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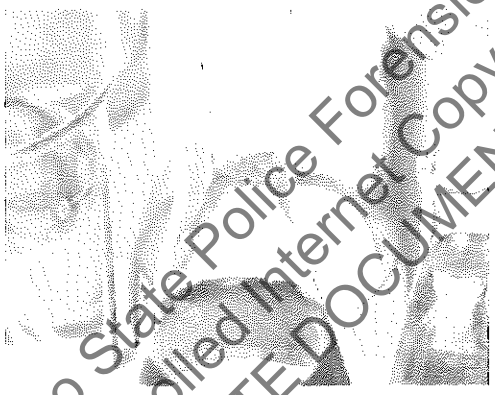

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2.3.6 Benzoylecgonine Extraction Employing United Chemical Techniologies (UTC) 200mg CLEAN SCREEN® DAU Extraction Column

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Section Two
Urine Toxicology

2.3 Solid Phase Extraction (SPE) Methods for Qualitative GC/MSD Confirmation

2.3.6 Benzoylecgonine Extraction Employing United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

2.3.6.1 BACKGROUND

Cocaine is a naturally occurring alkaloid derived from leaves of the South American shrub, Erythroxylon coca. Cocaine also can be produced synthetically. Cocaine is one of the most potent stimulants of the central nervous system due to its mechanism of action, which involves blocking reuptake of stimulatory neurotransmitters. Cocaine is used licitly as a local anesthetic in ophthalmology. The positive effects of cocaine include an increased mental awareness and alertness, a sense of clarity and feelings of elation. The fictional detective Sherlock Holmes used cocaine for its transcendently stimulating and mind clarifying properties to the displeasure of Doctor Watson. As with all drugs, the effects of cocaine depend on the dosage, the form in which it is taken and the route of administration. Other significant factors include the setting or circumstances in which the drug is used and the expectations of the user. Side effects can include pupillary dilation, restlessness, dizziness, dyskinesia, tremor, dysphoria, and paranoia. Additional major side effects of cocaine use are a consequence of discontinued use. If the user does not readminister the drug, they may experience increased anxiety, agitation, restlessness and the disturbance of normal sleep patterns, which leads to fatigue. Due to these effects following cocaine use, an individual's ability to operate a motor vehicle is impaired both during and following cocaine use.

Routes of administration include snorting, injection and smoking. The metabolism of cocaine and its metabolites involves hydrolysis, transesterification and n-demethylation. Cocaine metabolites detectable in urine include benzoylecgonine, ecgonine methyl ester, norcocaine and various arylhydroxy- and arylhydroxymethoxy- metabolites. The duration of the action of cocaine is limited by its rate of metabolism since its major metabolites are inactive. One of the active metabolites, cocaethylene is produced via transesterification when cocaine and ethanol are ingested concurrently.

2.3.6.2 SCOPE

This procedure outlines the use of the 200mg CLEAN SCREEN® DAU SPE column for the extraction of cocaine, methylecgonine and benzoylecgonine from urine. The CLEAN SCREEN® DAU column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering

substances.

The cation exchanger will allow the anionic sorbent (-) to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption. The nonpolar aspect of the column serves to extract nonpolar compounds from a polar sample matrix.[2] The cation exchanger component of the phase is effective for compounds which are present in the urine sample in a cationic form bonding ionically to the sorbent.

To maximize the ionic character of analytes, the urine is adjusted with a pH 6 100mM phosphate buffer, and loaded onto a pre-conditioned SPE column. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. Analytes are retained by ionic interaction of the amine functional groups present on the drug and the anionic sulfonic acid exchanger on the sorbent. The column is subsequently washed with water and a weak aqueous buffer, to selectively remove matrix components and interfering substances from the column. The wash also disrupts the hydrophobic and adsorption interactions but not the ionically bound material. Next, the column is dried to remove traces of aqueous and organic solvents. When the column is dry the analytes of interest are recovered from the column with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for qualitative confirmation on a gas chromatograph equipped with a mass selective detector (GC/MSD).

2.3.6.3 EQUIPMENT AND SUPPLIES

2.3.6.3.1 200mg CLEAN SCREEN® Extraction Column
2.3.6.3.2 Tube Rocker
2.3.6.3.3 Vortex Mixer
2.3.6.3.4 Drybath or Laboratory Oven
2.3.6.3.5 Evaporative concentrator equipped with nitrogen tank
2.3.6.3.6 Vacuum Manifold/pump
2.3.6.3.7 Laboratory centrifuge capable of ≥3200 rpm
2.3.6.3.8 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated
2.3.6.3.9 pH indicator strips
2.3.6.3.10 16 x 100mm Screw-top Glass Tube
2.3.6.3.11 Screw Cap for 16mm O.D. tube
2.3.6.3.12 {Optional} 16X144mm tapered tip centrifuge tubes
2.3.6.3.13 Automated Liquid Sample (ALS) vials
2.3.6.3.14 GC/MS Vial Microinsert
2.3.6.3.15 Gas Chromatograph equipped with a mass selective detector and a nonpolar capillary column with a phase composition

comparable to 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%-diphenyl

2.3.6.4 REAGENTS

Refer to Manual section 5.12 for solution preparation

2.3.6.4.1 Methylene Chloride (Certified ACS Grade)
2.3.6.4.2 Isopropanol (Certified ACS Grade)
2.3.6.4.3 Ammonium Hydroxide (Certified ACS Grade)
2.3.6.4.4 Methanol (Certified ACS Grade)
2.3.2.4.5 Ethyl Acetate (Certified ACS Grade)
2.3.6.4.6 Deionized/distilled (DI) water
2.3.6.4.7 100mM Phosphate buffer pH 6.0
2.3.6.4.8 100mM Monobasic Sodium Phosphate
2.3.6.4.9 100mM Dibasic Sodium Phosphate
2.3.6.4.10 100mM HCl
2.3.6.4.11 Elution Solvent
Mix 20mL isopropyl alcohol with 2mL ammonia hydroxide, QS to 100mL with methylene chloride.
2.3.6.4.12 BSTFA + 1% TMCS

2.3.6.5 QUALITY ASSURANCE MATERIALS

2.3.6.5.1 Positive Control
Positive Control can be prepared with the working solution described below and/or obtained commercially.

2.3.6.5.1.1 **Positive Control Stock Solution**
Obtain 1mg/mL (1µg/µL) stock drug reference material solutions through Cerilliant, Alltech, Sigma or other appropriate vendor.

2.3.6.5.1.2 **Positive Control Working Solution**
Add the designated volume of stock solution to 10mL methanol.

Stock Solution (1.0mg/mL)	Volume (µL)	ng/µL
Benzoylecgonine	100	10
Cocaine	100	10
Ecgonine methyl ester	100	10

Solution is stable for 1 year when stored under refrigeration.

2.3.6.5.2 Internal Standard
2.3.6.5.2.1 **Stock Solution**

1 mg/mL Mepivacaine

2.3.6.5.2.2 **Working Internal Standard Solution [10ng/µL]**
Add 100µL Mepivacaine stock solution to 10mL volumetric ball flask. QS with methanol.
Solution is stable for three months when stored at room temperature.

2.3.6.5.3 Negative Control
Commercially obtained or in-house urine verified to be negative for drugs of interest.

2.3.6.5.4 Non-extracted Reference Material

2.3.6.5.4.1 Reference material not included in extracted positive control should be prepared as necessary.

2.3.6.5.4.2 Obtain 1mg/mL stock drug reference material solutions through Cerilliant, Alltech, Sigma or other appropriate vendor.

2.3.6.5.4.3 Dilute 1mg/mL drug reference material as necessary. More than one compound may be added to this solution.

2.3.6.6 PROCEDURE

2.3.6.6.1 Initial set-up
Label extraction tubes in duplicate and ALS vials with microinserts for Negative Urine Control, Positive Urine Controls and with appropriate Laboratory Numbers.

2.3.6.6.2 Positive Control Sample Preparation

2.3.6.6.2.1 Add 5mL of negative urine to two screw top tubes.

2.3.6.6.2.2 Add indicated amount of 10ng/µL working mixed control solution.

Desired ng/mL	µL Working Control
400	200

2.3.6.6.2.3 Additional concentrations may be used at the discretion of the analyst.

2.3.6.6.3 Negative Control Sample Preparation
Add 5mL of negative urine to extraction tube.

2.3.6.6.4 Case Sample Preparation

2.3.6.6.4.1 Based on enzyme immunoassay screen results, samples may be diluted with distilled water prior to analysis.

2.3.6.6.4.2 The total volume of urine or diluted urine should be 5mL.

2.3.6.6.4.3 Add 5mL neat or diluted sample to labeled extraction tube.

2.3.6.6.5 Internal Standard Addition
Add 250µL of internal standard to control and case samples. This results in an internal standard concentration of 500ng/mL.

2.3.6.6.6 SPE
All aspirations must be at ≤3 in. Hg to prevent sorbent drying. Gravity flow may be used.

2.3.6.6.2.1 Transfer 5mL urine specimen, Negative Control or Positive Control to an appropriate labeled extraction tube.

2.3.6.6.2.2 Add 2mL pH 6 100mM phosphate buffer. Vortex.

2.3.6.6.2.3 Check pH. If pH is not 6.0 ± 0.5, adjust as necessary with 100mM monobasic or dibasic sodium phosphate.

2.3.6.6.2.4 Insert labeled CLEAN SCREEN® extraction column into vacuum manifold.

2.3.6.6.2.5 Add 3mL of methanol to column.

2.3.6.6.2.6 After methanol has flowed through, add 3mL of DI H_2O to column.

2.3.6.6.2.7 After water has flowed through, add 1mL 100mM phosphate buffer (pH 6.0) to column.

2.3.6.6.2.8 After buffer has flowed through, add buffered urine. Load sample onto column at ≤2 mL/minute.

2.3.6.6.2.9 Wash column with 2mL DI H_2O.

2.3.6.6.2.10 Wash column with 2mL of 100mM hydrochloric acid.

2.3.6.6.2.11 Wash column with 3mL of methanol.

2.3.6.6.2.12 Dry column by aspirating at ≥ 10 in. Hg for ≥ 5 minutes.

2.3.6.6.2.13 Open vacuum manifold, wipe collection tips, and insert collection holding rack containing collection tubes.

2.3.6.6.2.14 Add 3mL of elution solvent to column and aspirate slowly, < 3 in. Hg (10kPa).

2.3.6.6.2.15 Remove collection tubes with elutes from rack and place into evaporative concentrator.

2.3.6.6.2.16 Evaporate to dryness under a gentle stream of nitrogen at ≤ 37°C.

2.3.6.6.7 Derivatization

2.3.6.6.7.1 Add 50µL ethyl acetate, vortex.

2.3.6.6.7.2 Add 50µL BSTFA + TMCS.

2.3.6.6.7.3 Cap and vortex.

2.3.6.6.7.4 Heat tube for 20 minutes at 70°C.

2.3.6.6.7.5 Remove tube from dry heat. Allow to cool to room temperature.

2.3.6.6.7.6 Transfer extract to the appropriately labeled ALS vial.

2.3.6.6.8 Preparation for Analysis Run

2.3.6.6.8.1 Into Sequence log table, enter the sample case numbers, blanks and controls.

2.3.6.6.8.2 Load samples, reference material, blank and controls into the quadrant rack as noted in the sequence table.

2.3.6.6.9 GC-MSD Analysis Parameters

2.3.6.6.9.1 Refer to instrument METHOD printout for current analysis parameters.

2.3.6.6.9.2 Current analysis method must be stored centrally as a hard or electronic copy.

2.3.6.6.10 Detection and Identification Criteria

The presence of a drug compound is indicated if the retention time for the sample versus applicable reference material does not differ by more than ±0.2 minutes and there are no significant differences in the mass spectral data.

2.3.6.7 QUALITY ASSURANCE REQUIREMENTS

2.3.6.7.1 General

2.3.6.7.1.1 Urine samples are to be stored frozen until allowed to thaw prior to analysis.

2.3.6.7.1.2 Urine samples are to be stored under refrigeration after aliquots are removed for analysis.

2.3.6.7.1.3 Post analysis, urine samples are to be stored frozen until appropriate disposal date.

2.3.6.7.1.4 Refer to toxicology analytical methods 5.8 and 5.10 for additional quality assurance and reference material authentication requirements.

2.3.6.8 ANALYSIS DOCUMENTATION

2.3.6.8.1 Original data for controls will be prepared for each analysis run and stored centrally in the laboratory where the analysis was performed until archiving.

2.3.6.8.2 A copy of controls need not be included in individual case files. When necessary, a copy of control printouts can be prepared from the centrally stored document.

2.3.6.9 REFERENCES

2.3.6.9.1 UCT CLEAN SCREEN® Extraction Columns Application Manual.

2.3.6.9.2 Telepchak, M.J., August, T.F. and Chaney, G., Drug Methods for the Toxicology Lab, pp. 204 - 209. *in:* Forensic and Clinical Applications of Solid Phase Extraction, Humana Press: New Jersey, 2004.

2.3.6.9.3 Platoff, G.E., Gere, J.A. Solid Phase Extraction of Abuse Drugs from Urine, For. Sci. Review, 3 (2):117-132; 1991.

Revision History

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Revision No.	Issue Date	Revision/Comments
1	02-05-2002	Original Issue in SOP format
2	10-18-2002	Refinements
3	05-07-2007	Addition of internal standard and updated QA measures and reformatting.