CHROM. 5438

IMPROVED CHROMATOGRAPHIC TECHNIQUES AND THEIR INTERPRETATION FOR THE SCREENING OF URINE FROM DRUG-DEPENDENT SUBJECTS

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(Received April 23rd, 1971)

SUMMARY

A screening procedure is described for the identification of barbiturates, amphetamines and narcotics in urine. A simple pH adjustment and extraction with organic solvents is followed by a combination of thin-layer and gas–liquid chromatography. Interference and interpretation are discussed, with special reference to the metabolites of barbiturates, nicotine and phenothiazines.

INTRODUCTION

The problem of detecting and supervising drug-dependent persons in various communities has increased during the past decade. This has resulted in considerable research directed to perfecting screening tests that can be applied to urine[1–12]. The techniques described include gas chromatography (GC)[1,2,7,12] ion-exchange chromatography[3,10,11] thin-layer chromatography (TLC)[3,12] and fluorimetry[12], most of them being used for the identification of barbiturates, amphetamines and some of the narcotics.

An analyst establishing a urine-screening service *de novo* is presented, therefore, with a large number of procedures, some of them so similar, that it is difficult to evaluate the merits of any particular method. Moreover, little attention has been paid to the interpretation of results or any interferences that might be encountered. This paper describes and explains in detail the techniques selected and also indicates the reasons for preferring these methods. It attempts to clarify those features and interferences that may prove confusing in practice.

MATERIALS

Thin-layer chromatography

Plates were prepared from a slurry of 30 g of Silica Gel G (Anderman, London) and 65 ml of water. The thickness of the layer was 250 μ and after drying at 110° for 30 min, the plates were stored in an air-tight box containing self-indicating silica gel crystals (Hopkin & Williams, Ltd., Chadwell Heath, Essex).

Standard reference solutions of drugs were made up in ethanol or chloroform

to contain 1 mg/ml of drug, equivalent to 1 μg for each 1 μl spotted on the plate.

All solvents and chemicals were of analytical grade and were purchased from Hopkin & Williams Ltd.

Mercuric chloride diphenylcarbazone reagent. Equal volumes of a 2 % ethanol solution of mercuric chloride and a 0.2 % ethanolic solution of diphenylcarbazone were mixed together. The reagents were stored away from direct sunlight.

Mercurous nitrate reagent. Concentrated nitric acid was added to a 1 % solution of mercurous nitrate just until the solution cleared. This solution was stable.

F.P.N. reagent. This was prepared as described by FORREST AND FORREST[13]. 5 ml of aqueous 5 % ferric chloride solution were mixed with 45 ml of 70 % perchloric acid–water (1:5) and 50 ml of concentrated nitric acid–water (1:1). This solution will keep indefinitely.

Iodoplatinate reagent[14]. 3 ml of a 10 % solution of chloroplatinic acid was diluted with 97 ml of water; a 100 ml of an aqueous solution of 6 % potassium iodide was then added. This reagent will keep if stored in brown glass bottles.

Gas chromatographic conditions

A Pye 104, or Perkin-Elmer F-11 gas chromatograph was employed with flame ionization detector.

A 5 ft. glass column was silanised by filling with a 5 % dichlorodimethylsilane solution in benzene, and standing 24 h. The column was emptied, dried in an oven, then packed with 10 % Apiezon L, 10 % KOH on 80–100 mesh AW/DMCS Chromosorb W (Perkin-Elmer, Beaconsfield, Bucks.), and conditioned overnight at 180°.

The support was coated by dissolving KOH in methanol adding the Chromosorb W and leaving 2 h with occasional swirling. The solvent was then evaporated off with a Bucchi Rotavapour (Orme Scientific, Middleton, Manchester). The appropriate amount of Apiezon L, dissolved in methylene chloride was then added to the alkalinised support and the procedure repeated.

The instrument settings for the Pye 104 were: Injection heater 160°; column temperature 140°; air 400 ml/min; hydrogen 45 ml/min; nitrogen 50 ml/min; sensitivity 2×10^{-10} A. Similar settings were used for the F-11.

METHODS

Extraction procedures

Barbiturates. 10 ml of urine were made acid with 1 ml of *N* HCl and extracted with 10 ml of chloroform by shaking vigorously for 5 min (Griffin Flask Shaker, Griffin & George, Alperton, Middlesex). After centrifuging, the aqueous layer was aspirated off and the organic layer washed with 10 ml of a 5 % lead acetate solution. The aqueous layer was then removed, and the chloroform dried by passing through a Whatman No. 90 filter paper into a 10 ml conical tube (BC24/C14T, Quickfit & Quartz, Stone, Staffordshire). The organic layer was quickly evaporated to dryness by placing this tube in a 250 ml beaker containing hot water and passing a stream of air through the solution.

Amphetamines. 5 ml of urine were pipetted into a 10 ml conical tube. Two drops of 2 *N* NaOH were then added followed by 0.1 ml of chloroform. The contents were mixed for 1 min on a Whirlmix (Fisons, Loughborough) and then centrifuged hard for 5 min to separate the chloroform layer.

Narcotics. 10 ml of urine were saturated with solid sodium bicarbonate and extracted with an equal volume of chloroform–isopropanol (9:1) by shaking vigorously for 5 min. After centrifuging, the aqueous layer was aspirated off and the organic phase dried and evaporated in the manner described previously for barbiturates.

Detection

Barbiturates. The dried sample was reconstituted with 0.1 ml of chloroform and 30 μl of this solution were applied to the plate together with 20 μl of the standard reference solutions of amylo-, quinal- and phenobarbitone. The plate was run in an unsaturated tank containing chloroform–acetone in the proportions 9:1. It was found that better separations were obtained by drying the acetone over anhydrous sodium sulphate before use. After drying, the plate was sprayed with the mercuric chloride–diphenylcarbazone reagent. The barbiturates gave white spots on a lilac background.

Amphetamines. The separation of amphetamine, methylamphetamine and some related compounds on the gas chromatograph are shown in Fig. 1a. A standard mixture was made up in ether to contain 10 mg % of each drug and 3 μl of this solution was injected on the column prior to analysis of a sample.

A sample of the chloroform layer from the amphetamine extract was withdrawn by placing the syringe needle into the lower layer with the plunger slightly withdrawn. By depressing the plunger, the expelled air prevented any urine entering the syringe. 5 μl of the chloroform were then withdrawn and injected onto the column.

The retention times of any peaks appearing in the sample chromatogram were compared with those of the standard chromatogram.

Narcotics. The dried extract already described was reconstituted in 0.1 ml of ethanol and portions applied to two different plates as follows:

Plate A. This was divided into two halves. A single 30 μl spot of the specimen extract was applied to each half of the plate. 20 μl of morphine, codeine and methadone standards were applied to one half and chlorpromazine to the other. The plate was run in an unsaturated tank containing benzene–dioxan–ethanol–ammonia (50:40:5:5) (System A). This is mixed in a separating funnel, allowed to stand for 10–15 min and the lower layer rejected before use.

After drying under a cold air blower (H. J. Latham Ltd., Shoeburyness) until all the ammonia and solvent were removed, the phenothiazine half of the plate was sprayed with F.P.N. reagent and the narcotic half with iodoplatinate reagent. The two halves were then compared.

Plate B. A 30 μl spot of the sample extract was applied to a second plate, with 20 μl of each of the morphine, methadone and cocaine standards as reference. This was then run in an unsaturated tank with methanol–12 *N* ammonia (100:1.5) (System B) as the solvent. After running, the plate was dried and sprayed with iodoplatinate reagent.

DISCUSSION

Chromatographic development

We have used unsaturated tanks in all our thin-layer procedures. These were found to be more suitable for day-to-day work since it was not necessary to equilibrate the systems.

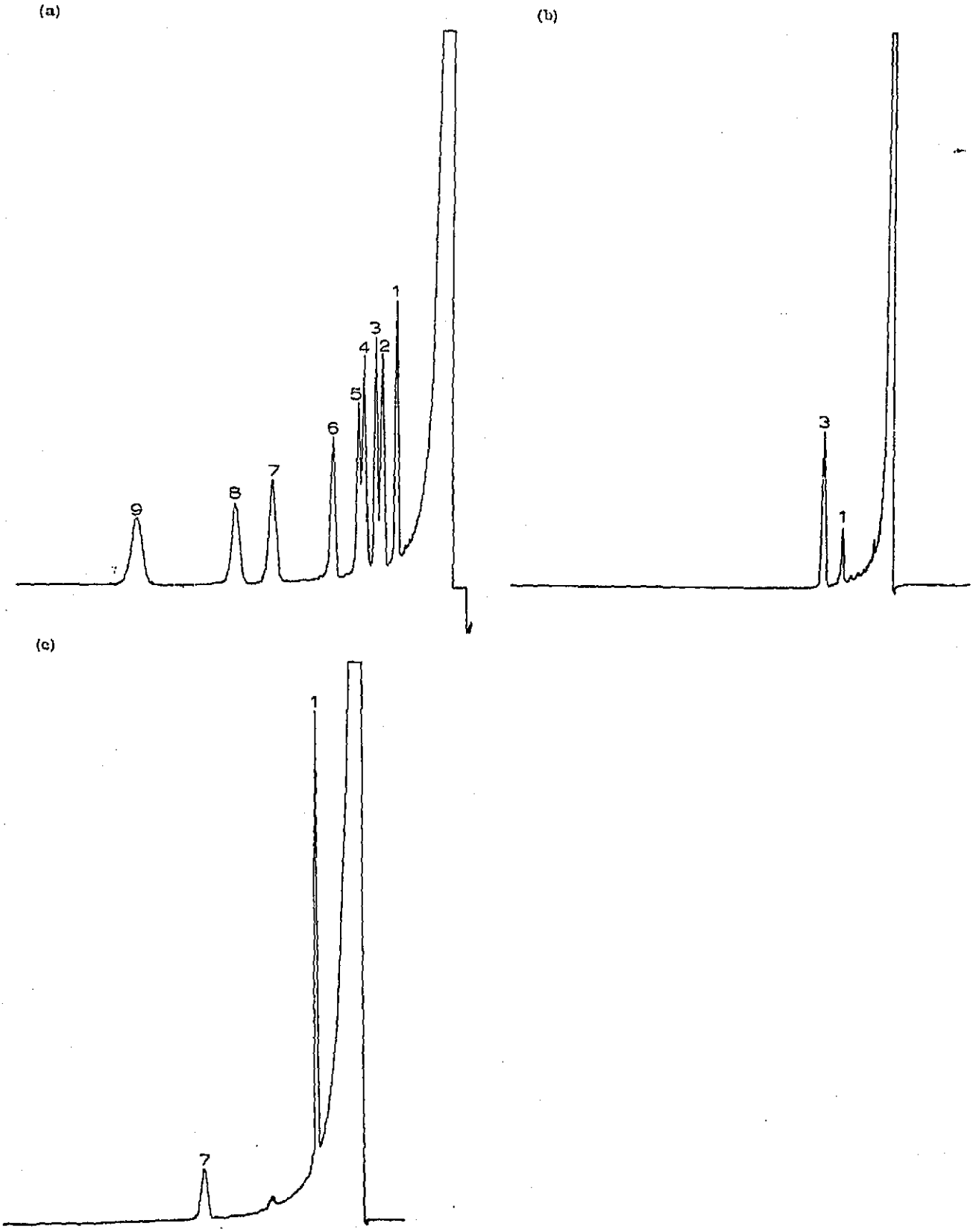


Fig. 1. (a) Separation of some amphetamine-like drugs. 1 = amphetamine, 2 = fenfluramine, 3 = methylamphetamine, 4 = pargyline, 5 = tranylcypromine, 6 = mephentermine, 7 = nicotine, 8 = chlorphentermine, 9 = phenmetrazine. (b) chromatogram of an extract of 5 ml of urine 24 h after taking 20 mg of methylamphetamine hydrochloride. (c) chromatogram from an extract of 5 ml of urine 24 h after taking 20 mg of amphetamine sulphate.

Reference standards were always applied to each plate since R_F values alone are not so reliable with hand-made plates. Variations in laboratory humidity can also affect the R_F reproducibility since it influences the amount of water the silica gel will adsorb from its surroundings during the time the extract is being applied to the plate.

Each 20 × 20 cm plate was divided vertically in ten equal columns and a line was drawn horizontally across the plate 10 cm from the origin. By waiting until the solvent reached this line in all the columns, the same elution distance was ensured and a greater reproducibility of R_F values across the plate achieved. However, co-extractable material in a few urines tended to hold back the morphine and methadone spots in System B. DAVIDOW *et al.*[5] reported an opposite effect in their solvent systems which they attributed to urea. MULÉ[12] has also noted the difference between R_F values of drugs extracted from urine and those from non-extracted reference standards.

Barbiturates

Most authors extract barbiturates at an acid pH. DAVIDOW[6] has, however, recommended a single extraction of the urine buffered at pH 9.6 followed by subsequent TLC for amphetamines, barbiturates and narcotics. Unfortunately, in our experience, alkaline extraction resulted in low recoveries for phenobarbitone and barbitone. When extracting with chloroform–isopropanol (9:1) at pH 9.6, recoveries from urine using our procedure were only 49.1 % for phenobarbitone and 31.4 % for barbitone. Extraction with chloroform alone lowered these recoveries to 5.1 % for phenobarbitone and 3.3 % for barbitone. Where barbiturate addiction is suspected, therefore, we feel that this procedure should be avoided.

Ion-exchange methods of extraction of drugs from urine have been reported by DOLE[10] and by MARKS AND FRY[11]. A subsequent examination of the DOLE method by both MULÉ[15] and MONTALVO *et al.*[3] emphasised the extremely low recoveries of barbiturates (pentobarbitone 2.4 ± 0.8 %)[15]. Hence in our opinion this procedure cannot be recommended for routine barbiturate screening.

The clean-up of the organic phase by washing with 5 % lead acetate has been reported by FRAHM *et al.*[16]. Their ether extract was found to take up larger amounts of urinary pigments than the chloroform which we preferred. Even so the treatment with lead acetate gave a much improved chromatogram. Fig. 2 shows the large streaks from the origin due to these urinary constituents, which after treatment with lead acetate, are completely removed. We found the mercuric chloride–diphenylcarbazone spray more sensitive than the more commonly used mercurous nitrate spray which gave white spots on a grey back-ground. No doubt this was due, in part, to the contrast of the white spots on a lilac background. Another advantage of lead acetate washing was the increased sensitivity with mercurous nitrate solution. This we were not able to explain, for not only did the mercuric chloride–diphenylcarbazone reagent give better results, but black spots were obtained with mercurous nitrate, although the standards (not treated with lead acetate) still gave their customary white spots.

Chloroform was also chosen in preference to ether since smaller quantities of barbiturate metabolites are extracted by this solvent (removing 4–10 % in contrast to the 40–50 % extracted by ether in the case of hydroxyamylobarbitone). Nevertheless, these metabolites were still evident in the chromatograms of a number of urines since the concentration of excreted unconjugated hydroxy-barbiturates is

many times that of the unchanged drug. The R_F values of these metabolites are, however, lower than phenobarbitone in chloroform–acetone (see Fig. 2).

Amphetamines

After extracting amphetamines from alkalinised urine, any concentration of the organic phase can involve extensive loss, especially in the case of amphetamine itself. The evaporation of 10 ml of ether containing 50 μg of amphetamine using the technique described above, even with the surrounding beaker filled with ice water resulted in a loss of 98 % of the amphetamine if the solvent was allowed to go to dryness. The addition of acid to the organic phase is therefore imperative if thin-layer techniques are to be considered. We have not employed such a procedure, since it is usually followed by visualisation with such sprays as ninhydrin, fast blue B or bromo-cresol purple which are non-specific in their reactions.

The use of GC for routine screening is generally not favoured because sample preparation procedures are often very time consuming. However, the extremely simple extraction procedure outlined above, which has been described by RAMSEY AND CAMPBELL[17], does not require any solvent concentration, and being more sensitive and specific than thin-layer procedures has much to commend it. Our recovery of amphetamine from urine was 76–80 % at the 1 μl/ml level. The technique was able to detect both methylamphetamine (Fig. 1b) and amphetamine (Fig. 1c) for 24 h in the urine of volunteers receiving single 20 mg oral doses. Fig. 1b (volunteer taking methylamphetamine) has two peaks in the chromatogram, one due to methyl- amphetamine and the other due to amphetamine formed metabolically. Fig. 1c (volunteer taking amphetamine) has one peak for amphetamine and a second peak at retention time 12 min due to nicotine absorbed from tobacco smoke. This nicotine peak must be allowed to elute when performing numerous analyses to avoid confusion in subsequent chromatograms. Alternatively, having once established its retention time by timing the next injection, it can be arranged to "lose" this peak under the solvent peak of the second sample, thus saving the time needed for each analysis.

The statement by MULÉ[12] that phenmetrazine has a shorter retention time than amphetamine is erroneous. In fact as expected on the basis of their chemical structures phenmetrazine elutes after amphetamine (Fig. 1a).

Narcotics

In practice, the detection of heroin abuse is of paramount importance so that our procedure has been designed with special emphasis on the extraction of its metabolite, morphine, from urine.

It is essential also to differentiate between the two closely related compounds, morphine and codeine and in System A these are clearly separated, running at R_F 0.12 and 0.28, respectively. However, in this system cocaine, methadone and its metabolite N-demethylmethadone run together on the solvent front. System B was chosen therefore specifically to separate these three compounds, although morphine and codeine now have the same R_F of approximately 0.33. The R_F values of the drugs commonly encountered in these systems are shown in Table I. In our experience, identification of narcotics in one TLC system is unreliable and we prefer to obtain R_F values in two systems before making a positive identification.

System A is a two phase system and removal of the lower aqueous phase

Fig. 2

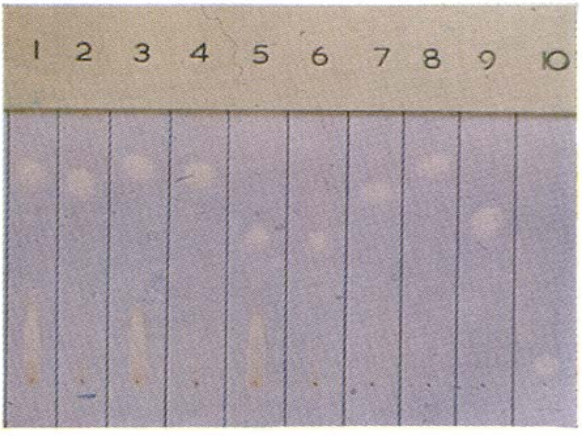
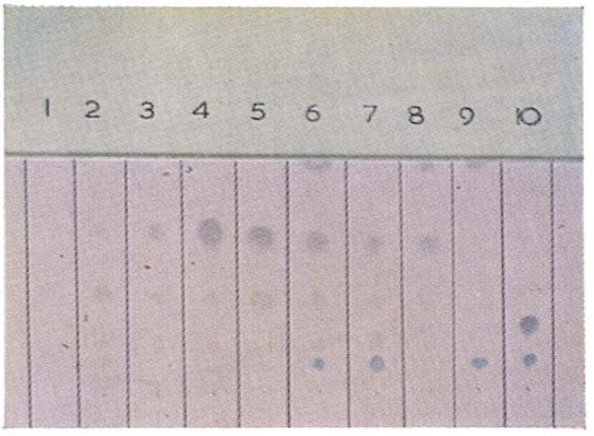


Fig. 3



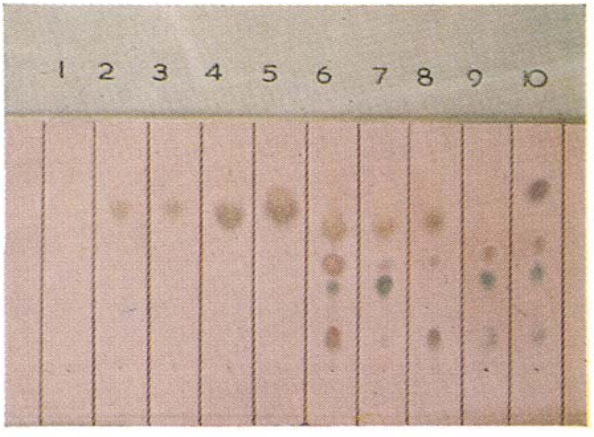


Fig. 4

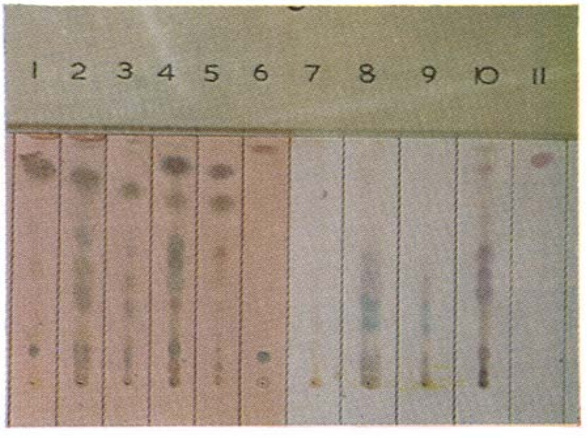


Fig. 5

Fig. 2. Barbiturate plate. Columns 1, 3 and 5 show extracts from urine containing pentobarbitone, quinalbarbitone and phenobarbitone, respectively. Columns 2, 4 and 6 show extracts from the same urines after lead acetate clean-up. Columns 7, 8, 9 and 10 are reference standards of pentobarbitone, quinalbarbitone, phenobarbitone and hydroxyamylobarbitone.

Fig. 3. Narcotic plate, System A. Column 1 is an extract from a non-smoker. Columns 2, 3 and 4 are extracts from smokers. Column 5 has reference standards of nicotine and cotinine. Columns 6, 7 and 8 are extracts from addicts who smoke while column 9 shows an extract from a non-smoking addict. 6 = morphine and methadone–cocaine, 7 = morphine, 8 = methadone–cocaine 9 = methadone–cocaine and morphine. Column 10 has reference standards of morphine and codeine.

Fig. 4. Narcotic plate, System B. Chromatogram of the same extracts shown in Fig. 3. The spots at the solvent front in columns 6, 8 and 9 in Fig. 3 are now shown to be methadone and its metabolite and not cocaine. Column 10 now has reference standards of cocaine, methadone, morphine and N-demethylmethadone.

Fig. 5. Narcotic plate, System A. Columns 1–6 sprayed with iodoplatinate and columns 7–11 sprayed with FPN reagent. Columns 1 and 7 are extracts from the urine of an addict not receiving phenothiazines. Columns 2, 3, 4, 5, 8, 9 and 10 are extracts from the urine of addicts treated with phenothiazines. Columns 6 and 11 have the reference standards morphine and chlorpromazine.

necessary to avoid double running of the plate. Occasionally a further complication can arise in this system if peroxides are found in the dioxan. These produce a yellowish-orange background, rather than the normal pale pink and must be removed by distillation of the dioxan over sodium wire.

TABLE I

R_F VALUES OF DRUGS IN SYSTEM A AND SYSTEM B

Drug	*Solvent system A*	*Solvent Solvent B*
Morphine	0.12	0.33
Desmethylcotinine/hydroxycotinine	0.21	0.58
Codeine	0.28	0.33
Quinine	0.38	0.48
Cotinine	0.41	0.58
Nicotine	0.70	0.58
Pethidine	0.82	0.48
Chloropromazine	0.91	0.40
N-Demethylmethadone	0.98	0.13
Methadone	0.98	0.42
Cocaine	0.98	0.72

The colour of the background obtained with the iodoplatinate reagent is most important when assessing a plate. We have found that if the plate is properly prepared, the colours obtained with this reagent can be an excellent aid to discrimination.

This differentation by colour is enhanced by the use of the iodoplatinate reagent described, giving distinct light-blue (morphine), dark-blue (codeine), grey-blue (cocaine) and reddish-purple (methadone and N-demethylmethadone) spots against a pale pink background (Figs. 3 and 4).

With neutral iodoplatinate it is imperative to remove any trace of ammonia. This is achieved by subjecting the plate to a cold air stream until both ammonia and solvent are removed, otherwise the spots fade rapidly after spraying. It is also important to use a cold stream of air, both in spotting and drying the plate since methadone can be volatilised by hot air.

Acidified iodoplatinate reagent is less discriminating because the blues become darker and more difficult to distinguish from those of other basic drugs that may react. We do, however, sometimes overspray the phenothiazine side of plate A with iodoplatinate after the F.P.N. reagent, since this gives a slightly higher sensitivity for morphine.

The colours obtained with Dragendorff's reagent were found to be insufficiently contrasted to aid identification and reliance has then to be placed on R_F values.

Interferences

The most common interference encountered in the narcotic fraction is that from nicotine and its metabolites. These metabolites have been described by BOWMAN *et al.*[18] and recently the interference of nicotine in TLC of morphine has been investigated by GOENECHEA AND BERNHARD[19]. Heavy smoking is very prominent amongst addicts and this can lead to difficulties with interpretation of the plate.

Fig. 3 shows the spots obtained from the urines of laboratory personnel run

in System A. Column 1 being an extract from the urine of a non-smoker, is clean. Column 2, 3 and 4 are extracts from smokers of 5–20 cigarettes a day and show spots for nicotine (R_F 0.70), cotinine (R_F 0.41) and desmethylcotinine and hydroxycotinine (R_F 0.21). Column 5 has reference spots of nicotine and cotinine. Columns 6, 7 and 8 show the chromatographic pattern obtained from urines of addicts who smoke and column 9 shows that from an addict who does not smoke. Column 10 has standards of morphine and codeine.

In System A, cotinine lies very near to the position of codeine, while desmethylcotinine and hydroxycotinine lie very close to that of morphine. The colours of these spots are greyish-brown but in large concentrations they tend to become darker and blacker and, as shown in the addict columns 6 and 7 these large spots can render interpretation of the plate difficult. Fortunately in the methanol–ammonia of System B nicotine and its metabolites coalesce and chromatograph at an R_F of 0.58. Fig. 4 shows the same samples as those of Fig. 3 run in System B with column 10 now having standards of cocaine, methadone, morphine and N-demethylmethadone. Note the colours of the drugs and nicotine moieties in both of these plates.

Another interference, commonly found in our screening is that of phenothiazine derivatives and their metabolites, especially as these drugs are favoured at a treatment by some clinicians. These interferences have already been noted by DAVIDOW[5,6].

The FORREST AND FORREST[13] colour test directly on the urine is not very sensitive, but on a TLC plate is excellent. Accordingly we have divided the plate run in System A into two halves and spotted 30 μl of sample extract on each half as previously described. After development and drying, one half is lightly sprayed with FPN reagent and shows any phenothiazine and metabolites which by this solvent system are separated extremely well. Then by spraying the other half with iodoplatinate reagent, one can often differentiate between a spot due to morphine or other narcotic from those of the phenotiazines, (Fig. 5). Final confirmation must always be sought on the second plate.

Confirmatory tests

A specific test for morphine has been described by YOSHIMURA *et al.*[20]. The thin-layer plate is placed in a chromatographic tank containing a beaker of concentrated ammonia solution until the colour of the iodoplatinate has faded. Pseudomorphine thus formed from any morphine present exhibits a blue fluoresence when viewed under light of 254 nm. This can be a useful test in doubtful cases, although we have noted that some confusion can arise from naturally occurring fluorescent materials. System A is more satisfactory to carry out this test since these materials are moved away from the origin while the R_F of morphine is much lower.

Fluorescence is also used by many authors to demonstrate the presence of quinine, especially in the U.S.A. where illicit heroin is often contaminated with this substance. In Britain, however, quinine is rarely encountered and after three years' work we have never identified it in any of the addict urines screened by this laboratory. Nevertheless, should the need to confirm quinine arise it can be readily done by viewing the phenothiazine half of plate A under UV light after spraying with FPN reagent.

In man the principal metabolite of methadone is N-demethylmethadone and its identification is extremely useful when confirming the presence of methadone. The

two compounds run together on the solvent front in System A, but in System B methadone is just above morphine at R_F 0.42 while the N-demethylmethadone runs to R_F 0.13 (see Fig. 4). Both the parent drug and the metabolite produce reddish-purple spots with iodoplatinate reagent. However, in cases of doubt (particularly when the plate is overcrowded) a useful confirmatory step is to continue spraying with iodoplatinate. A characteristic white halo will then appear around the methadone spot. If this halo fails to appear, then further confirmation on the gas chromatograph can be sought with the amphetamine extract using the gas–liquid chromatographic (GLC) conditions described below for cocaine. Some workers use only the metabolite to identify methadone, although we report methadone when the unchanged drug or the metabolite are present. In the great majority of instances both of these compounds will be found together.

A promising procedure for the confirmation of cocaine has been based on the quick extraction method described for amphetamine. Alkalinisation with 1 ml of 0.880 ammonia solution was preferred in this instance. 5 μl of the extract were injected onto a column packed with 2 % OV-225 on Gas-Chrom Q (Field Instruments, Richmond, Surrey). The oven and injection temperatures were 190° and 230°, respectively, with a nitrogen flow rate of 35 ml/min. Cocaine eluted after 5 min while pethidine and methadone had retention times of 0.75 and 2 min, respectively. The recovery of of cocaine was quantitative in the range 0.5–10 μg/ml and the extracts prepared by

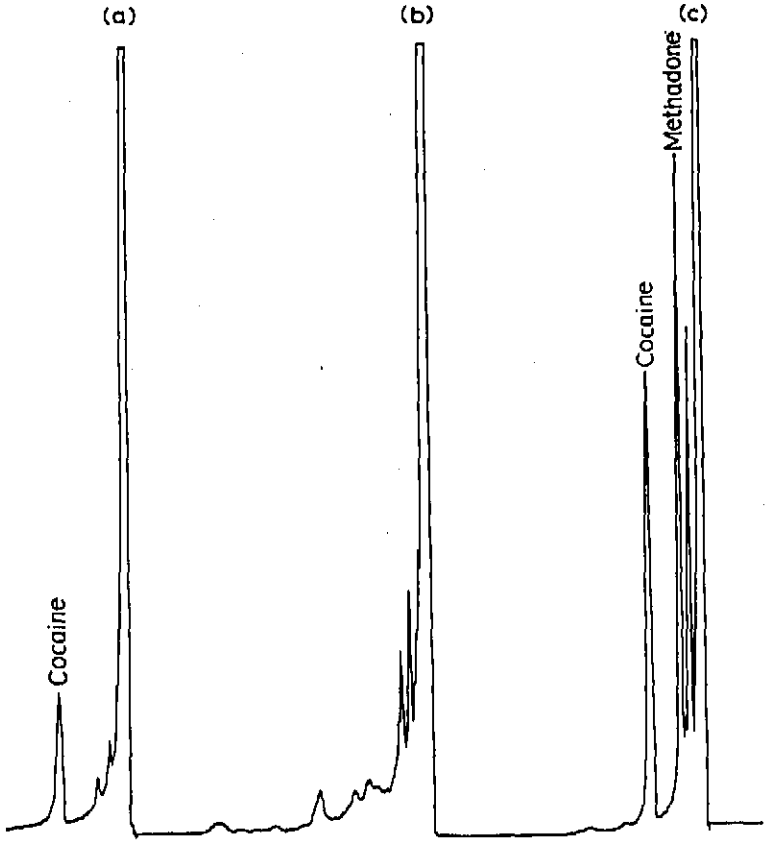


Fig. 6. (a) Chromatogram of an extract of 1.0 μg of cocaine added to 5 ml of water. (b) Chromatogram of an extract from 5 ml of smoker's urine. (c) Chromatogram of an extract from 5 ml of urine of a cocaine addict, also receiving methadone.

this procedure were very clean with no interfering peaks in the region of cocaine, (Fig. 6).

The presence of very large amounts of phenothiazine derivatives can make interpretation of the narcotic plates extremely difficult. In these cases a further purification step is recommended. This consists of extracting the urine as described for barbiturates but rejecting the chloroform layer which will contain a large proportion of the phenothiazines. The urine is then carefully neutralised with caustic soda solution, saturated with sodium bicarbonate and the narcotic extract worked up as described above. It was found that methadone was lost in this purification, but that codeine, morphine and cocaine were extracted with no decrease in sensitivity.

An alternative procedure for these urines containing phenothiazines was developed with the aid of electrophoresis. After extracting the urine, as described above, under narcotics, 50 μl were spotted onto a pre-coated cellulose plate (Kodak Ltd., Liverpool) and electrophoresis carried out in a 0.2 *M* borax–caustic soda buffer of pH 10 for 45 min at 500 V (BERRY[21]). The morphinate ion then moved towards the anode, codeine and cocaine to the cathode, while phenothiazine derivatives and methadone remained at the origin. After drying the paper, visualisation of the drugs was made by spraying initially with FPN reagent to neutralise the buffer and then overspraying with iodoplatinate reagent.

Finally, when screening urines by TLC procedures the analyst must be aware that many other basic drugs, besides those already discussed, may be present, *e.g.*, tricyclic antidepressants or antihistamines. In cases where the presence of these are suspected more specific sprays can be employed *e.g.* MANDELIN's reagent[22]. Similarly the acid extract may contain drugs other than barbiturates that would also give a positive result, *e.g.* glutethimide and hydantoins.

Limits of detection

The establishment of these detection limits is difficult, since the comparison of oral doses and intravenous injections are not strictly correct. Nevertheless they do serve as a useful guide. The ability of our techniques to identify amphetamine and methylamphetamine 24 h after ingestion of oral doses of 20 mg has been mentioned already. It has also been established that barbiturates are detectable in the early morning urine of patients receiving therapeutic doses[23]. 20 mg oral doses of heroin were detected at a maximum of 8 h after ingestion by our direct extraction method. We have not routinely used acid hydrolysis or β-glucuronidase hydrolysis since these are time consuming and in the case of β-glucuronidase, expensive, although higher sensitivity is undoubtedly obtained with both procedures.

Occasionally when requested to check a finding on an addict receiving low doses of heroin we have used the acid hydrolysis described by PARKER AND HINE[4].

The detection limit of methadone was checked by laboratory personnel taking 10 mg of physeptone linctus. We were able to detect either the unchanged drug, the metabolite, or both compounds quite easily for 24 h, but towards the end of this period, only the N-demethylmethadone appeared in the urine.

We were unable to detect free cocaine by our TLC procedure in the urines of volunteers following a single oral dose of 20 mg. However, the extracts from these urines did contain an iodoplatinate reacting compound (R_F 0.3 in System A) between 2–8 h after the ingestion which may be the metabolite benzoylecgonine.

Our GLC procedure has a limit of detection of 0.1 μg/ml and cocaine was found by this technique in the urines collected from 0–2 h after a single 20 mg dose.

FISH AND WILSON[24] have described a GC method for determining morphine and cocaine in urine. They also studied one in patient receiving intramuscularly 120 mg cocaine and 180 mg heroin daily[25] and found a considerable fluctuation in the urine out-put of cocaine which at times fell below 0.1 μg/ml. This would suggest that one would fail to detect some addicts if the samples were collected during a period of low urinary concentration. However, we have found all of our cocaine addict urines to have concentrations in the region 1–80 μg/ml and were thus easily analysed by our procedure. We are currently investigating the possibility of looking for the metabolite benzoylecgonine in a similar manner to the metabolite of methadone.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. R. GOULDING for his advice and encouragement during the course of this work. We also wish to thank Miss G. L. THORNLEY for her invaluable technical assistance, and Dr. D. M. TURNER of the Tobacco Research Council Laboratories for the gift of cotinine.

REFERENCES

1 K. D. PARKER, C. R. FONTAN AND P. L. KIRK, *Anal. Chem.*, 35 (1963) 356.
2 A. H. BECKETT AND A. C. MOFFAT, *J. Pharm. Pharmacol.*, 20 (1968) 48S.
3 J. G. MONTALVO, E. KLEIN, D. EYER AND B. HARPER, *J. Chromatogr.*, 47 (1970) 542.
4 K. D. PARKER AND C. H. HINE, *Bull. Narcotics*, xix (1967) 51.
5 B. DAVIDOW, N. LI PETRI, B. QUAME, B. SEARLE, B. FASTLICH AND J. SAVITSKY, *Amer. J. Clin. Pathol.*, 46 (1965) 58.
6 B. DAVIDOW, N. LI PETRI AND B. QUAME, *Amer. J. Clin. Pathol.*, 50 (1968) 714.
7 H. V. STREET, *J. Chromatogr.*, 37 (1968) 111.
8 D. A. L. BOWEN, D. M. GURR AND G. B. OPPENHEIMER, *Clin. Toxicol.*, 3 (1970) 89.
9 M. L. BASTOS, G. E. KANANEN, R. M. YOUNG, J. R. MONFORTE AND I. SUNSHINE, *Clin. Chem.*, 16 (1970) 931.
10 V. P. DOLE, W. K. KIN AND I. EGLITIS, *J. Amer. Med. Ass.*, 198 (1966) 115.
11 V. MARKS AND D. FRY, *Proc. Ass. Clin. Biochem.*, 5 (1968) 95.
12 S. J. MULÉ, *J. Chromatogr.*, 55 (1971) 255.
13 I. S. FORREST AND F. M. FORREST, *Clin. Chem.*, 6 (1960) 11.
14 E. SHITTLER AND J. HOHL, *Helv. Chim. Acta*, 35 (1952) 29.
15 S. J. MULÉ, *J. Chromatogr.*, 39 (1969) 302.
16 V. M. FRAHM, A. GOTTESLEBEN, K. SOEHRING, *Pharm. Acta Helv.*, 38 (1963) 785.
17 J. D. RAMSEY AND D. B. CAMPBELL, to be published.
18 E. R. BOWMAN, L. B. TURNBULL AND H. MCKENNIS, *J. Pharm. Exptl. Therap.*, 127 (1959) 92.
19 S. GOENECHEA, W. BERNHARD, *Z. Anal. Chem.*, 246 (1969) 130.
20 H. YOSHIMURA, K. ORGURI AND H. TSUKAMOTO, *Chem. Pharm. Bull.*, 14 (1966) 62.
21 D. J. BERRY, unpublished work.
22 W. W. FIKE AND I. SUNSHINE, *Anal. Chem.*, 37 (1965) 127.
23 J. COCHIN AND J. W. DALY, *J. Pharm.*, 139 (1963) 154.
24 F. FISH AND W. D. C. WILSON, *J. Chromatogr.*, 40 (1969) 164.
25 F. FISH AND W. D. C. WILSON, *J. Pharm. Pharmacol.*, 21 (1969) 135S.