If the extract, prepared as described, is too contaminat-
ed for reliable analysis, a double small-volume extraction
can be used. In this modification, the buffered urine is ex-
tracted with 1.0 ml of extraction solvent. After centri-
fugation, an aliquot of this extract is transferred to a tube
containing 100 μl of HCl (0.4 mol/liter). After the mixture
has been shaken and centrifuged, the HCl layer is re-
moved and made alkaline with 50 μl of NaOH (1 mol/
liter). This mixture is then extracted with 50 μl of extrac-
tion solvent, an aliquot of which is injected into the chro-
matograph.

We have used the described procedure effectively and
reliably for longer than a year as a confirmatory test for
organic bases, other than morphine and codeine, in urine.
We favor the described procedure over other techniques
for several reasons. (a) Evaporation of large volumes of
solvents is eliminated. (b) Sample manipulation is mini-
mized, since a purification of the initial extract is not
generally required. (c) Most importantly, this procedure's
brevity does not affect the quality of the extract, and
highly reliable information may be gained from its use.

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Single-pH Extraction Procedure for Detecting Drugs of Abuse

Ramon E. Stoner and Connie Parker

A method is presented for determining certain drugs
of abuse in a single extract of urine. The urine sam-
ples is adjusted to pH 6.0 with a buffer containing
bromcresol purple. Basic drugs, such as amphet-
amines and narcotics, form an organic salt with the ion-
ized bromcresol purple, which is extractable with
a mixture of chloroform and 2-propanol (3:1 by vol-
ume). At pH 6.0, weak acids such as barbiturates
and neutral drugs such as glutethimide are also sol-
uble in this solvent. Consequently, the major classes
of drugs are extracted simultaneously. The extract is
then concentrated and the individual drugs are de-
termined by thin-layer chromatography in a solvent
that will separate bromcresol purple from the drugs.

Additional Keyphrases: drug screening  •  toxicology
  •  thin-layer chromatography  •  screening procedure

We needed a means of testing urine samples that
would save time without sacrificing proficiency. The pro-
cedure described here was developed to extract acid, neu-
tral, and basic drugs from urine at pH 6, with a buffer
containing bromcresol purple. At this pH, acid and neu-
tral drugs are soluble in the organic solvent. The basic
drugs form an organic salt with bromcresol purple, and
this salt (but not bromcresol purple) is also soluble in the
solvent. Consequently, the major classes of drugs are ex-
tracted simultaneously, and may then be concentrated
and determined by (e.g.) use of thin-layer chromato-
graphy (TLC).

Materials and Methods

Materials

Silber-Porter 50-ml centrifuge tubes with stoppers (A.
H. Thomas, Philadelphia, Pa. 19105; No. 2619-B25
and 2619-F20, respectively).

TLC development apparatus (No. 6071; Eastman Kodak
Co., Rochester, N.Y. 14650).

TLC sheets, Eastman no. 6061 silica gel.

Micropipets, 10-μl (Camag Inc., New Berlin, Wis.
53151).

Ultraviolet view box, containing lamps emitting light at
254 and 366 nm.

Spectrofluorometer, MK I (Farrand Optical Co., Val-
halla, N.Y. 10595).

Gas chromatograph, (Hewlett Packard No. 5700A) fit-
ted with 6 ft. × ½ in. stainless-steel column packed with
"3% OV 17 on HP Chromosorb W" (Supelco, Inc., Belle-
fonte, Pa. 16823)

Evaporating bath, To handle a large number of samples
per day, one needs a method of evaporating the solvent
quickly. Such a device may be constructed by building an
aluminum tube rack to fit into a water bath (set at 70
°C). A 12-unit hematocrit tube cleaner is mounted over
this rack and a latex rubber tube, 3 mm (I.d.) by 30 cm, is
connected to each U-tube of the cleaner. To the other end
of each of the rubber tubes is connected a 5-in. "Pharma-
seen" connector (Scientific Products, McGaw Park, Ill.
60085; No. T5300). The free end of this connector is loose-
ly inserted into the sample tube. The outlet of the hema-
tocrit tube cleaner is connected to a vacuum line. Such a
unit produces a decreased pressure that is the same for all
tubes.
Reagents

Extracting solvent: Chloroform:2-propanol (3:1, by vol).
Brom cresol purple buffer: Dissolve 1.7 g of anhydrous dibasic sodium phosphate, 12.0 g of monobasic potassium phosphate, and 0.4 g of the sodium salt of brom cresol purple in 500 ml of water, and dilute the mixture to 1 liter with water. The pH should be 6.0.


Developers for TLC: For barbiturates (1), mix 90 ml of chloroform and 10 ml of aceton immediately before use. For basic drugs (2), 85 ml of ethyl acetate, 10 ml of methanol and 5 ml of concentrated ammonium hydroxide are mixed immediately before use.

Mercury reagent: (1) Suspend 10 g of mercuric oxide in 200 ml of water, add 40 ml of sulfuric acid, mix, and dilute to 500 ml with water.

Diphenylcarbazone reagent: (2) Add 25 mg of diphenylcarbazone to a solution of 125 ml of water and 125 ml of aceton.

Ninhydrin: 50 mg/dl aceton.
Sulfuric acid, 0.09 mol/liter.
Iodoplinate reagent (3): Add 5.0 ml of platinic chloride (0.96 mol/liter) to 45 ml of potassium iodide (0.6 mol/liter). This solution is added to 100 ml of water.

Drangendorff reagent: (2) Dissolve 1.3 g of bismuth subnitrate in a solution consisting of 60 ml of water and 15 ml of glacial acetic acid. Dissolve 12.0 g of potassium iodide in 30 ml of water. Mix these two solutions and add 100 ml of water and 25 ml of glacial acetic acid.

Tollen’s reagent: Add dropwise, ammonium hydroxide (6 mol/liter) to 5 ml of silver nitrate (0.59 mol/liter) until only a very faint precipitate remains. This reagent should be prepared just before use and should not be stored, since the solution decomposes on standing and deposits a highly explosive precipitate.

Method

Extraction. Add 15 ml of urine and 2.0 ml of brom cresol purple buffer to a Silber–Porter centrifuge tube. The mixture should now have a pH of 6.0 ± 0.3, as evidenced by a reddish color. If necessary adjust the pH with either dilute sodium hydroxide or hydrochloric acid. Almost fill the tube with extracting solvent (about 25 ml), stopper, and mix vigorously on a mechanical shaker for 5 min. Centrifuge for 10 min at 400 × g, aspirate the aqueous layer, and discard it. Place the tube in a water bath at 70 °C and insert a vacuum line into the mouth of each tube. When all the solvent is evaporated, dissolve the residue by adding 100 μl of methanol down the sides of the tube while it is being rotated.

Chromatography. The chromatogram sheets are prepared by drawing a line 2.0 cm from the bottom of the sheet and marking pips every 1.5 cm. Another line is drawn 10 cm above this line to mark what will be the solvent front. Before spotting, place the sheets in a 90 °C oven for 30 min (any humidity causes poor migration). Spot 20 μl of each sample on a sheet by making a double application with a 10-μl Camag pipette. For complete analysis, run two such sheets, one for the barbiturates and one for the basic drugs. Apply 5 μl of the Gelman I standard to the barbiturate sheet and 5 μl each of the Gelman II and III standards to the basic drug sheet. Develop the sheets in their respective solvents, and remove them from the chamber when the solvent front reaches the 10-cm line. Spray them after all solvent has evaporated.

Detection. Spray the sheet for barbiturates with the mercury reagent. Barbiturates, diphenhydantoin, meprobamate, and glutethimide appear as white, chalky spots. The sheet is partially dried and sprayed with diphenylcarbazone. All of the above except meprobamate will appear as a blue or purple spot.

Spray the basic drug sheet for detection of basic drugs with ninhydrin and place it under a 254-nm ultraviolet light for 15 min. Amphetamines will appear as purple spots. Next, spray the sheet lightly with the dilute sulfuric acid and examine it under 254 nm ultraviolet light for fluorescing quinine spots. Overspray the sheet with iodo-platininate. Methadone and its metabolite will appear near the top of the sheet as a pink-purple spot. Morphine will appear as a blue spot with an Rf of about 0.4. Morphine should not be considered absent until 15 min after spraying, as low concentrations will develop slowly. The sheet is then oversprayed with Drangendorff reagent. Morphine spots will remain dark, while other drugs will fade or become pink or orange.

Morphine and quinine. In drug-abuse centers, many samples require only an analysis for morphine and quinine. This can be performed conveniently by spotting the samples on a half-sheet of paper and running the developing solvent to within 2 to 4 mm of the top of the sheet. Spray these sheets with the dilute sulfuric acid and then overspray with freshly prepared Tollen’s reagent, and place the sheets in a 90 °C oven for 5 min. Morphine will appear as black spots. Tollen’s reagent is slightly more sensitive than the iodoplinate reagent for the detection of morphine.

Results and Discussion

The efficacy of the screening procedure was tested as follows: Morphine was confirmed by spectrofluorimetry, by using an excitation wavelength of 390 nm and an emission wavelength of 420 nm (4). Barbiturates and glutethimide were determined by gas–liquid chromatography of an acid to neutral, chloroform extract (5). The drugs were eluted from the column at 200 °C. Amphetamines were acetylated and the derivative determined by gas–liquid chromatography, with a column temperature of 140 °C. Phenothiazine was confirmed by use of a ferric chloride reagent (6). Corroboration was obtained on the following urine samples, found to be positive by the screening procedure: 50 contained morphine, 21 phenobarbital, 5 secobarbital, 6 glutethimide, 15 amphetamine, and 6 were from overdose of chlorpromazine.

Because the extraction is performed at pH 6.0, weak acids and neutrals will also be extracted. Consequently, most drugs of abuse are extracted simultaneously.

The strong colors produced when indicators, such as brom cresol purple, are used as the acidic component for salt formation with basic drugs have been used to spectrophotometrically determine drugs (7–9). We chose to use brom cresol purple, which forms salts very easily with most basic drugs, because more morphine was extracted than with brom cresol green, our other choice, as evidenced by darker spots on thin-layer chromatography sheets.

A solvent was needed that would dissolve the organic salts. Chloroform and benzene worked well for a number of the drugs, but the morphine salt was insoluble. The best solvent system we found is that described. All of the brom cresol–drug salts that we tested were soluble in this
Table 1. $R_f$ Value for Drugs Detected with Mercury Reagent

<table>
<thead>
<tr>
<th>Drug</th>
<th>$R_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meprobamate</td>
<td>0.21</td>
</tr>
<tr>
<td>Diphenylhydantoin</td>
<td>0.45</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>0.57</td>
</tr>
<tr>
<td>Amobarbital</td>
<td>0.70</td>
</tr>
<tr>
<td>Secobarbital</td>
<td>0.73</td>
</tr>
<tr>
<td>Glutethimide</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Solvent: 90 ml chloroform and 10 ml acetone.

Table 2. $R_f$ Value of Drugs Detected with Iodoplatinate

<table>
<thead>
<tr>
<th>Drug</th>
<th>$R_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>0.92</td>
</tr>
<tr>
<td>Codeine</td>
<td>0.55</td>
</tr>
<tr>
<td>Chloralazine</td>
<td>0.70</td>
</tr>
<tr>
<td>Morphin</td>
<td>0.45</td>
</tr>
<tr>
<td>Methaqualone</td>
<td>0.87</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>0.85</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>0.60</td>
</tr>
<tr>
<td>Quinine</td>
<td>0.63</td>
</tr>
<tr>
<td>Trihexyphenidyl</td>
<td>0.93</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.87</td>
</tr>
<tr>
<td>Dosepin</td>
<td>0.77</td>
</tr>
<tr>
<td>Methadone</td>
<td>0.84</td>
</tr>
<tr>
<td>Imipramine</td>
<td>0.41</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>0.90</td>
</tr>
<tr>
<td>Desipramine</td>
<td>0.18</td>
</tr>
<tr>
<td>Meperidine</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Solvent: 85 ml ethyl acetate, 10 ml methanol, and 5 ml concd ammonium hydroxide.

The following drugs have been added to drug-free urine and detected at a concentration of 0.1 mg/liter: morphine, cocaine, codeine, methadone, meperidine, quinine, procaine, dioxazepam, and chloraloxazines. d-Ampheta- mine can be detected at a concentration of 0.2 mg/liter, barbiturates at 50 µg/liter.

We do not imply that a knowledge of drug metabolites is not needed for a thorough toxicologic determination. We selected the drugs because they represent a wide variety of solubility classes, and illustrate the utility of the extraction procedure.

In practice, we sometimes rely on the TLC preparative technique of Sunshine (1), whereby 50 µl of the residue is streaked across the bottom of the plate. After development, a strip of the outside column is cut off and sprayed to locate the drugs. This strip is then placed alongside the untreated portion of the plate and the corresponding segment is cut out, eluted with methanol into a cuvet, and the ultraviolet absorption spectrum is obtained.

One drug addict was hospitalized with severe convulsions. A reddish-brown spot, with an $R_f$ of 0.45 was obtained with iodoplatinate reagent. The corresponding eluted segment gave an ultraviolet absorption maximum of 253 nm and a minimum of 229 nm, which suggests the presence of strychnine. Strychnine and the sample were co-chromatographed, and had identical $R_f$'s and color. Moreover, the ultraviolet absorption spectrum of strychnine was identical with the material eluted from the patient's urine.

Our extraction method has several advantages. It is relative fast in terms of time spent in actual procedure; one technician has performed as many as 100 urine analyses in 8 h. It is inexpensive, requiring very little glassware and few manipulations. Because the samples are extracted at an acid pH with a low salt concentration, there is seldom trouble with emulsions. The bromcresol purple is a useful marker for the point of origin and for the presence of ammonia on the sheets when they are removed from the basic development solvent. This is important because the first spray is ninhydrin. If ammonia is still present, the spots at the origin will be blue but become yellow when the sheet is free of ammonia. We think that the most important feature of the procedure is that sufficient extract is left over for repeating the chromatography, for confirming the results with another solvent system, or for increasing the detection limit by applying more than the recommended 20 µl of sample on the original sheet.

References