Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

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

2.3.6 Extraction of Benzoylecgonine 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 is also can be produced synthetically. Cocaine is one of the most potent stimulants to 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 PRINCIPLE

This procedure outlines the use of the 200mg CLEAN SCREEN® DAU SPE column for the extraction of cocaine 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 in the urine sample.

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.

For the extraction of cocaine and benzoylecgonine, the urine is adjusted to pH 6 with a phosphate buffer to maximize the ionic character of the analyte, 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. The analyte is 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 confirmation on the GC/MSD.

2.3.6.3 EQUIPMENT AND SUPPLIES

2.3.6.3.1 200 mg CLEAN SCREEN® Extraction Column (ZSDAU020 OR ZCDAU020 or equivalent)

2.3.6.3.2 Drybath (Fisher or equivalent)

2.3.6.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.

2.3.6.3.4 Vacuum Manifold/pump

2.3.6.3.5 Glassware

16X100 Test Tubes (Fisher 14-961-29 or equivalent)
16X144mm tapered tip centrifuge tubes (Fisher 05-538-41C or equivalent)
Snap Caps (Fisher 05-538-41N or equivalent)
GC/MS Automated Liquid Sampler (ALS) vials (HP 5182-0865 or equivalent)

GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.3.6.3.6 pH paper (Fisher 09-876-17 or equivalent)

2.3.6.3.7 Gas chromatograph equipped with a mass selective detector (HP 6890/5973 or equivalent) and a nonpolar capillary column with a phase composition capable of efficiently separating amines, alkaloids, drugs compounds and other analytes encountered in toxicological specimens (e.g. 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5% diphenyl)

2.3.6.3 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.6.4.1 Methylene Chloride (Fisher D37-4 or equivalent)

2.3.6.4.2 Isopropanol (Fisher A416-1 or equivalent)

2.3.6.4.3 Ammonium Hydroxide (Fisher A669-500 or equivalent)

2.3.6.4.4 Methanol (Fisher A412-4 or equivalent)

2.3.2.4.5 Ethyl Acetate (Fisher E145-4 or equivalent)

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 HCl

2.3.6.4.9 Elution Solvent

Mix 70mL methylene chloride, 20mL isopropyl alcohol, and 2mL ammonia hydroxide.

2.3.6.4.10 Silylating Agent (select from)

- MSTFA/1% TMCS (Pierce#48915 or equivalent)
- MSTFA (Pierce#48910 or equivalent)
- BSTFA/1% TMCS (Pierce#38831 or equivalent)
- BSTFA (Pierce#38830 or equivalent)

2.3.6.5 CONTROL

2.3.6.5.1 UTAK 66812-C or an equivalent control which contains benzoylecgonine in the appropriate concentrations.

2.3.6.6 STANDARDS

2.3.6.6.1 Run necessary analytical standards as indicated by examination of GC/MSD data.

Standards (in solution)	*Potential Vendors*
Benzoylecgonine	Cerilliant B-004, Alltech 018203
Cocaine	Cerilliant C-008, Alltech 018003
Ecgonine methyl ester	Cerilliant E-001, Alltech 014553
Norcocaine	Cerilliant N-005, Alltech 6015353
Cocaethylene	Cerilliant C-010, Alltech 6015363

2.3.6.7 PROCEDURE

2.3.6.7.1 Initial set-up

Label the test tubes and GC/MS vials with microinserts.

- Negative Control
- Positive Control
- Appropriate Laboratory Numbers

2.3.6.7.2 Extraction Procedure Utilizing 200mg CLEAN SCREEN® DAU Column

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

2.3.6.7.2.2 Add 2mL 100mM phosphate buffer and Vortex. pH should be 6.0 ± 0.5. Adjust pH as necessary with 100mM monobasic or dibasic sodium phosphate.

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

2.3.6.7.2.4 Add 3mL of methanol to column and aspirate at ≤3 in. Hg to prevent sorbent drying.

2.3.6.7.2.5 Add 3mL of DI H_2O to column and aspirate and aspirate at ≤3 in. Hg.

2.3.6.7.2.6 Add 1mL 100mM phosphate buffer (pH 6.0) to column and aspirate at ≤3 in. Hg.

2.3.6.7.2.7 Load sample onto column at 1 to 2 mL/minute.

2.3.6.7.2.8 Wash column with 2mL DI H_2O and aspirate at ≤3 in. Hg.

2.3.6.7.2.9 Wash column with 2mL of 100mM hydrochloric acid and aspirate at ≤3 in. Hg.

2.3.6.7.2.10 Wash column with 3mL of methanol and aspirate at ≤3 in. Hg.

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

2.3.6.7.2.12 Open vacuum manifold, wipe collection tips, and insert collection holding rack containing the 16X144mm tapered tip centrifuge tubes.

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

2.3.6.7.2.14 Remove collection vials with elutes from rack.

2.3.6.7.2.15 Evaporate to dryness under a gentle stream of nitrogen at ≤ 40°C.

2.3.6.7.2.16 Add 50µL ethyl acetate.

2.3.6.7.2.17 In fume hood, add 50µL silylating agent.

2.3.6.7.2.18 Cap.

2.3.6.7.2.19 Vortex.
2.3.6.7.2.20 Heat for 20 minutes 70°C dry bath.
2.3.6.7.2.21 Remove from dry bath and cool to room temperature.
2.3.6.7.2.22 Transfer to the appropriately labeled ALS vial.

2.3.6.7.3 Automated Extraction Procedure Utilizing 200mg CLEAN SCREEN® extraction column.
2.3.6.7.3.1 Refer to the following attached methods/printouts.

2.3.6.7.4 Gas Chromatography/Mass Spectrometry (GC/MS) Analysis
2.3.6.7.4.1 Inject 1 µL into GC/MS using the ALS.

2.3.6.7.4.2 Analyze sample extract in full scan acquisition. Refer to attached GC/MSD method printout for current analysis parameters.

2.3.6.7.5 Detection and Identification Criteria
2.3.6.7.5.1 The presence of a drug compound can be established if there are no significant differences in the retention time and mass spectra for the sample versus that of an authenticated standard.
2.3.6.7.5.2 Acceptable retention time window is ±5%.

2.3.6.8 REFERENCES

2.3.6.8.1 UCT CLEAN SCREEN® Extraction Columns Application Manual.

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2.3.5.8 REFERENCES

2.3.5.8.1 Automated SPEC® · Solid Phase Extraction Protocols for Drugs of Abuse Using the RapidTrace™ SPE Workstation, ANSYS, 1997.

2.3.5.8.2 SPEC·PLUS™·3ML·DAU Drugs of Abuse in Urine Extraction Applications, Ansys, 1999