Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

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

2.3.7 Extraction of Codeine and Morphine Employing the SPEC·PLUS™·DAU Extraction Column.

2.3.7.1 BACKGROUND

Morphine and codeine are natural derivatives of the opium poppy, *Papaver somiferum*. Opium contains several alkaloids including morphine, codeine and papaverine. Codeine is the phenolic methyl ether of morphine while heroin is a diacetyl derivative. Classified as opiates, codeine and morphine are used therapeutically primarily as analgesics and antitussives. While both morphine and codeine have potential for abuse, the illicit drug, heroin continues to be the second most widely abused drug in the United States (DAWN 2000). The effect these opiates exhibit is dependent upon their 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
Codeine	Tylenol 3®	μ agonist, δ agonist	morphine, norcodeine
Morphine	MSIR Roxanol MS Contin	μ agonist, κ agonist, δ agonist	minor quantities normorphine
Heroin	---------	μ agonist, κ agonist, δ agonist	6-monoacetylmorphine, morphine, small quantities of codeine (addicts)

2.3.7.2 PRINCIPLE

This procedure outlines the use of the ANSYS® Diagnostics, Inc SPEC·PLUS™ 3ml SPE column for the extraction of codeine and morphine 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. Codeine and morphine 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 codeine and morphine, the urine is adjusted with a low pH acetate buffer to maximize the ionic character of the analytes and the sorbent. 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.

2.3.7.3 EQUIPMENT AND SUPPLIES

2.3.7.3.1 SPEC·PLUS™·DAU extraction column. (Ansys 532-DAU or equivalent)

2.3.7.3.2 Drybath (Fisher or equivalent)

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

2.3.7.3.4 Vacuum Manifold/pump

2.3.7.3.5 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)

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

2.3.7.3.7 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)

2.3.7.3 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.7.4.1 Methanol (Fisher A412-4 or equivalent)

2.3.7.4.2 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.7.4.3 Ammonium Hydroxide (Fisher A669-500 or equivalent)

2.3.7.4.4 1.0M Acetate Buffer (pH 3.8)

2.3.7.4.5 0.1M Acetic Acid

2.3.7.4.6 1.5M Acetic Acid

2.3.7.4.7 1.5M Phosphate Buffer (pH 10.8)

2.3.7.4.8 Elution solvent

Mix 80mL of ethyl acetate, 20mL methanol and 2 mL ammonium hydroxide.

2.3.7.4.9 β-Glucuronidase Options

- Prepare from Patella vulgata Type L-II powder (Sigma G8132 or equivalent)
- Prepared Helix pomatia Type H-2 Solution (Sigma G0876 or equivalent)

2.3.7.4.10 Silylation Reagent Options

- MSFTA (Pierce 48910 or equivalent)
- BSTFA (Pierce 38830 or equivalent)

2.3.7.5 CONTROL

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

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

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

2.3.7.6 STANDARDS

2.3.7.6.1 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

2.3.7.7 PROCEDURE

2.3.7.7.1 Standard Preparation

Prepare the following non-extracted standards.

TMS derivative: Morphine, Codeine.

Add 10μL of stock solution to labeled tapered bottom centrifuge tube.

2.3.7.7.2 Initial set-up

Label SPEC·PLUS™·DAU 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.

2.3.7.7.3 Sample Preparation

- Transfer 1.0mL urine specimen, negative urine or positive control to extraction tube.
- Add 200µL of 1.0M acetate buffer (pH 3.8)
- Vortex.
- pH should be approximately 4, adjust if necessary using 0.1M acetic acid or KOH.

2.3.7.7.4 Sample Hydrolysis

- To each extraction tube add:
  - 200 µl β-Glucuronidase solution
- Cap and vortex *gently* to mix.
- Place in 60°C laboratory oven or waterbath for two hours.
- Allow samples to cool
- Add 1.0mL of phosphate buffer (pH 10.8)
- The resulting pH should be approximately 9.
- Adjust pH as necessary.
- Centrifuge for 5 minutes at 3000-3500 rpm

2.3.7.7.5 Extraction

- Insert labeled SPEC·PLUS™·DAU extraction column in the vacuum manifold.
- Add 200µL of methanol to the column and wait for one minute.
- Decant sample into column and aspirate at 3-5 in. Hg (10-17kPa).
- Wash column with the following and aspirate aspirate at 3-5 in. Hg (10-17kPa)
  - 500µL of deionized water.
  - 500µL 0.1 M acid.
  - 500µL methanol
- Increase vacuum to 10-20 in. Hg (34-68 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 800µL elution solvent to the column and aspirate at < 3 in. Hg (<10 kPa) to aspirate solvent through disc into collection vial.
- Increase vacuum to 5in. Hg (17kPa) to assist final amount of elution solvent through disc.
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in TurboVap at approximately 60°C.

2.3.7.7.6 Derivatization

- In fume hood, add 50µL of silylating agent and 50µL ethyl acetate.
- Cap tubes with snap caps.
- Vortex.
- Heat tube for 20 minutes in 60°C dry bath.
- Remove from heat and allow to cool.
- Transfer derivative to labeled GC/MSD ALS vial with microinsert.

2.3.7.7.7 Automated Extraction Procedure Utilizing SPEC·PLUS™ - 3ml DAU column.

2.3.8.7.7.1 Refer to the following attached method/printouts.

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

2.3.7.7.1 Analysis Parameters

2.3.8.7.1.1 Inject 1 µL into GC/MSD 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.

2.3.7.7.2 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%.

2.3.7.8 REFERENCES

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

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

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

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