1.0 PRINCIPLE

Cocaine is rapidly metabolized in the body by hydrolysis of the methyl ester group, to form benzoylecgonine (BE) with a cocaine half-life of ~90 min. Because of the rapid breakdown, BE is usually the major detectable product of cocaine ingestion in biological fluids. In many cases, the parent compound, cocaine, will not be detected at all. This procedure details the process by which the presence of cocaine and/or BE may be confirmed in urine, blood or other aqueous fluids. Cocaine or BE present in the sample is extracted onto a solid phase extraction column. Co-extracting materials are washed from the column, and extracted cocaine and/or BE is subsequently eluted. The eluent is evaporated, and any BE present is derivatized with BSTFA (N,O-bis trimethylsilyl trifluoroacetamide)/1% TCMS (Trimethylchlorosilane). The presence of any cocaine and/or derivatized BE in the sample is qualitatively evaluated by 3-ion SIM, using retention time and 2 qualitative ions ratios as the basis for identification, in comparison to co-extracted calibrator and control samples.

2.0 SPECIMEN

A. Urine and Blood can be analyzed by this method. Other sample matrices may be also be appropriate to run, consult with Unit Lead for guidance.
B. JusticeTrax can be used to generate a worklist for specimens requiring confirmation for BE/Cocaine.
C. All evidence transfers, either between individuals or between an individual and storage location must be documented on the Chain of Custody for the case, either in the LIMS, or on hard-copy COC document maintained in the Case Jacket.
D. When not in the sampling or aliquot process samples will be maintained in locked storage within the Toxicology unit.
E. Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.
F. When analysis of samples in the Toxicology unit is complete, they must be maintained “Under Proper Seal.” Refer to SOP TX-19 for further guidance.
G. Samples are maintained in the Toxicology Section for a minimum of 8 weeks after case is completed, in the absence of notification of any legal action, or other reason to maintain the samples. Samples from fatalities and sexual assaults are maintained indefinitely by the DSS. Cases with requests for retention are maintained by the laboratory based on the request. Upon request from the courts or submitting agency the evidence will be returned to the submitting agency.

3.0 EQUIPMENT:

A. GC/MS and associated data station/computer (HP6890/5973, Agilent Technologies 7890A, AT-7890B, AT-5975 or 9577 or equivalent)
B. General laboratory glassware and equipment
C. Solid phase extraction manifold and associated vacuum equipment
D. Analytical evaporator
E. UCT; ZSDAU020 “Clean Screen” extraction columns (or equivalent)

4.0 **REAGENTS**:

A. **Reagents available as stock items:**
   1. Methanol (MeOH; Baker HPLC grade or better)
   2. Deionized water (DIW; 2-megohm In-House supply)
   3. Hydrochloric Acid (HCl; Baker Reagent Grade, or equivalent)
   4. Isopropanol (IPA) (Baker HPLC grade or better)
   5. Methylene Chloride (Baker HPLC grade or better)
   6. Ammonium Hydroxide(NH_4OH; Baker Reagent Grade, or equivalent)
   7. Ethyl acetate (ETOAc; Baker HPLC grade or better)
   8. BSTFA with 1% TMCS
   9. Blank Blood (may be acquired from a hospital blood bank, or the American Red Cross or similar source)
   10. Sodium Phosphate Dibasic (Na2HPO4).
   11. Sodium Phosphate Monobasic (NaH2PO4).Drug free urine

B. **Reagents prepared in the Toxicology Laboratory:**
   1. <u>Methylene chloride/isopropanol/ammonium hydroxide</u> (39/10/1mL = 50mL total; or equivalent ratio; Prepared fresh each day of use)
   2. <u>0.1 M Phosphate Buffer pH 6</u>
      a. Combine 48.6 g Na_2HPO_4 and 6.8 g NaH_2PO_4
      b. QS to 4000 ml using DWI0.1 M HCl (8.4 ml conc. HCl/1000 mL H2O)
   3. <u>0.1 M HCl</u>
      a. To 400 mL DIW in a volumetric flask
      b. Add 8.4 mL concentratred HCl
      c. Mix and QS to 1000 ml
      d. Storage: room temperature in glass or plastic. Stability: 12 months
         a. Note: Reagent Preparation and Validation is documented in the Toxicology “Reagent Preparation/Validation Logbook” maintained in the Toxicology unit.

C. **Stock Calibrator and Control Solutions:** Comprised of benzoylecgonine and cocaine (1 mg/mL; Cerilliant, Lipomed, Grace, or equivalent manufacturer)
   1. Working Standard Solution 10ug/mL:
      a. Into a 10 mL volumetric flask partially filled with methanol
      b. Pipette 100 ul of each reference standard (1mg/mL)
      c. QS with methanol and mix
      d. Store in freezer or refrigerator
      e. Stability for ~1 year, it must be revalidated after that year
   2. Diluted Working standard solution 1.0 ug/mL:
      a. Into a 10 mL volumetric flask partially filled with methanol
      b. Pipette 1 mL of working stock solution 10 ug/mL
      c. QS with methanol and mix
      d. Store in freezer or refrigerator
      e. Stability for ~1 year, it must be revalidated after that year

D. **Internal Stock Standards:** Comprised of benzoylecgonine and cocaine (1 mg/mL; Cerilliant, Lipomed, Grace, or equivalent manufacturer)
   1. Working Internal Standard: (10 ug/mL Cocaine D3, 25 ug/mL Benzoylecgonine D3)
      a. Into a 10 mL volumetric flask partially filled with methanol
      b. Pipette 100 ul of each Cocaine D3 (1mg/mL)
      c. Pipette 250 ul of Benzoylecgonine D3 (1 mg/mL)
      d. QS with methanol and mix
      e. Store in freezer or refrigerator
      f. Stability for ~1 year, it must be revalidated after that year

E. **Controls:**
   1. Urine Qualitative: the target concentration for the positive urine control is 250 ug/ml.
      a. Pipette 25 uL of the working standard solution (10 ug/mL) into 1.0 mL blank matrix
      b. Continue to follow sample preparation procedure
   2. Blood Quantitative:
      a. Refer to table in procedure section, continue to follow sample preparation procedure

F. **Quality Controls:**

Calibrators and controls must be independently prepared from a separate initial dilution or obtained from other sources. When available commercial reference controls will be purchased from an outside vendor. If commercial controls are not available, In-house controls should be prepared from a different provider. When only one supplier is available, a lot different from the calibrator should be used. At the least, when there is only one source, a separate preparation, different from the

calibration standard (prepared by a 2nd analyst) should be used.

1. Urine Control DAU LC 2, Product # 50703, Utak Laboratories Valencia, CA 91355
    a. Remove cap from vial
    b. Reconstitute control material by adding exactly 10.0 mL of DIW, using a 10 mL volumetric pipette
    c. Replace cap and let sit 10 – 15 minutes
    d. Swirl gently 3 – 4 minutes to ensure homogeneous mixture
    e. Swirl gently each time an aliquot is removed to ensure a homogeneous mixture
    f. Handle and test the control material in the same manner as case specimens
    g. For quantitative assays, record the results of the controls obtained on the Excel Quality Control Chart on the DSS S drive, that describes statistical limits for the test method and the particular lot of the control material
    h. Store reconstituted control material refrigerated at 2-8°C, stable for 25 days after reconstitution

G. **Validation of Reagents**:

1. Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Validated reagents are marked with a green dot/sticker, detailing the specific procedure for which the reagent was validated, and the batch on which that process was documented. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample results Reagents so validated are marked with a green sticker as noted above. Preparation of reagents, and their validation is documented in the Toxicology unit Reagent Preparation Validation Logbook, maintained in the Toxicology laboratory.

5.0 PROCEDURE

A. **Sample Preparation:**

1. Blood:
    a. Pipette 1 mL of sample into an appropriately labeled 16 x 100 borosilicate culture tube.
    b. Add 20 uL of the Working Internal Standard
    c. Add 3 mL phosphate buffer (pH 6.0)
    d. Sonicate for 15 minutes

e. Centrifuge 10 minutes at ~ 5000 rmp

2. Urine (1mL)
   a. Pipette 1 ml of sample into an appropriately labeled 16 x 100 borosilicate culture tube
   b. Add 20 uL of the Working Internal Standard
   c. Add 3 mL phosphate buffer (pH 6.0)
   d. Mix

3. Urine (100 uL)
   a. Pipette 100 ul of sample into a 2nd appropriately labeled 16 x 100 borosilicate culture tube
   b. Add 20 uL of the Working Internal Standard
   c. Add 3 mL phosphate buffer (pH 6.0)
   d. Mix

4. Blood Quantitation: The chart below may be used as a guide, pipette 1 mL of sample, blank, calibrator and control to each appropriately labeled 16 x 100 screw top culture tube.

Calibration Concentration ng/mL	uL Working Standard (10 ug/mL)	uL diluted Working Standard (1.0 ug/mL)	Blank blood pipette uL
0 blank	0	0	1000
20	--	20	980
50	--	50	950
100	--	100	900
200	20		980
500	50		950
1000	100		900
QC Concentration	uL Stock Control (10 ug/mL)		
250	25		975
	Utak Control		
Utak low	500		500

5. Label SPE tubes to correspond with each culture tube. Load into the manifold.

6. Condition the columns:
   a. 1 x 3 ml methanol; aspirate
   b. 1 x 3 ml DIW; aspirate
   c. 1 x 1 ml 0.1 M phosphate buffer pH 6; aspirate
   d. DO NOT LET COLUMN GO DRY!

7. Transfer contents of each tube to the appropriately labeled SPE tube, and allow gentle drop wise flow until the level reaches the top of the column bed.

8. Wash column:
   a. 1 x 2 ml DIW; drain (vacuum assist)
   b. 1 x 2 ml 0.1 M HCL; drain (vacuum assist)
   c. 1 x 3 ml methanol; drain (vacuum assist)
   d. Dry column (10 minutes at > 10 inches Hg)

9. Position appropriately labeled 13x100 test tubes under each SPE column, with clean thru tip inside collection test tube.

10. Elute analytes from SPE columns by adding 3 ml methylene chloride/ Isopropanol/ammonium hydroxide (39/10/1) to each SPE tube. Collect at 1-2 ml/minute.

    a. Note: Elution solvent must be prepared fresh the day of use.

11. Remove receiver tubes and evaporate to dryness at < 40° C using a gentle flow of Nitrogen.

12. Reconstitute residue with 150 ul of ethyl acetate and transfer to GC/MS vial with limited volume insert. Add 25 micro liters BSTFA (with 1% TMCS), cap and reserve for GC/MS analysis (no evaporation).

13. Note: Cases requiring reanalysis due to high concentrations (i.e. initial analysis is greater than the high control), should be diluted with 0.1 M pH 6.0 Phosphate buffer as appropriate. The initial quantitative values may be used as a guide for the dilution process the dilution process shall be documented in the case jacket.

14. Note: Departure from procedures as specified in this SOP is not

anticipated. Should an issue arise that may require such a departure, the issue must be reviewed and approved by with the Unit Lead and Deputy Director. Depending on the nature of the deviation DSS customers may need to be consulted, see GL-20 for guidance.

6.0 CHROMATOGRAPHY AND MASS SPECTROSCOPY

A. Instrument and Setup:
   1. GC/MS/Auto sampler: (Hewlett-Packard 6890/5973, or equivalent)
   2. Column: 30M RTX-5MS (0.25 mm ID; 0.25 micron film)
   3. Injector Temp. 250 ^{o}
   4. Detector Temp. 160 ^{o}
   5. Oven (initial): 160^{o}, 25 ^{o} /min to 280 ^{o}, (4.50 min hold).
   6. 9.30 minutes total run time
   7. 1 ul injection
   8. Method: cocbe20.M
      a. Method Details Appended (Appendix I)

B. Injection sequence: Samples are injected on the GC/MS in the following general sequence:
   1. <u>Urine Qualitative</u>:
      a. Solvent blank
      b. Positive urine calibrator/standard 250 ng/mL (i.e. In-house control)
      c. Urine blank
      d. Utak control
      e. Case samples
      f. End batch with calibrator or control

   2. <u>Blood Quantitation Multipoint Calibrators:</u>
      a. Solvent blank
      b. Priming calibrator
      c. Blood blank
      d. Calibrator 1 (50 or 20 ng/mL)
      e. Calibrator 2 (100 ng/mL)
      f. Calibrator 3 (200 ng/mL)
      g. Calibrator 4 (500 ng/mL)
      h. Calibrator 2 (1000 ng/mL)
      i. Blood blank
      j. UTAK Low control
      k. Case samples
      l. Run set of low and high controls midway through the batch or approximately after 10 case samples (when appropriate for longer

batches)

m. End batch with a set of controls

3. File name – Include case number (including year) in each sample data file.

4. Verify the sequence, verify the vial positions in the auto sampler tray match the sequence.

C. Detection and Identification:

1. Determination of the presence of Cocaine and/or BE in the sample extract are identified by appearance and ratio of the 3 ions characteristic of each species at the appropriate retention time, hence both retention time (a GC characteristic) and fragmentation pattern and ratio (MS characteristics) are used as the basis of qualitative identification.
2. For a positive identification of either cocaine or BE, the retention time must be within 5% of the corresponding analyte in the calibrator injection, and the ion ratios for both qualitative ions must be within 20% of the corresponding ratio calibrator sample.
3. Qualitative identification of each analyte is independent.

D. Calibration:

1. Calibration is not performed on urine samples
2. Calibration for each batch is done independently through the use of method specific controls.

E. Quantitation:

1. Quantitation is accomplished by the comparison of the response ratio of the analyte in a specific sample, to the response ratios of the calibrators as expressed as a “standard curve”. The concentration of the analyte in the sample is then extrapolated, from the standard curve, and corrected for any dilution that may have been performed to facilitate the analysis of relatively concentrated blood samples.

2. For multi-point calibrations, the criteria for acceptability of the calibration, and for individual calibrators is that when the values are read back against the final calibration curve they should generally be within +/- 20% of their value. A slightly wider acceptance value (e.g. +/-25% or 30%) is acceptable for calibrators that approach the LOQ of the assay.

3. Note: The available quantitative range of this procedure is (in terms of amount of drug injected on the instrument (thereby allowing for appropriate calculation of diluted samples) is defined for , and validated on each batch by the high and low control, and the acceptable performance of each.

4. The criteria for a valid calibration GC/MS linear regression "r_2" value for a 3 point curve is ≥ 0.98 using non-deuterated internal standards. A significant change in the slope of the calibration line, monitored between runs, may indicate that corrective action needs to be taken.

5. When more than 3 calibration points are used, one point may be removed if it failed to fall into the acceptable quantitative range. If two or more points need to be removed consult with the Unit Lead if any results can be accepted. On a case-by-case basis, results may be reported qualitatively or semi-quantitative.

7.0 QUALITY CONTROL AND RUN EVALUATION

A. Verification of Vial Sequence; Vial sequence is checked both before and after the injection of samples. The check after the samples are injected is documented on the run summary sheet.

B. Chromatography - Evaluation and Acceptance Criteria: Chromatographic quality is evaluated for each peak. While general guidelines are that a peak should be symmetrical and be resolved to baseline on at least one side with 90% resolution on the other side, significant departures from those guidelines may be experienced with forensic samples. In many cases, chromatographic quality will warrant rejection of the chromatographic run, or specific samples, by the operator. Any such action should be clearly documented on the batch summary sheet. Questionable chromatographic peak shape, resolution, or other problems with chromatography can be discussed with the Unit Lead or Deputy Director.

C. Evaluation of Potential Carryover: Carryover in the chromatographic quality is evaluated by injection of a blank sample immediately following the calibrator. Carryover of greater than 2% of cocaine or BE requires batch rejection, and remedial action for the instrument (e.g. replacement of injector insert, new septum and perhaps column trim or even replacement. Demonstrated carryover,

if less than 2%, will require operator consideration with regard to the potential for effects on specific samples, and may require re-extraction of specific samples. Carryover is further evaluated on a per sample basis by the requirement that quantitative results between replicates agree within 20%. Any significant carryover effect should cause the first of the two replicate samples to exceed the second by an amount in excess of the 20% differential. If not, any carryover may be considered inconsequential. In practice, when a question of potential carryover exists, the potentially affected sample replicates may be repeated at the end of the batch.

D. Evaluation of Controls: Positive and negative controls are evaluated to allow for procedural batch acceptability.

E. Qualitative Results: Controls must demonstrate the target analyte with acceptable chromatography and spectral characteristics.

F. Quantitative: Each batch must have at least 10% controls including a positive and negative. The controls can be re-injected in the middle and end of the batch to demonstrate the stability of the calibration. Acceptable results are the mean or target +/- 20% or +/- 2 standard deviations.

1. Levy-Jennings charts will be used monitor assay performance precision over time.

G. Internal standard abundance should be similar throughout the batch.

H. Linearity: Linearity of the calibration curve is demonstrable in each batch, for each analyte as a function of linear regression and quantitative results of control materials.

I. Sensitivity – Limits of Detection (LOD) and Quantitation (LOQ): For the purposes of this procedure, the LOD and LOQ are defined as equal to the lowest concentration of the lowest control. Qualitative Identification and/or Quantitative analysis below the concentration of the low control may be accepted on a case-by-case basis with the concurrence of the analyst, technical reviewer, Unit Lead and Deputy Director. LOD should be at least 3 times the signal to noise ratio, LOQ should be at least 10 times the signal to noise ratio.

J. Accuracy and Precision: Precision of the procedure is evaluated on a yearly basis, by repeat analyses of control or PT materials. Accuracy is expressed as a mean (absolute value) percentage difference between mean quantitative value of

10 reps of the specific control, and the target value. Precision is expressed as the CV of that value.

K. Specificity: Specificity is a function of both the resolution of target analyte during the analytical process, and the mass spectral fragmentation that analyte molecules undergo during the instrumental analysis. There has been no report of any material other than WAN and Basic drugs that elutes within 5% if the retention time of known standard materials, and produce the same fragmentation ions and ratios.

8.0 REPORTING OF RESULTS:

A. Once the batch is completed and the data is complied, the batch undergoes a batch review (refer to SOP TX-5 for guidance). Once the batch is accepted the results are entered into JusticeTrax.
B. Wherever possible, analytical results must be reviewed with reference to whatever case history or other information is available.
C. Procedural Uncertainty is reported with all quantitative results, and is calculated and tabulated annually for each analytical method, (See SOP TX-19 section 6.3).

9.0 SOURCES OF ERROR: The utilization of 3-ion SIM methodology, with reference to procedural, controls and calibrators yields qualitative drug identification with essentially no uncertainty. Urine drug analyses are reported only as qualitative results.

10.0 REFERENCES

A. Clarke's Isolation and Identification of Drugs. 2nd Edition
B. UCT United Chemical Technologies: Solid phase extraction methods

Appendix I:

GC/MS temperature program specifications

Temperature program	COCBE20.M		
Parameter			
Initial temp	160° C		
Initial Time	0.00min		
Ramps Rate/final temp/final time	rate	temp	time
	25.0	280	3.5
Post temp	280°c		
Post time	0.50		
Run Time	8.3min		
Front inlet			
Mode	Pulsed split less		
Initial temp	250°c		
Pressure	14.2psi		
Pulse pressure	30psi		
Pulse time	1.0 min		
Total flow	54.1mL/min		
Gas type	Helium		
Injection volume	1 microliter		
Post injection washes Solvent A / Solvent B	Solvent A -3 Solvent B - 3		
Tune file	ATTUNE		
Acquisition mode	SIM		

Solvent delay	3.8 minutes
M/Zs	182, 185, 198, 201, 303, 306
M/Zs	240, 243, 256, 259, 361, 364
Dwell	20
MS Quad	150°c max 200
MS Source	230°c max 250