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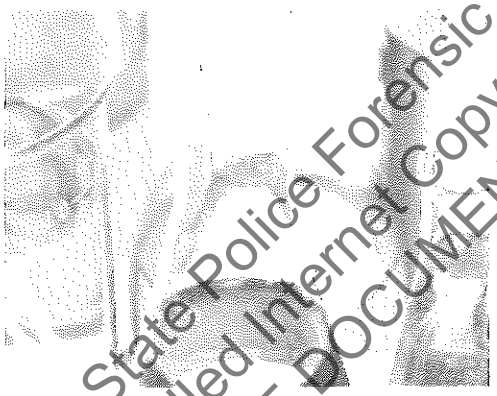

Discipline/Name of Document: Toxicology
3.10.3 – Extraction and Quantitation of Free (Unbound) Codeine and Morphine in Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column **(FOR QUALITATIVE USE ONLY)**

Revision Number: 0

Issue Date: 11/21/2006

APPROVED BY: Corinna C Owsley — Quality Manager

6/26/07 — Date Signed

Original Certificate did not document that the approval was only for reporting qualitative results.

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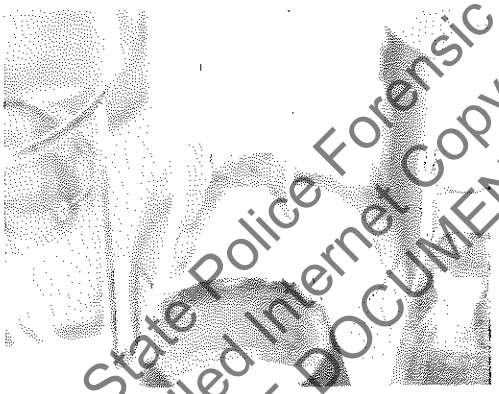

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Idaho State Police
Forensic Services
Toxicology Discipline

Section Three
Blood Toxicology

3.10 Manual Solid Phase Extraction (SPE) Methods

3.10.3 Extraction and Quantitation of Free (Unbound) Codeine and Morphine in Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

3.10.3.1 BACKGROUND

Refer to qualitative opiate analytical method 3.4.4 and provided references for information regarding the background and pharmacology of these compounds.[2-5]

3.10.3.2 PRINCIPLE

This procedure is based on a method developed by United Chemical Technology (UCT) which applies the UCT 200 mg CLEAN SCREEN® extraction column for the extraction of opiates from blood.[1] 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 blood sample. The cation exchanger utilizes an anionic sorbent to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption.

For the extraction of opiate class drugs, the blood sample is diluted and centrifuged, adjusted to pH 6 with a phosphate buffer, and loaded onto a pre-conditioned SPE column. The blood pH is adjusted to maximize the ionic character of the analyte. 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, a weak aqueous buffer and methanol 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. Quantitation is accomplished with a 5 to 6 point calibration curve using the

corresponding deuterated standard to establish the response factor.

3.10.3.3 EQUIPMENT AND SUPPLIES

3.10.3.3.1 200mg CLEAN SCREEN® Extraction Column (ZSDAU020 or ZCDAU020 or equivalent)
3.10.3.3.2 Laboratory oven (Fisher or comparable)
3.10.3.3.3 Evaporative concentrator equipped with nitrogen tank.
3.10.3.3.4 Tube Rocker
3.10.3.3.5 Vortex Mixer
3.10.3.3.6 Laboratory centrifuge capable of 3200rpm
3.10.3.3.7 Vacuum Manifold/pump
3.10.3.3.8 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
3.10.3.3.9 pH indicator strips
3.10.3.3.10 16 x 100mm silanized glass tubes
3.10.3.3.11 Screw Cap for 16mm O.D. tube
3.10.3.3.12 GC/MS Automated Liquid Sample (ALS) vials
3.10.3.3.13 Silanized GC/MS Vial Microinsert
3.10.3.3.14 Gas Chromatograph (GC) equipped with a mass selective detector (MSD) and a nonpolar capillary column with a phase composition comparable to 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane with 5%-diphenyl.

3.10.3.4 REAGENTS

Refer to analytical method 5.12 for solution preparation instructions.

3.10.3.4.1 Acetonitrile (Certified ACS Grade)
3.10.3.4.2 Deionized/distilled (DI) water
3.10.3.4.3 Methanol (Certified ACS Grade)
3.10.3.4.4 Methylene Chloride (Certified ACS Grade)
3.10.3.4.5 Ethyl Acetate (Certified ACS Grade)
3.10.3.4.6 Isopropanol (Certified ACS Grade)
3.10.3.4.7 Ammonium Hydroxide (Certified ACS Grade)
3.10.3.4.8 100mM Phosphate Buffer (pH 6.0)
3.10.3.4.9 100mM Acetate Buffer (pH 4.5)
3.10.3.4.10 100mM Monobasic sodium phosphate
3.10.3.4.11 100mM Dibasic sodium phosphate
3.10.3.4.12 Elution Solvent
Mix 20mL Isopropanol and 2mL Ammonium Hydroxide QS to 100mL with methylene chloride. pH should be 11-12.
Make fresh.
3.10.3.4.13 BSTFA + 1% TMCS

3.10.3.5 QUALITY ASSURANCE MATERIAL

3.10.3.5.1 Drug Stock Solutions

3.10.3.5.1.1 1mg/mL Codeine
1mg/mL Morphine

3.10.3.5.2 Working Drug Solutions

3.10.3.5.2.1 10ng/µL
Add 100.0µL each Codeine and Morphine Stock Solution to ≅9mL Methanol in a 10mL volumetric class A flask. QS to 10mL. Store remaining stock solution in ALS vial in freezer.

3.10.3.5.2.2 1ng/µL
Add 1.0mL 10ng/µL working drug solution to ≅5mL Methanol in a 10mL volumetric class A flask. QS to 10mL.

3.10.3.5.2.3 Working solutions are stable for 6 months when stored at 4°C.

3.10.3.5.3 Internal Standard Stock Solutions

Codeine-D_3
Concentration: 1mg /mL or 100µL/mL

Morphine-D_3
Concentration: 1mg /mL or 100µL/mL

3.10.3.5.4 1.0ng/µL Working Internal Standard Solution
Add 10.0µL each 1mg/mL or 100.0µL each 100µL/mL Codeine-D3 and Morphine-D3 Stock Solution to ≅9mL Methanol in a 10mL volumetric class A flask. QS to 10mL. Store remaining stock solution in ALS vial in freezer.

Working solution is stable for 6 months when stored at 4°C.

3.10.3.5.5 Commercial Whole Blood Controls

3.10.3.5.5.1 **Negative Whole Blood**

3.10.3.5.5.2 **Positive Whole Blood**
Control containing Amphetamine and Methamphetamine each at a specified target concentration. Refer to package insert for verified value and expected range.

3.10.3.6 PROCEDURE

3.10.3.6.1 Initial set-up

Label extraction tubes, 200mg CLEAN SCREEN® extraction columns, eluate collection tubes and GC/MSD vials with microinserts for calibrators, controls and case samples.

3.10.3.6.2 Calibration Standard Preparation

3.10.3.6.2.1 Add 1mL of negative whole blood to six screw-top extraction tubes.

3.10.3.6.2.2 Add the volume of working Codeine-Morphine 1ng/µL mixed standard to appropriate tube as indicated in the chart below.

Level	ng/mL	µL Working Standard
1	25	25
2	50	50
3	100	100

3.10.3.6.2.3 Add the volume of working Codeine and Morphine 10ng/µL mixed standard to the appropriate tube as indicated in the chart below.

Level	ng/mL	µL Working Standard
4	250	25
5	500	50
6	1000	100

3.10.3.6.2.4 Additional or alternative concentrations may be used as necessary as long as the requirements in 3.10.3.6.15.1 are met.

3.10.3.6.3 Positive Control Sample Preparation

3.10.3.6.3.1 Add 1mL of negative whole blood to two screw-top tubes.

3.10.3.6.3.2 Add indicated amount of 1ng/µL working mixed control solution.

ng/mL	µL Working Control
75	75

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

ng/mL	µL Working Control
750	75

3.10.3.6.3.4 Additional or alternative concentrations at the discretion of the analyst may be used as long as the requirements in 3.10.3.10.2 are met.

3.10.3.6.4 Negative Control Sample Preparation

Add 1mL of negative whole blood to a screw top tube.

3.10.3.6.5 Case Sample Preparation

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

3.10.3.6.5.2 The total volume of blood or diluted blood should be 1mL.

3.10.3.6.5.3 Add 1mL neat or diluted sample to a labeled 13X100mm screw top tube.

3.10.3.6.6 Internal Standard Addition

3.10.3.6.6.1 To prepare 100ng/mL internal standard add 10µL of 1mg/mL (1000ng/µL) or 100µL of (100ng/µL) 1ng/µL of internal standard mix to calibrators, controls and casework samples.

3.10.3.6.6.2 Cap tube and vortex tube briefly.

3.10.3.6.6.3 Allow tubes to stand 15 to 30 minutes for sample equilibration.

3.10.3.6.7 Protein Precipitation

3.10.3.6.7.1 While vortexing, add 2mL cold acetonitrile

to case, calibrator and control samples.

3.10.3.6.7.2 Cap tubes and rock samples for approximately 15 minutes. Tubes should be at room temperature. Remove from rocker and place samples into centrifuge and let stand for 5 minutes.

3.10.3.6.7.3 Centrifuge at 3200-3400 rpm for 10 minutes.

3.10.3.6.7.4 Transfer organic supernatant into second labeled tapered bottom centrifuge tube.

3.10.3.6.7.5 Transfer tube to TurboVap and evaporate under nitrogen at approximately 37°C to approximately 1mL. *Do not allow extract to go to dryness.*

3.10.3.6.7.6 To evaporated extract add 2mL 100mM phosphate buffer (pH 6). Vortex to mix. pH should be 6.0 ±0.5. Adjust pH as necessary with 100mM monobasic or dibasic sodium phosphate.

3.10.3.6.7.7 If needed, centrifuge an additional 5 minutes to remove blood fragments or foam.

3.10.3.6.8 SPE Column Preparation

No vacuum is necessary except for drying step, however, if desired, aspirate at ≤ 3 in. Hg to prevent sorbent drying.

3.10.3.6.8.1 Insert labeled 200mg CLEAN SCREEN® Extraction column in the vacuum manifold.

3.10.3.6.8.2 Add 3mL methanol to the column.

3.10.3.6.8.3 Add 3mL DI water to the column.

3.10.3.6.8.4 Add 1mL 100mM Phosphate buffer (pH 6.00) to the column.

3.10.3.6.9 Blood Extract Loading

Load buffered blood onto column and allow to gravity flow or apply minimal vacuum.

3.10.3.6.10 Column Clean-up

3.10.3.6.10.1 Add 2mL DI water to the column.

3.10.3.6.10.2 Add 2mL 100mM Acetate buffer (pH 4.5) to the column. Aspirate.

3.10.3.6.10.3 Add 3mL Methanol.

3.10.3.6.10.4 Increase vacuum to ≥10 in. Hg (≥34 kPa) for ≥5 minutes (disc should be dry).

3.10.3.6.11 Compound Elution

3.10.3.6.11.1 Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.

3.10.3.6.11.2 Add 3mL elution solvent (3.10.3.4.12) to the column. Collect eluate with gravity flow or apply minimal vacuum.

3.10.3.6.12 Eluate Evaporation

Transfer centrifuge tube to TurboVap. Take solvent to dryness under a gentle stream of nitrogen at approximately 37°C.

3.10.3.6.13 Derivatization

3.10.3.6.13.1 In fume hood add 50µL ethyl acetate. Vortex for ≅15 seconds.

3.10.3.6.13.2 Add 50.0µL BSTFA + 1% TMCS.

3.10.3.6.13.3 Cap tubes and vortex briefly.

3.10.3.6.13.4 Place tubes in 70°C oven for 20 minutes.

3.10.3.6.13.5 Remove from heat and allow to cool.

3.10.3.6.13.6 Transfer derivative to labeled GC/MSD ALS vial with microinsert.

3.10.3.6.14 Preparation for GC-MS Run

3.10.3.6.14.1 Perform an AUTOTUNE and TUNE EVALUATION. Evaluate applying acceptance criteria outlines in analytical method 5.3.1.

3.10.3.6.14.2 When tune values are acceptable, program SEQUENCE TABLE with sample, calibrator and control information.

3.10.3.6.14.3 Load ALS vials into quadrant racks as indicated in the SEQUENCE TABLE.

3.10.3.6.15 GC-MS Calibration Curve

3.10.3.6.15.1 The calibration curve should be established with a minimum of four data points.

3.10.3.6.15.2 All reported results must be bracketed by calibrators.

3.10.3.6.15.3 Calibrators should be analyzed in order of increasing concentration.

3.10.3.6.15.4 The least squares line resulting from the analysis of calibrators must have a coefficient of correlation of ≥0.98.

3.10.3.6.15.5 If calibration standards are run in duplicate, it is not required that duplicate calibration points are included as long as the linearity requirement is met.

3.10.3.7 GC and MSD ACQUISITION PARAMETERS

Critical parameters are specified below. Parameters not specified are at the discretion of the analyst and should be optimized for the particular GC-MSD instrument. Each laboratory should maintain a centrally stored printed or electronic copy of current and past GC-MSD methods. The data supporting the GC-MSD method should be stored centrally.

3.10.3.7.1 GC Temperature Parameter

Injection Port: 250° or 260°C

3.10.3.7.2 MSD Instrument Parameters

Detector/Transfer Line: 280°C

3.10.3.7.3 ALS Parameters

Injection Volume: 1µL (1 stop)
Viscosity Delay: A minimum of 3 seconds
Solvent Washes (A & B): A minimum of 4 pre- and post-wash rinses.

3.10.3.7.4 MS SIM Parameters

Analyte	Target Ion	Qualifier Ion 1	Qualifier Ion 2
Morphine	429	287	324
Morphine-D3	432	290	327
Codeine	371	234	343
Codeine-D3	374	237	346

3.10.3.8 REPORTING CRITERIA

3.10.3.8.1 Qualitative Chromatographic and SIM Criteria

3.10.3.8.1.1 Qualitative results can be accepted when the following two criteria are met.

1. The retention time falls within the ±0.2 minute window established by calibrators.
2. Ion ratios for the analyte and its corresponding internal standard, established by calibrators for target and qualifier ions, do not differ by more than ±20%.

3.10.3.8.2 Quantitative Mass Spectral Criteria

3.10.3.8.2.1 Quantitative results can be accepted if the calculated concentration of all calibration standards and control samples are within ±20% of their respective concentrations.

3.10.3.8.2.2 Quantitation is achieved through the plotting of the target ion response ratio versus the concentration for each calibrator.

3.10.3.8.2.3 Quantitative values for case samples, calibrators and controls will be truncated for reporting purposes.

3.10.3.8.2.4 Administrative limit of detection (LOD) for Codeine and Morphine is 50ng/mL. Results < this LOD should be reported as negative unless there are extenuating circumstances. The Toxicology Discipline Leader must be consulted to evaluate exceptions.

3.10.3.8.2.5 If the concentration exceeds the calibration

range, the sample can either be appropriately diluted with DI water for reanalysis or reported as greater than 1000ng/mL.

3.10.3.9 REPORTING OF RESULTS

3.10.3.9.1 Quantitative Value

Analysis results should be truncated and reported out without decimal places.

3.10.3.9.2 Uncertainty Value

Based on the current uncertainty assessment, the +/– range should be included on the analysis report. Refer to quality monitoring spreadsheet for current uncertainty figure.

3.10.3.10 QUALITY ASSURANCE REQUIREMENTS

3.10.3.10.1 General

3.10.3.10.1.1 Blood samples are to be stored under refrigeration after aliquots are removed for analysis.

3.10.3.10.1.2 Refer to toxicology analytical method 5.1 for pipette calibration options.

3.10.3.10.1.3 Refer to toxicology analytical method 5.2 for balance calibration requirements.

3.10.3.10.1.4 Refer to toxicology analytical method 5.3.1 for GC-MSD maintenance guidelines.

3.10.3.10.1.5 Refer to toxicology analytical methods 5.8 and 5.10 for reference standard authentication and additional GC-MSD quality assurance requirements.

3.10.3.10.2 Per Analysis Run Quality Requirements

3.10.3.10.2.1 Solvent blank should follow the highest calibrator as well as each case sample.

3.10.3.10.2.2 A minimum of two blood commercially obtained controls and the spiked controls described in section 3.10.3.6.3 must be run per batch of samples.

3.10.3.10.2.3 In addition to the four blood controls indicated above, for each additional 10 case

samples, one control must be run. The preparation of controls is outlined in section 3.10.3.6.3. Additional concentrations may be used.

3.10.3.10.3 Monitoring of Control Values
Upon the completion of analysis, input blood control values on spreadsheet used to assess uncertainty for this method.

3.10.3.11 ANALYSIS DOCUMENTATION

3.10.3.11.1 A packet containing original data for controls and standards will be prepared for each analysis run and stored centrally in the laboratory where the analysis was performed until archiving.

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

3.10.3.12 REFERENCES AND RECOMMENDED READING

3.10.3.12.1 Telepchak, M.J., August, T.F. and Chaney, G., Drug Methods for the Toxicology Lab, pp. 227 - 230. *in:* Forensic and Clinical Applications of Solid Phase Extraction, Humana Press: New Jersey, 2004.

3.10.3.12.2 Stout, P.R. and Farrell, L.J., *Opioids - Effects on Human Performance and Behavior,* Forensic Science Rev. 15(1): 29 - 60, 2003.

3.10.3.12.3 Drummer, O.H., *Opioids* pp. 219 - 265. *in:* The Forensic Pharmacology of Drugs of Abuse, Arnold: London, 2001.

3.10.3.12.4 Kerrigan, S. and Goldberger, B.A., *Opioids.* pp. 187 - 206. *in:* Principles of Forensic Toxicology. Levine, B. ed., AACC, 2003.

3.10.3.12.5 Baselt, R.C., *Codeine*, pp. 262 - 265. and *Morphine*, pp. 759 - 763. *in:* Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications: Foster City, CA. Seventh ed., 2004.

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Discipline Leader: _____ **Date:** _____
Susan C. Williamson

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Alan C. Spanbauer