Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

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

2.3.8 Extraction of Opiates Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Extraction Column.

2.3.8.1 BACKGROUND

Morphine and codeine are natural derivatives of the opium poppy, *Papaver somiferum*. Opium contains several alkaloids including morphine, codeine and papaverine. These natural products lead to the development of numerous synthetic analgesics. Narcotic analgesics are divided into 3 classes, the phenanthrenes (morphine, codeine, oxycodone, pentazocine), phenylpiperidines (meperidine, fentanyl), and the phenylheptanes (methadone, propoxyphene). As illustrated in the chart below, the effects of opiate class drugs are dependent upon interactions with specific receptor sites within the central nervous system (CNS). In addition to analgesia and cough suppression, effects of opiate use include euphoria, respiratory depression, sedation, reduced GI motility/constipation, hypothermia, dysphoria, miosis, bradycardia, nausea, and physical tolerance and dependence.

Compound	Trade Name	Receptor/ Action	Metabolites	Therapeutic uses
Buprenorphine	Buprenex®	μ agonist, κ antagonist	Norbuprenorphine	moderate-severe pain
Butorphanol	Stadol®, Stadol NS®	κ agonist, μ antagonist	3-hydroxybutorphanol, norbutorphanol	moderate-severe pain
Codeine	Tylenol 3®	μ agonist, δ agonist	morphine, norcodeine	mild-moderate
Dihydrocodeine	Paracodin®	μ agonist	dihydromorphine, nordihydrocodeine	mild-moderate
Fentanyl	Sublimaze®	μ agonist	despropionylfentanyl, norfentanyl, hydroxyfentanyl, hydroxynorfentanyl	moderate-severe
Heroin	NA in US	μ agonist	6-acetylmorphine, morphine, normorphine	----
Hydrocodone	Hycodan®, Vicodin®, Codone®, Lortab®	μ agonist	hydromorphone, norhydrocodone, dihydrocodeine hydromorphol	moderate-severe
Hydromorphone	Dilaudid®	μ agonist	hydromorphol	moderate-severe
Levorphanol	levo-dromoran®	μ agonist, κ agonist	norlevorphanol	moderate-severe
Meperidine	Demerol®	μ agonist	normeperidine	moderate-severe

Compound	Trade Name	Receptor/ Action	Metabolites	Therapeutic uses
Methadone	Dolophine®, Methadose®	μ agonist	methadol, normethadol, EDDP, EMDP	Detoxification
Morphine	MS-IR Roxanol	μ agonist, κ agonist, δ agonist	normorphine	moderate-severe
Nalbuphine	Nubain®	κ agonist, σ agonist, μ antagonist	nornalbuphine	moderate-severe
Oxycodone	Percolone®, Roxicodone®, Oxycontin®, Oxy®	μ agonist	oxymorphone, noroxycodone	moderate-severe
Oxymorphone	Numorphan®	μ agonist	6-oxymorphol	moderate-severe
Pentazocine	Talwin®	μ agonist, κ agonist, σ agonist	cis- and trans-hydroxypentazocine, trans-carboxypentazocine	moderate-severe
Propoxyphene	Darvon®, Darvocet®	μ agonist	norpropoxyphene,	mild-moderate
Tramadol	Ultram®	μ agonist	nortramadol, O-desmethyltramadol, N-desmethyltramadol	moderate

2.3.8.2 **PRINCIPLE**

This procedure outlines the use of the UCT 200 mg CLEAN SCREEN® extraction column for the extraction of Opiates 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 utilizes an anionic sorbent (-) to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption.

Opiates form glucuronide conjugates to facilitate their excretion. Prior to extraction, an enzymatic hydrolysis is required to free them from the glucuronide sugar moiety. For the extraction of opiates, the hydrolyzed urine is adjusted with a low pH acetate buffer, to maximize the ionic character of the analytes and the sorbent. The sample is then loaded onto a pre-conditioned SPE column. The conditioning creates an environment that allows for optimal interaction between the sorbent and the analytes of interest. The analyte is retained by ionic interaction of the cationic 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.

2.3.8.3 EQUIPMENT AND SUPPLIES

2.3.8.3.1 200mg CLEAN SCREEN® extraction column (ZSDAU020 OR ZCDAU020 or equivalent)

2.3.8.3.2 Drybath (Fisher or equivalent)

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

2.3.8.3.4 Vacuum Manifold/pump

2.3.8.3.5 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)

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

2.3.8.3.7 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)

2.3.8.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.8.4.1 Methanol (Fisher A412-4 or equivalent)

2.3.8.4.2 Methylene Chloride (Fisher D37-4 or equivalent)

2.3.8.4.3 Isopropanol (Fisher A416-1 or equivalent)

2.3.8.4.4 Ammonium Hydroxide (Fisher A669-500 or equivalent)

2.3.8.4.5 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.8.4.6 Deionized/distilled water

2.3.8.4.7 1.0M Acetate Buffer (pH 5.0)

2.3.8.4.8 100mM Phosphate Buffer (pH 6.0)

2.3.8.4.9 100mM Acetate Buffer (pH 4.5)

2.3.8.4.10 1N NaOH

2.3.8.4.11 Elution Solvent

Mix 78mL Methylene Chloride, 20mL Isopropanol and 2mL Ammonium Hydroxide. ***Make fresh.***

2.3.8.4.12 β-Glucuronidase (Patella vulgata)

2.3.8.4.13 Silylation Reagent Options

- MSFTA (Pierce 48910 or equivalent)
- MSFTA + 1% TMCS (Pierce 48915 or equivalent)
- BSTFA (Pierce 38830 or equivalent)
- BSTFA + 1% TMCS (Pierce 38831 or equivalent)

2.3.8.5 CONTROL

2.3.8.5.1 Liquid Urine Control containing a minimum of Morphine and/or Codeine (BioRad 478, Utak 66812-C or equivalent)

2.3.8.5.2 Drug Mix (Alltech 601827 {Codeine, Morphine, Hydromorphone, Oxycodone, Nalorphine and Diacetylmorphine }or similar)

2.3.8.5.3 Morphine-3β-D-glucuronide (Alltech [1mg/ml] M-031, [100µg/ml] M-018, or equivalent)

2.3.8.6 STANDARDS

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

Standards (in solution)	*Potential Vendors*
Codeine	Cerilliant C006, Alltech 018013
Dihydrocodeine	Cerilliant D-019, Alltech 017773
Fentanyl	Cerilliant F-013, Alltech 013993
Heroin	Cerilliant H-038, Alltech 013653
Hydrocodone	Cerilliant H-003
Hydromorphone	Cerilliant H-004, Alltech 013553
Methadone	Cerilliant M-007, Alltech 018023
Morphine	Cerilliant M-005, Alltech 018033
Oxycodone	Cerilliant O-008, Alltech 013543
Oxymorphone	Cerilliant O-004, Alltech 013983

2.3.8.7 PROCEDURE

2.3.8.7.1 Standard Preparation

Prepare a minimum of the following non-extracted standards. Additional standards should be prepared as necessary indicated by *current drug therapy*.

- TMS derivative: Morphine, Codeine, and Hydrocodone. Add 10µL of stock solution to labeled tapered bottom centrifuge tube.

2.3.8.7.2 Initial set-up

Label 200 mg CLEAN SCREEN® Extraction Column, test tubes, tapered-bottom derivatization tubes and GC/MS

vials with microinserts as follows for derivatized extractions (TMS) for the negative control (NC), positive control (PC), Morphine-3β-D-glucuronide control, Standards, and appropriate laboratory numbers without prefix.

2.3.8.7.3 Sample Preparation

- Transfer 5.0mL of urine specimen, negative urine or positive control to extraction tube.

2.3.8.7.4 Sample Hydrolysis

- To each extraction tube add:
  - 2 mL β-Glucuronidase solution (pH 5.0)
- Cap and vortex *gently* to mix.
- Place in 65°C laboratory oven or waterbath for three hours.
- Allow samples to cool
- Centrifuge for 10 minutes at 2000 rpm and discard pellet
- Adjust pH to 6.0 ± 0.5 with approximately 700µl of 1.0N NaOH

2.3.8.7.5 Extraction

- Insert labeled 200mg CLEAN SCREEN® Extraction column in the vacuum manifold.
- Add 3mL of methanol to the column and aspirate and aspirate at ≤ 3 in. Hg to prevent sorbent drying.
- Add 3mL of deionized water to the column and aspirate at ≤ 3 in. Hg.
- 1mL of 100mM phosphate buffer (pH 6.0) and aspirate at ≤ 3 in. Hg.
- Load sample into column at 1 to 2mL/minute.
- Wash column with the following and aspirate at ≤ 3 in. Hg
  - 2mL of deionized water
  - 2mL 100mM acetate buffer (pH 4.5)
  - 3mL methanol
- Increase vacuum to ≥ 10 in. Hg (≥34 kPa) and dry extraction disc for ≥ 5 minutes.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 3mL elution solvent to the column and aspirate at < 3 in. Hg (<10 kPa).
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in TurboVap at ≤ 40°C.

2.3.8.7.6 Derivatization

- In fume hood add the following:
  - 50µL ethyl acetate.
  - 50µL silylating agent.
- Cap tubes with snap caps.
- Vortex.
- Heat tube for 20 minutes in 70°C dry bath.
- Remove from heat and allow to cool.
- Transfer derivative to labeled GC/MSD ALS vial with microinsert.

2.3.8.7 GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) ANALYSIS

2.3.8.7.1 Analysis Parameters

2.3.8.7.1.1 Inject 1 µL into GC/MS using the ALS.

2.3.8.7.1.2 Analyze sample extract in full scan acquisition.

2.3.8.7.1.3 Refer to attached GC/MSD method printout for current analysis parameters.

2.3.8.7.2 Detection and Identification Criteria

2.3.8.7.2.1 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 standards.

- Acceptable retention time window is +/- 5%.

2.3.8.8 REFERENCES

2.3.8.8.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

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

2.3.8.8.3 Baselt RC, Disposition of Toxic Drugs and Chemicals in Man 5th ed., Chemical Toxicology Institute, 2000.

2.3.8.8.4 Hutchison TA & Shahan DR (Eds): DRUGDEX® System. MICROMEDEX, Inc., Greenwood Village, Colorado (Edition expires [12/01]).