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SINGLE- AND TWO-STEP EXTRACTION AND THIN-LAYER DETECTION PROCEDURES FOR BENZOYLECGONINE (COCAINE METABOLITE) ALONE OR IN COMBINATION WITH A WIDE VARIETY OF COMMONLY ABUSED DRUGS IN URINE SCREENING PROGRAMS

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SUMMARY

Three extraction procedures for the detection of benzoylecgonine (a major metabolite of cocaine) in urine are presented. Each technique has its advantages, depending on the needs of a clinical operation.

Procedures I and II involve the use of ion-exchange resin-loaded paper. Procedure I has a sensitivity of 1 μg/ml and requires 20 ml of urine, and is recommended when the aim is to test for the abuse of cocaine only. Procedure II is a two-step extraction method in which a wide variety of abused drugs are extracted by the first step and the benzoylecgonine left in the aqueous buffer phase is extracted in the second step. The sensitivity for benzoylecgonine using this procedure is 2 μg/ml and it requires 20–50 ml of urine.

Procedure III involves the direct extraction of benzoylecgonine using 5 ml of urine and has a sensitivity of 0.5 μg/ml.

INTRODUCTION

Drug abuse appears to be a continuing phenomenon, but the pattern varies depending on the availability of particular drugs in the illicit drug market. Different groups of people use different drugs, and it is therefore necessary to make distinctions among these drugs. Recently, the Director of North Central Regional Laboratory of Drugs Enforcement Administration (DEA) told Industrial Research[1] that of the total drugs identified by the laboratory each month, about 20% of the specimens were cocaine. Cocaine (methylbenzoylecgonine) is an alkaloid obtained from the leaves of *Erythroxylon coca* or by synthesis from ecgonine. It used to be considered a very expensive drug, but today it is cheaper and readily available to drug addicts.

McIntyre[2] reported urinary excretion of unchanged cocaine to the extent of 54% (12 h after ingestion) in the case of accidental peritonsillar injection of 800 mg of cocaine. Woods *et al.*[3] found that the dog excreted only 1–12% (24 h after ingestion) of unchanged cocaine of the various injected doses. Fish and Wilson[4] published qua-

* Reprints available at US$ 2.00 each.

titative data on the excretion of unchanged cocaine and benzoylecgonine in addict's urine after administration of intramuscular doses of cocaine hydrochloride (1–9% unchanged cocaine and 49–54% benzoylecgonine). Their data on unchanged cocaine were in agreement with the results reported earlier by Woods *et al.*[3]. The abuse of cocaine remains undetected by commonly used thin-layer chromatographic procedures, owing to its rapid biotransformation into the primary metabolite (benzoylecgonine) and a minor metabolite (ecgonine). These metabolites are highly soluble in water and are minimally extracted by traditional solvents such as chloroform and diethyl ether.

Syva Corporation was the first to market a reliable immunological procedure for the detection of benzoylecgonine at a concentration of 1–1.6 μg/ml in urine[5]. The assay also detects ecgonine at significantly higher detection limits but does not detect unchanged cocaine. Roche Diagnostics[6] have developed a radioimmunoassay technique using [^{125}I]benzoylecgonine derivative with a sensitivity of 100 ng/ml in urine, but the kit is not yet commercially available. Fish and Wilson[4] proposed a gas chromatographic (GC) procedure for the detection of benzoylecgonine by its prior methylation with diazomethane. Koontz *et al.*[7] also prepared the methyl derivative of benzoylecgonine in dimethylformamide dimethyl acetal prior to GC analysis. Jain *et al.*[8] proposed a similar GC procedure by converting benzoylecgonine into its methyl, ethyl, propyl, *n*-butyl or *tert.*-butyl ester by treatment with the appropriate dialkyl acetal in dimethylformamide. Jatlow and Bailey[9] described a GC procedure for the detection of cocaine in plasma using a nitrogen detector. The propyl ester of benzoylecgonine was used as an internal standard. Moore[10] suggested that the presence of ecgonine and benzoylecgonine in illicitly manufactured cocaine could be quantitated by their silylation prior to GC analysis using N,O-bis(trimethylsilyl)-acetamide (BSA).

Valanju *et al.*[11] described a thin-layer detection procedure with a sensitivity of 3–5 μg/ml of benzoylecgonine in urine. A 25-ml aliquot of urine was extracted twice with 75 ml of a polar solvent mixture, the organic solvent evaporated to dryness and the residue chromatographed. The TLC plate was developed in two different solvent systems. Bastos *et al.*[12] published a TLC procedure in which the major metabolites of cocaine were extracted from human urine, butylated, subsequently isolated by extraction and then identified by TLC using a double solvent system. The use of a double solvent system or two-dimensional chromatography was found to be necessary in order to avoid interference from urinary impurities and from the possible presence of morphine. Although the sensitivity of the reported technique for the cocaine metabolites was 3–5 μg/ml in urine, it is cumbersome for routine use. Meola and Brown[13] proposed a modification of their charcoal TLC procedure[14] and reported the detection of benzoylecgonine at a concentration of 4 μg/ml in urine.

Wallace *et al.*[15] reported a TLC procedure, claiming a sensitivity of 0.1 and 0.25 μg/ml of cocaine and benzoylecgonine, respectively, in urine. They recommended that owing to the influence of co-extraction on the R_F value of benzoylecgonine in urine specimens, standards of benzoylecgonine for comparison with unknown specimens should be carried through the assay procedure. The method specified that the extracted solvent should be dried to about 50–100 μl in a water-bath at 55° under a gentle stream of air. They observed that the evaporation of solvent completely to dryness resulted in a decreased sensitivity. We were not able to reproduce the results

satisfactorily for benzoylecgonine even at a sensitivity level of 0.5 µg/ml. Furthermore, in some cases it was virtually impossible to differentiate between a spiked and blank urine.

This paper describes three procedures for the detection of benzoylecgonine in human urine. Procedures I and II involve the use of cation-exchange resin-loaded paper to absorb benzoylecgonine and other drugs of abuse. Procedure I is a single-step extraction method with a sensitivity of 1 µg/ml in urine, and is recommended when the aim is to test for the abuse of cocaine only. Procedure II is a two-step extraction method in which a wide variety of commonly abused drugs except benzoylecgonine are first extracted at a pH of 10.1[16–18] and chromatographed using a two-stage solvent system[18,19]. The aqueous buffer phase left after the extraction of commonly abused drugs is used for the extraction of benzoylecgonine. This aqueous buffer phase is brought to a pH of 1–2 by the addition of concentrated hydrochloric acid and then saturated with sodium hydrogen carbonate (resulting in a pH of 8.1–8.4). The benzoylecgonine is extracted with a polar solvent mixture and chromatographed as described under *Detection Procedure A for benzoylecgonine*. The sensitivity of this procedure is 2 µg/ml in urine. Procedure III is a single-step method and involves the direct extraction of benzoylecgonine from a 5-ml aliquot of urine. The sensitivity of this method is 0.5 µg/ml in urine.

METHODS

Procedure I. Single-step ion-exchange extraction of benzoylecgonine

A 6 × 6 cm piece of H. Reeve Angel (Clifton, N.J., U.S.A.) SA-2 cation-exchange resin-loaded paper (identified by the patient's identification number or name with a lead pencil) is soaked in 20 ml of fresh undiluted urine in a 4-oz wide-mouthed screw-capped jar. The urine is discarded after shaking for 20–30 min on a reciprocating shaker (Eberbach table model). (The urine, if desired, may be transferred into another bottle and saved for acid hydrolysis of water-soluble conjugates of morphine and other drugs. This urine can also be used for re-checks as a small portion of drugs if available in high concentration are left in this spent urine and can be re-absorbed by adding another ion-exchange resin-loaded paper.) The ion-exchange paper left in the bottle is rinsed with 10 ml of deionized or distilled water (the rinsings are discarded), then 5 ml of water are added and water is saturated with sodium hydrogen carbonate. The contents are shaken for 20 min on a reciprocating shaker with 30 ml of chloroform–isopropanol–dichloroethane (4.5:0.9:4.5); undissolved sodium hydrogen carbonate must be visible after shaking. The lower organic phase is allowed to separate and is then pipetted into a 50-ml non-graduated conical centrifuge tube. The solvent is evaporated in an oven having a horizontal air flow and maintained at 85–90°. The residue along the sides of the tube is washed with about 1 ml of dichloroethane, the contents are vortexed, and the sides of the tube are again washed with a few drops of dichloroethane. The solvent is evaporated to dryness as above. The residue is spotted on the chromatographic plate as described under *Detection Procedure A for benzoylecgonine.*

Procedure II. Two-step ion-exchange extraction of poly-drugs and benzoylecgonine

A wide variety of drugs of abuse as reported earlier[17–19] and benzoylecgonine

are absorbed on a 6 × 6 cm piece of Reeve Angel SA-2 cation-exchange resin-loaded paper. The paper is soaked in 20–50 ml of fresh undiluted urine and shaken for 20–30 min as described above under *Procedure I*. After rinsing the ion-exchange resin paper with 10 ml of water (the rinsings are discarded), the extraction for poly-drugs and benzoylecgonine is accomplished in two steps as follows.

(a) Poly-drugs (opiates, amphetamines, barbiturates and other drugs of abuse). To the jar containing the ion-exchange resin paper, 3 ml of ammonium chloride–ammonium hydroxide buffer of pH 10.1[17–19], 5 ml of water and 15 ml of chloroform–isopropanol (5:2) are added. After shaking for 20 min on a reciprocating shaker (Eberbach table model)*, the lower organic phase is pipetted into a 15-ml non-graduated conical centrifuge tube containing 5 drops of 0.5% sulfuric acid in methanol (the upper aqueous buffer is saved and used for the extraction of benzoylecgonine). The solvent is evaporated in an oven as described under *Procedure I*. The residue along the sides of the tube is washed with 0.5–1 ml of methanol, the contents are vortexed and the sides of the tube are again washed with a few drops of methanol. The methanol is evaporated to dryness as above. The residue thus obtained is chromatographed for either opiates or poly-drugs as described under *Detection Procedure B for opiates* and *Detection Procedure C for poly-drugs.*

(b) Benzoylecgonine. To the aqueous buffer phase (left after pipetting the lower organic phase) containing the ion-exchange resin paper, concentrated hydrochloric acid (about 1.8 ml) is added until a pH of 1–2 is obtained. The solution is saturated with sodium hydrogen carbonate and then the method as described under *Procedure I* is followed from "The contents are shaken for 20 min on a reciprocating shaker with 30 ml of chloroform–isopropanol–dichloroethane".

Procedure III. Single-step direct extraction of benzoylecgonine

Five milliliters of urine are transferred into a 50-ml round-bottomed non-graduated centrifuge tube, the urine is saturated with sodium hydrogen carbonate and 25 ml of chloroform–isopropanol–dichloroethane (4:5:0.9:4.5) are added. The tube is placed in a test-tube rack, which is then shaken for 20 min on a reciprocating shaker (Eberbach table model) at low speed (the tube is not capped; if a cap is to be used, the lower acetate lining should be removed). The lower organic layer is allowed to separate and then pipetted into a 40-ml non-graduated conical centrifuge tube. The solvent is evaporated to dryness in a water-bath at 55–65° under a gentle stream of dry air. The residue along the sides of the tube is washed with 1 ml of dichloroethane, the contents are vortexed and the sides are again washed with 1 ml of dichloroethane. The solvent is evaporated to dryness as above. The residue thus obtained is chromatographed as described under *Detection Procedure A for benzoylecgonine.*

* The utility carrier available with the Eberbach shaker accomodates 55–60 4-oz wide-mouthed screw-capped jars. Each jar has to be labelled with a laboratory number identifying the identification number of the client. The labelling of each bottle and subsequent removal of these labels, loading and unloading of the shaker each time and arranging the bottles in numerical order after shaking required 30–40 min of each technician's time per 100 specimens. To increase our daily production load, the laboratory has introduced the use of a shaker basket which has 60 holes to accomodate 60 4-oz wide-mouthed screw-capped jars and each hole is pre-numbered. This step has increased the daily production per technician by 8–10%. The shaker basket and the bracket to fit the Eberbach shaker was designed by Mid-West Wire Specialties Co. (Chicago, Ill., U.S.A.).

Thin-layer chromatography (TLC)

Gelman pre-coated silica gel glass microfiber sheets (ITLC Type SA) with a layer thickness 250 μm were used throughout. These sheets are preferred for their convenience in handling and in cutting to the desired size. The superiority of Gelman glass microfiber sheets lies in their capacity to endure varying heat treatments necessary for the specific detection of various drugs, *e.g.*, detection of phenmetrazine (Preludin) and methylphenidate (Ritalin). Furthermore, the sensitivity of a test can be increased by spraying both sides of the plate, by which the colors of minute concentrations of drugs are enhanced.

Developing solvents. For benzoylecgonine, chloroform–methanol–water–concentrated ammonia (70:30:0.5:1) is used. This solvent should preferably be used after overnight storage at room temperature to achieve optimal separation of benzoylecgonine from all urinary impurities.

For opiates, barbiturates and poly-drugs, solvent systems C, D, E, and F as reported by Kaistha and co-workers[17–19] are used.

For opiates (morphine, codeine, methadone and quinine), solvent C consisting of ethyl acetate–cyclohexane–concentrated ammonia–methanol–water (70:15:2:8:0.5) is used. For barbiturates, solvent D consisting of ethyl acetate–cyclohexane–methanol–concentrated ammonia (56:40:0.8:0.4) is used. For poly-drugs (opiates, amphetamines, barbiturates and other abused drugs), solvent E consisting of ethyl acetate–cyclohexane–methanol–concentrated ammonia (70:15:10:5) or solvent F consisting of ethyl acetate–cyclohexane–concentrated ammonia (50:40:0.1) is used.

It is recommended that solvents D and F be used fresh or within 24 h. Solvents C and E should preferably be used after overnight storage at room temperature and can be used up to 4 weeks after preparation.

Detection reagents. For benzoylecgonine, the following reagents are used.

(1) Munier's modified Dragendorff reagent[20]. Solution A is prepared by dissolving 17 g of bismuth subnitrate and 200 g of tartaric acid in 800 ml of water, and solution B by dissolving 160 g of potassium iodide in 400 ml of water. A stock solution is prepared by mixing 1 part by volume of solution A with 1 part by volume of solution B. This can be kept for a few months. A working dilute solution is prepared by dissolving 100 g of tartaric acid in 50 ml of stock solution and 500 ml of water.

(2) Iodine–potassium iodide solution[17]. Add 2 g of iodine to 50 ml of 95% ethanol and shake; dissolve 2 g of potassium iodide in 16.2 ml of water, mix both solutions together and shake until a clear solution is formed, then add 33.8 ml of concentrated hydrochloric acid and mix to form a final solution. Store at room temperature.

(3) Dragendorff's reagent[21]. Solution A is prepared by dissolving 1.7 g of bismuth nitrate in 100 ml of 20% acetic acid, and solution B by dissolving 40 g of potassium iodide in 100 ml of water. A working solution is prepared by mixing 20 ml of solution A with 5 ml of solution B and adding 70 ml of water.

(4) Sulfuric acid 20% (v/v) solution in water[15]. For poly-drugs (opiates, amphetamines barbiturates and other abused drugs), the following detection reagents are used each as described by Kaistha and coworkers[17–19]:

(a) ninhydrin, 0.5% (w/v) solution in *n*-butanol; this solution can be used for 24–48 h if stored in a refrigerator;

(b) diphenylcarbazone (DPC), 0.01 % (w/v) in equal parts of acetone and water;
(c) silver acetate, 1 % (w/v) solution in water;
(d) mercury(II) sulfate solution;
(e) sulfuric acid, 0.5 % (v/v) solution in water;
(f) iodoplatinate;
(g) iodine–potassium iodide.

Detection procedures

Procedure A for benzoylecgonine. The residue obtained as described under *Procedure I* or *II(b)* or *III* is dissolved in 30–50 μl of dichloroethane, vortexed and spotted on a 20 × 20 cm Gelman pre-coated silica gel glass microfiber sheet (Gelman ITLC Type SA). Three standards (1½–2 capillaries* of benzoylecgonine reference standard in methanol at a concentration of 1 mg/ml) are spotted along with samples on the same TLC plate. The spots are air dried and the plate is then dried for 5 min in an oven at 85–90° before it is placed in a standard rectangular tank containing 100 ml of developing solvent consisting of chloroform–methanol–water–concentrated ammonia (70:30:0.5:1). After the solvent has travelled a distance of about 14.5 cm (40–45 min), the plate is taken out and air dried for 10 min or until the smell of ammonia disappears. The plate is sprayed on both sides with Munier's modified Dragendorff reagent and then with iodine–potassium iodide reagent. Benzoylecgonine gives a yellow coloration with the former reagent and a rusty brown color with the latter (low concentrations give a color only after the latter spray). The spots seen in the samples at the level of benzoylecgonine standard (R_F value of about 0.33–0.42) should be circled immediately as they fade within 1 min (spots due to minute concentrations of benzoylecgonine disappear in less than 1 min; however all spots due to benzoylecgonine** reappear on re-spraying with iodine–potassium iodide reagent. Additional spots due to urinary constituents and other drugs appear at higher R_F values than benzoylecgonine and are not circled.

Alternatively, the plate may be sprayed as proposed by Wallace *et al.*[15] by spraying first with Dragendorff reagent[21] and, after a further 2 min, with 20 % (v/v) sulphuric acid. The spots due to benzoylecgonine, urine constituents and other drugs appear as distinct yellowish orange spots. However, using our proposed thin-layer developing solvent system, the spots due to urine constituents and other drugs travel further than benzoylecgonine thus no interference is observed. It is recommended that spots appearing at the level of benzoylecgonine standard should be circled immediately as spots due to weaker concentrations disappear in less than 1 min.

Procedure B for opiates. This procedure is recommended when the aim is to test for opiates alone. In this instance the residue obtained as described under *Procedure II(a)* is dissolved in 30–50 μl of methanol, vortexed, and the entire extract is spotted on a 10 × 20 cm Gelman pre-coated silica gel glass microfiber sheet (ITLC

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* We use 5-μl capillary tubes for routine spotting of more than 4,000 specimens and prefer these to a Hamilton syringe. We are interested in the progress of a treatment program and therefore mainly in qualitative information. These 5-μl capillaries can be purchased from Sherwood Medical Industries (St. Louis, Mo., U.S.A.).

** Benzoylecgonine was purchased from Technam Inc., Park Forest South, Ill., U.S.A., and contained no water of crystallization.

Type SA (a 20 × 20 cm sheet is cut into two 10 × 20 cm pieces). Three standards* (one at each edge and one in the center) and 10–12 samples are spotted on each 10 × 20 cm piece of TLC plate. After the standards and samples have been applied to the TLC plate, the spots are air dried and the plate is placed for 3–7 min in an oven at 85–90° prior to its development in 100 ml of solvent C (two 10 × 20 cm plates are placed in each tank). The solvent is allowed to travel a distance of 7–8 cm, then the plate is air dried for 10 min or until the smell of ammonia disappears. The plate is then sprayed with sulfuric acid followed by iodoplatinate as reported earlier[17–19].

Procedure C for poly-drugs. This procedure is recommended when the entire array of abused drugs is to be tested. In this instance the residue obtained as described under *Procedure II(a)* is dissolved in 30–50 μl of methanol, vortexed, and the entire extract is spotted on a 20 × 20 cm Gelman pre-coated silica gel glass microfiber sheet (ITLC Type SA). Four standards** (one at each edge and two interspaced in the center between urine specimens) and 10 samples are spotted on each sheet. The spots are air dried and the plate is dried for 5 min in an oven at 85–90° before it is placed in the solvent (drying for 3–7 min is needed, depending on the humidity of the air). A two-stage solvent system is used to achieve the optimal separation of a wide variety of abused drugs. The plate is first developed up to 9.0 cm in 100 ml of solvent E (150 ml of solvent E are used if two plates are placed in the tank). The plate is air dried for about 10 min and then dried in an oven at 85–90° for 3–5 min. Solvent E is discarded and the plate is then developed in the same direction up to 14.5–15.0 cm in 100 ml of Solvent F (180 ml of solvent F are used if two plates are placed in the tank)[18,19]. After the plate has been developed in solvent F, it is air dried for 10 min or until the smell of ammonia disappears. Detection reagents (a)–(g) are applied in succession[18,19].

Performance evaluation of detection techniques. The Center for Disease Control, Department of Health, Education and Welfare, Atlanta, Ga., U.S.A., has been conducting a Proficiency Testing Program in Toxicology Drug Abuse since 1972 for screening a wide variety of drugs. This laboratory has been participating for the last 3½ years and has been consistently achieving an average of 100% grade points.

RESULTS AND DISCUSSION

A double-blind study was conducted to validate the accuracy of the proposed extraction and detection procedures for benzoylecgonine. The urines for this study were supplied by Drs. C. R. Schuster and Marian W. Fishman of the Department of Psychiatry, University of Chicago, Chicago, Ill., U.S.A. These urines were collected

* An opiate standard is prepared by dissolving morphine hydrochloride, codeine phosphate, quinine sulfate and methadone hydrochloride in methanol (each at a concentration of 1 mg/ml). Methapyrilene (histadyl) may be included in this standard in areas where it is used to cut heroin along with quinine.

** Poly-drug standards are prepared as follows. Standard No. 1: morphine hydrochloride, codeine phosphate, quinine sulphate, methadone hydrochloride, phenmetrazine hydrochloride and methamphetamine hydrochloride dissolved in methanol (each 1 mg/ml). Standard No. 2: trifluorperazine hydrochloride, chlorpromazine hydrochloride, thioridazine hydrochloride, glutethimide and diphenylhydantoin dissolved in methanol (each 1 mg/ml). Standard No. 3: cocaine hydrochloride, amphetamine sulphate and methylphenidate hydrochloride dissolved in methanol (each 1 mg/ml). Standard No. 4: morphine hydrochloride, codeine phosphate, quinine sulphate, methadone hydrochloride, phenobarbital and secobarbital dissolved in methanol (each 1 mg/ml).

from individuals who were administered 16 and 32 mg of cocaine intravenously at 24-h intervals. Eight urines were collected: two after 3–8 h and three after 24 h after administering 16 mg of cocaine; two after 24 h after administering 32 mg of cocaine; and one after 24 h from the individual who was administered a placebo. These specimens were analyzed by Procedure II using 40–50 ml of urine. All urine specimens except the placebo urine were strongly positive for benzoylecgonine.

The accuracy of the proposed technique was also validated by giving the technician a set of 10 urine specimens specially obtained from the Center for Disease Control. The results obtained had an accuracy of 100% for the three urines that contained benzoylecgonine. Proficiency specimens received from the Center for Disease Control are tested using a two-step extraction procedure (Procedure II), poly-drugs are extracted first at a pH of 10.1 and benzoylecgonine is then extracted from the remaining aqueous alkaline phase as described in this paper. The results reported by this Laboratory for benzoylecgonine for four specimens of Survey II and three of Survey III, 1976, Proficiency Testing had an accuracy of 100%. The sensitivities for poly-drugs as now established[22] using 20–50 ml of urine are as follows: morphine base, 100 ng/ml of urine (volume of urine 50 ml) and 150–190 ng/ml (volume of urine 20 ml); methadone hydrochloride, 0.5–1.0 μg/ml (0.45–0.9 μg of methadone base); amphetamine sulfate, 1.0 μg/ml (0.87 μg of base); methamphetamine hydrochloride, 0.5 μg/ml (0.40 μg of base); phenmetrazine hydrochloride (Preludin), 0.5 μg/ml (0.41 μg of base); methylphenidate hydrochloride (Ritalin), 1.0 μg/ml (0.87 μg of base); cocaine hydrochloride, 0.5 μg/ml; codeine phosphate, 0.5 μg/ml; phenobarbital 0.5 μg/ml; and secobarbital 0.36 μg/ml. The thin-layer developing solvent systems that proved to be satisfactory for weekly monitoring of more than 3000 urines for opiates and 1000 urines for poly-drugs are solvent C for opiates (single-stage development system) and solvents E and F for poly-drugs (two-stage development system). Solvent D has been found to be suitable for confirmation of barbiturates, methadone, propoxyphene (Darvon) and unchanged cocaine. Solvent F has proved useful for the confirmation of methadone, methadone major metabolite, unchanged cocaine and propoxyphene (Darvon). Solvent C can also separate barbiturate mixtures such as phenobarbital, diphenylhydantoin (Dilantin) and seconal from glutethimide (Doriden).

The costs of setting up a Toxicology Laboratory Facility using TLC techniques and detection procedures currently used in drug abuse screening programs were discussed earlier[23,24]. A technician can analyse 120–135 urine specimens for opiates and 80–100 specimens for poly-drugs per day. Our overall cost of analysis, including chemicals, supplies, technical and support services, supervisory salary (one Chief Toxicologist, one Laboratory Manager and one Chief Chemist), laboratory rental and overhead charges is approximately $1.38 per specimen when monitoring 3500–4000 specimens per week. A single specimen tested for opiates (4–5 tests per specimen) cost $0.58 and for poly-drugs (9–14 tests per specimen) $0.82. This cost includes labor, chemicals and TLC supplies only[25].

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