Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

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

2.3.5 Extraction of Benzoylecgonine Employing the SPEC·PLUS™·DAU Extraction Column.

2.3.5.1 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 of cocaine 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.

2.3.5.2 PRINCIPLE

This procedure outlines the use of the SPEC·PLUS™ DAU SPE column for the extraction of Benzoylecgonine from urine. ANSYS Technologies' SPEC™ Solid Phase Extraction products are manufactured with polypropylene plastic and bonded-silica impregnated on a glass fiber disc. The DAU column utilizes a sorbent which combines a strong cation exchanger with a non-polar phase (reversed phase) to interact effectively, physically and chemically, with benzoylecgonine and minimally with interfering substances in the urine sample. The cation exchanger component of the phase is effective for compounds which are present in the urine in a cationic form. The sample pretreatment with 0.1M HCl ensures that the nitrogen group on the ecgonine portion of the cocaine molecule (pKa 8.6) bonds ionically to the sorbent. For the extraction of benzoylecgonine the urine is adjusted with a phosphate buffer to maximize the ionic character of the analyte. The sample is then applied to a pre-conditioned SPE column. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The column is subsequently washed with the aqueous solvent, to selectively remove matrix components and interfering substances from the column. Next, the column is dried to remove traces of solvent. 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.

2.3.5.3 EQUIPMENT AND SUPPLIES

2.3.5.3.1 SPEC·PLUS™ 3ml DAU column (Ansys 532-DAU or equivalent)

2.3.5.3.2 Drybath (Fisher or equivalent)

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

2.3.5.3.4 Vacuum Manifold/pump

2.3.5.3.5 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)

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

2.3.5.3.7 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)

2.3.5.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.5.4.1 Methanol (Fisher A412-4 or equivalent)

2.3.5.4.2 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.5.4.3 50% Methanol/water

2.3.5.4.4 0.1M Hydrochloric Acid

2.3.5.4.5 Elution Solvent

Mix 80mL ethyl acetate with 20mL methanol.

2.3.5.4.6 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)

2.3.5.5 CONTROL

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

2.3.5.6 STANDARDS

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

Standard (in methanol)	*Potential Vendors*
Benzoylecgonine	Cerilliant B-004, Alltech 018203

2.3.5.7 PROCEDURE

2.3.5.7.1 Initial set-up

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

- Negative Urine Control
- Positive Urine Control
- Appropriate Laboratory Numbers

2.3.5.7.2 Extraction Procedure Utilizing SPEC·PLUS™·DAU Extraction Column

2.3.5.7.2.1 Transfer 1mL of urine specimen, Negative Control or Positive Control to an appropriate labeled test tube.

2.3.5.7.2.2 Add 1mL 0.1M HCl and Vortex.

2.3.5.7.2.3 Insert labeled SPEC·PLUS™·DAU extraction column into vacuum manifold

2.3.5.7.2.4 Add 200µL of methanol to column and wait for one minute.

2.3.5.7.2.5 Pour sample onto column and aspirate at 3-5 in. Hg (10-17 kPa)

2.3.5.7.2.6 Add 500µL of 0.1M HCl to column and aspirate at 3-5 in. Hg (10-17 kPa).

2.3.5.7.2.7 Add 500µL of 50% methanol/water to column and aspirate at 3-5 in. Hg (10-17 kPa).

2.3.5.7.2.8 Increase vacuum to 10-20 in. Hg. (34-68 kPa) and dry extraction disc for ≥5 minutes.

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

2.3.5.7.2.10 Add 800µL of elution solvent to column and aspirate slowly at < 3 in. Hg (10kPa).

2.3.5.7.2.11 Increase vacuum to 5 in. Hg (17 kPa) to assist final amount of elution solvent through the disc.

2.3.5.7.2.12 Remove collection vials with elutes from rack.

2.3.5.7.2.13 Evaporate solvent to dryness under a gentle stream of nitrogen at approximately 60°C.

2.3.5.7.2.14 Add 25µL of ethyl acetate.

2.3.5.7.2.15 In the hood add 25µL of silylating agent.

2.3.5.7.2.16 Cap.

2.3.5.7.2.17 Vortex.

2.3.5.7.2.18 Heat for 15 minutes at 90°C.

2.3.5.7.2.19 Cool to room temperature

2.3.5.7.2.20 Transfer to the appropriately labeled ALS vial.

2.3.5.7.3 Automated Extraction Procedure Utilizing SPEC·PLUS™·DAU Extraction Column

2.3.5.7.3.1 Refer to the following attached methods/printouts.

2.3.5.7.4 Gas Chromatography/Mass Spectrometry (GC/MSD) Analysis

2.3.5.7.4.1 Inject 1µL into GC/MSD using the ALS.

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

2.3.5.7.5 Detection and Identification Criteria