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Note

Preadsorbent thin-layer chromatography

III. Direct detection of quinine in urine as a presumptive test for heroin

J. A. VINSON*, M. A. PACZKOWSKI and C. PRONKO
Chemistry Department, University of Scranton, Scranton, PA 18510 (U.S.A.)
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Many methods are currently available for detecting morphine in the urine of heroin users. Those which have proven useful in screening large numbers of samples are thin-layer chromatography (TLC)[1,2], gas–liquid chromatography[3,4], fluorometry[5–7], radioimmunoassay[8], enzyme multiplied immunoassay[9] and hemagglutination inhibition[10,11]. These methods have recently been critically reviewed[12,13].

The immunoassays are highly sensitive, detecting from 30–400 ng/ml of total morphine for a preliminary screen[13]. They are easy to use, rapid and do not require sample treatment. However, these assays suffer from cross-reactivity with a number of drugs and thus give rise to false positives as compared with TLC of the hydrolyzed extract. In addition, the cost is quite high for immunoassays.

Fluorometry is also quite sensitive and reliably detects 0.22 μg/ml of free morphine[6]. A new manual method is sensitive to 0.04 μg/ml[7]. However, fluorometry requires considerable sample pre-treatment, and is subject to non-specific endogenous background interference. In addition, the extract for morphine analysis by fluorometry cannot then be used to screen for other drugs.

Gas chromatography is also very sensitive but the expense and relatively long retention times for morphine with many liquid stationary phases limits gas chromatography to confirmation rather than initial screening.

TLC suffers from two faults, a lack of sensitivity and the need for sample treatment, although the extract can be utilized for screening other drugs. The sensitivity of TLC for free morphine is 1 μg/ml at the 100% detection level[12]. Since the amount of free morphine excreted varies from 5 to 20% of the total, only an acid-hydrolyzed urine is suitable for efficient screening using TLC. The big advantages of TLC are its low cost and selectivity. It is the least expensive and thus the most widely used of all the screening methods.

Quinine is a major diluent of heroin, especially in the eastern part of the United States. It is used chiefly as a camouflage to prevent user determinations of purity by taste. It is readily detectable by the same fluorometric methods used to detect morphine in urine. The limit of detection of quinine by fluorometry after extraction is 0.1 μg/ml[5]. A New York City study in 1973[12] showed that in 42 urine samples from methadone patients, of those positive for quinine, 78.5% were positive for morphine by TLC after acid hydrolysis. Mule and Hushin[5] found only 1.3% of urines were

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positive for morphine and negative for quinine by fluorometry. The false negatives were 6.6%, *i.e.*, quinine positive and morphine negative. However, some of these samples were probably positive for morphine but below the detection limit of the assay. Thus, it appears quinine is an excellent marker for prediction of heroin use.

This work describes a simple, rapid procedure for the detection of quinine in urine by TLC without extraction.

MATERIALS

Microcaps, Drummond, 10 μl disposable; developing tank, Kontes Glass, 22 × 10 × 23 cm; developing solvent: chloroform–methanol–acetic acid (80:20:3); ultraviolet visualization chamber, Ultraviolet Products, Model CC-20; silica gel plates (Whatman, Clifton, NJ, U.S.A.), precoated LKD-5, 20 × 20 cm glass plates with nineteen 1-cm channels containing a 250-μm layer of silica gel and a 3-cm preadsorbent area.

Urine samples from a methadone program were obtained from the Allegheny County Coroner Drug Urine Screening Program.

PROCEDURE

Urine samples were stored frozen until use. After thawing, they were filtered or centrifuged to remove solids. A 50-μl volume of each urine is spotted with a 100-μl syringe along with 10 μl of a solution of quinine sulfate standard (1 mg/ml) in methanol. These were spotted in the middle of the preadsorbent area of each channel. A maximum of eighteen samples and one standard can be spotted on each plate. The samples are applied with the TLC plates on a hot plate at a maximum temperature of 65°C or with the aid of a hair dryer. No attempt is made to keep the spots small but the preadsorbent area should not be completely flooded vertically with sample for optimum chromatography. After the plate is dried, it is placed in a developing tank which has been equilibrated with solvent for 30 min using a sheet of filter paper wet with solvent. The plate was developed at 20°C for 10 cm from the top of the preadsorbent area. After removal, the plate was air-dried for 5 min. The plate was then observed for the blue fluorescence of quinine and its metabolites in long (366 nm) or short wave (254 nm) UV light. Those samples positive for quinine and/or its metabolites should then be confirmed for morphine by other methods such as TLC, gas chromatography or immunoassay. The samples negative for quinine should then be screened for drugs and morphine by the usual TLC method.

RESULTS AND DISCUSSION

In a study of quinine metabolism, 325 mg of quinine sulfate (Lilly, Indianapolis, IN, U.S.A.) was ingested and urine samples obtained daily for twelve days. The R_F values (measured from the top of the preadsorbent area) for the bands due to quinine and its two metabolites are shown in Table I. M_1 and M_2 are probably hydroxy metabolites of quinine (Q) and are more polar thus having lower R_F values than quinine. Q and M_1 are detected the day after ingestion and decrease rapidly thereafter. M_2 is seen the day after ingestion and then increases to a maximum five days after

TABLE I

R_F VALUES OF QUININE AND ITS METABOLITES

Compound	*R_F range*
Quinine Q (standard)	0.77–0.79
Quinine Q (urine)	0.74–0.77
Metabolite M_1 (urine)	0.73–0.75
Metabolite M_2 (urine)	0.46–0.53

ingestion. It can still be seen ten days later. No spots were visible eleven days after ingestion. A similar detection limit was seen by Mule and Hushin[5] using fluorometry. A sensitivity study using a blank urine spiked with quinine gave a limit of detection of 0.4 ng in 50 μl which is equivalent to 8 ng/ml of urine. This is much lower than the 100 ng/ml found for the solution fluorometric method. The limitation of solution fluorometry is due to the endogenous background fluorescence in all urines which must be "blanked out" using drug-free urine. The acidic nature of the developing solvent in the TLC method appears to increase the fluorescence. Complete removal of the developing solvent by heating in an oven greatly diminishes the fluorescence.

The specially manufactured preadsorbent area retains the highly polar compounds in urine and allows the less polar materials to move with the developing solvent until the top of the preadsorbent area is reached. Then the developing solvent in contact with silica gel separates these compounds as bands. Bands are preferable to spots, which are the norm for conventional TLC, because improved resolution and sensitivity result. Endogenous fluorescent spots are often seen below an R_F of 0.3 and appear as blue, yellow and purple bands.

A study was made of thirteen drugs which were fluorescent in a published TLC street drug procedure[14] and thus, possible interferences. The results are shown in Table II. Due to the excellent TLC separation, no drug constitutes an interference to detecting quinine or its metabolites by this method. No fluorescence was seen for morphine, phenmetrazine, diphenhydramine, chloroquine, adrenaline, thiopropazate and perphazine in this TLC procedure.

Fifty urines were obtained from a Pennsylvania methadone clinic and subjected to the direct TLC procedure. They were also analyzed by conventional extraction and TLC for quinine, morphine, amphetamines, barbiturates and methadone. Of the 50 samples, 11 were found positive for morphine by conventional methodology and 9 of these 11 or 82% were found to be positive for quinine by direct TLC (Table III). Ten of these 11 samples were found to contain quinine by conventional TLC. The

TABLE II

$R_{Quinine}$ VALUES OF QUININE METABOLITES AND DRUGS RELATIVE TO QUININE

Drug	*Color*	*$R_{Quinine}$*
Trifluoperazine	Blue-green	0.43
Morphine	Non-fluorescent	0.45
Mephentermine	Blue	0.51
Metabolite M_2	Blue	0.68
Metabolite M_1	Blue	0.96
Quinine	Blue	1.00
Thioridiazine	Blue	1.10
Pentazocine	Blue	1.28
Quinidine	Blue	1.31

false negative rate was 1/11 or 9%. Interestingly, unchanged quinine rather than its metabolites appears to be the most prevalent form in these urines.

TABLE III

A COMPARISON OF THE STANDARD EXTRACTION/TLC METHOD AND THE DIRECT TLC METHOD FOR THE DETECTION OF QUININE (Q), ITS METABOLITES M_1 AND M_2, AND MORPHINE

Sample number	*Results*	
	Conventional TLC	*Direct TLC*
1	+Q, +Morphine	+Q, +M_1, +M_2
2	+Q, +Morphine	+Q, +M_1, +M_2
3	+Q, +Morphine	+Q, +M_1, +M_2
4	+Q, +Morphine	+Q, +M_1, +M_2
5	+Q, +Morphine	+Q, +M_1, −M_2
6	+Q, +Morphine	+Q
7	+Q, +Morphine	+Q
8	+Q, +Morphine	+Q
9	+Q, +Morphine	+Q
10	+Q, +Morphine	+Q
11	−Q, +Morphine	−Q

CONCLUSION

This paper describes a simple, rapid TLC procedure for the detection of quinine in urine. Quinine is a common diluent of heroin and its presence in the urine is an indicator of heroin abuse. Urine is directly spotted on a commercial TLC plate equipped with a preadsorbent area and detected by native fluorescence.

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