissolve the residue. The sample and reference are spotted on a coated glass plate with a

in ethyl acetate–methanol–ammonium hydroxide (85:10:1.5). When the solvent has traveled 10–12 cm from the spot, the plate is removed and air dried.

The chromatogram plate is then sprayed with ninhydrin–acetone and placed under UV light for approximately 10 min. The D,L-amphetamines are noted as pink spots. The plate is then sprayed with ninhydrin–acid and placed in the oven at 105° for 10 min to visualize the methamphetamine. This also improves the resolution of the D,L-amphetamines. The plate is next sprayed with potassium iodoplatinate. Methadone, a methadone metabolite, morphine, codeine, and cocaine become visible. The methadone, methadone metabolite, and cocaine are recorded at this point. To confirm the presence of morphine and codeine, the plate is sprayed with concentrated hydrochloric acid and allowed to dry for at least an hour. All other drugs fade and the morphine and codeine intensify in color as blue-black spots.

To confirm the presence of any alkaloids and amphetamines that show in the

TABLE I

DRUG $R_F$ VALUES

Solvent 1 = chloroform–methanol–ammonium hydroxide (85:10:1); solvent 2 = chloroform–acetone (9:1); solvent 3 = ethyl acetate–methanol–ammonium hydroxide (85:10:1.5).

| *Drug* | $R_F$ | | |
|---|---|---|---|
| | *Solvent 1* | *Solvent 2* | *Solvent 3* |
| Morphine sulfate | 0.22 | | 0.11 |
| Codeine phosphate | 0.59 | | 0.21 |
| Methadone hydrochloride | 0.80 | | 0.67 |
| Methadone metabolite | | | 0.60 |
| Benzadrine | 0.56 | | 0.31 |
| Methamphetamine | 0.47 | | 0.22 |
| Cocaine hydrochloride | 0.96 | | 0.76 |
| Seconal | | 0.77 | |
| Amobarbital | | 0.73 | |
| Phenobarbital | | 0.53 | |

TABLE II

LIMITS OF DETECTION AND EXTRACTION EFFICIENCY

| *Drug* | *Minimum amount detectable on the TLC plate (μg)* | *Extraction efficiency (%)* |
|---|---|---|
| Morphine sulfate | 2 | 57 |
| Codeine phosphate | 2 | 40 |
| Methadone hydrochloride | 1 | 91 |
| Benzadrine | 3 | 71 |
| Methamphetamine | 5 | 62 |
| Cocaine | 1 | 33 |
| Seconal | 1 | 50 |
| Amobarbital | 2 | 67 |
| Phenobarbital | 1 | 50 |

ethyl acetate–methanol–ammonia solvent, the suspected samples are re-extracted from another 10 ml of fresh urine, spotted on a new plate and developed in chloroform–methanol–ammonium hydroxide (85:10:1). The same spray sequence as above is followed and the drugs identified. This confirmation eliminates many false positives that sometimes appear with a single solvent system.

*$R_F$ values*

$R_F$ values are very dependent upon the solvent concentration in the developing tanks. Table I lists the $R_F$ values for drugs for which this lab screens. Table II gives the minimum amounts detectable on TLC plates along with the extraction efficiency of the procedure.

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