Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

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

2.3.2 Extraction of Amphetamines Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® DAU Extraction Column

2.3.2.1 BACKGROUND

Amphetamine and methamphetamine are sympathomimetic drugs that mimic the actions of naturally occurring stimulatory neurotransmitters. Although still prescribed for the treatment of attention deficit disorder (ADD), narcolepsy, and obesity, these compounds have a high potential for abuse. Methamphetamine is produced clandestinely often through the reduction of ephedrine/pseudoephedrine. Psychological side effects may include agitation, nervousness, restlessness, and paranoia. Physiological effects may include mydriasis, insomnia, increased blood pressure and heart rate. The manifestation of adverse affects is dependent on the time since drug administration.

2.3.2.2 PRINCIPLE

This procedure outlines the use of the UCT 200mg CLEAN SCREEN® DAU column for the extraction of Amphetamines 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 will allow the anionic sorbent (-) to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption. The nonpolar aspect of the column serves to extract nonpolar compounds from a polar sample matrix.[2] The cation exchanger component of the phase is effective for amines which are present in the urine sample in a cationic form bonding ionically to the sorbent.[2]

For the extraction of amphetamines; the urine is adjusted to pH 6 with a phosphate buffer to maximize the ionic character of the analyte, and loaded onto a pre-conditioned SPE column. 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

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.

2.3.2.3 EQUIPMENT AND SUPPLIES

2.3.2.3.1 200 mg CLEAN SCREEN® extraction column (ZSDAU020 or ZCDAU020 or equivalent)

2.3.2.3.2 Drybath (Fisher or equivalent)

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

2.3.2.3.4 Vacuum Manifold/pump

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

2.3.2.3.6 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 Sample (ALS) vials (HP 5182-0865 or equivalent)

GC/MS vial microinsert (HP 5183-2088 or equivalent)

2.3.2.3.7 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)

2.3.2.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.2.4.1 Methanol (Fisher A412-4 or equivalent)

2.3.2.4.2 Methylene Chloride (Fisher D37-4 or equivalent)

2.3.2.4.3 Dimethylformamide (DMF) (Fisher D119-500 or equivalent

2.3.2.4.4 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.2.4.5 Isopropanol (Fisher A416-1 or equivalent)

2.3.2.4.6 Ammonium Hydroxide (Fisher A669-500 or equivalent)

2.3.2.4.7 100mM Phosphate Buffer (pH 6.0)

2.3.2.4.8 100mM Acetic Acid

2.3.2.4.9 Elution Solvent

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

2.3.2.4.10 Derivatizing Agents - Select from the following:

Heptafluorobutyric Acid Anhydride (HFAA) (Pierce 63164 or equivalent)

Pentafluoropropionic Acid Anhydride (PFAA) (Pierce 65193 or equivalent)

2.3.2.5 CONTROL

2.3.2.5.1 Toxi-Control No. 2, UTAK 98814, or an equivalent control which contains both Amphetamine and Methamphetamine in the appropriate concentrations.

2.3.2.6 STANDARDS

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

Standard (1 mg/mL)	Potential Vendors
Methamphetamine	Cerilliant M-009, Alltech 010013
Amphetamine	Cerilliant A-007, Alltech 010023
MDMA	Cerilliant M-013, Alltech 014093
MDA	Cerilliant M-012, Alltech 014603
Phenylpropanolamine	Cerilliant P-038, Alltech 6017803
Phentermine	Cerilliant P-023, Alltech 017833
Ephedrine	Cerilliant E-024, Alltech 017403
Pseudoephedrine	Cerilliant P-035, Alltech 6013213
PMA	Cerilliant P-050

2.3.2.7 PROCEDURE

2.3.2.7.1 Initial set-up

Label test tubes and GC/MSD vials with microinserts.

- Negative Control
- Positive Control
- Appropriate Laboratory Numbers

2.3.2.7.2 Extraction Procedure Utilizing the 200 mg CLEAN SCREEN® Extraction Column

2.3.2.7.2.1 Transfer 5mL of urine specimen, negative urine or appropriate Toxi-Control to the appropriate labeled test tube.

2.3.2.7.2.2 Add 2mL 100mM phosphate buffer and Vortex. Verify that pH is 6.0 ± 0.5, adjust

pH with 100mM monobasic or dibasic sodium phosphate, as necessary.

2.3.2.7.2.3 Insert labeled CLEAN SCREEN® extraction column into vacuum manifold.

2.3.2.7.2.4 Add 3mL of methanol to column and aspirate at ≤3 in. Hg.

2.3.2.7.2.5 Add 3mL of DI H_2O to column and aspirate at ≤3 in. Hg.

2.3.2.7.2.6 Add 1mL of 100mM phosphate buffer (pH 6.0) to column and aspirate at ≤3 in. Hg.

2.3.2.7.2.7 Pour sample onto column and aspirate at ≤3 in. Hg.

2.3.2.7.2.8 Wash column with 3mL DI H_2O and aspirate at ≤3 in. Hg.

2.3.2.7.2.9 Wash column with 1mL 100mM acetic acid and aspirate at ≤3 in. Hg.

2.3.2.7.2.10 Wash column with 3mL methanol and aspirate at ≤3 in. Hg.

2.3.2.7.2.11 Dry column for ≥ 5minutes at ≥10 inches Hg.

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

2.3.2.7.2.13 Add 3mL of elution solvent to column and aspirate slowly, < 3 in. Hg.

2.3.2.7.2.14 Add 30µL of DMF to eluate.

2.3.2.7.2.15 Evaporate eluate to ~30µL at ≤40°C under a gentle stream of nitrogen.

2.3.2.7.2.16 Add 50µL of PFFA or HFFA, cap, and vortex.

2.3.2.7.2.17 Heat for 20 minutes at 70°C.

2.3.2.7.2.18 Evaporate to dryness at ≤40°C.

2.3.2.7.2.19 Reconstitute with 100µL ethyl acetate.

2.3.2.7.2.20 Transfer to the appropriately labeled ALS vial.

2.3.2.7.3 Automated Extraction Procedure Utilizing 200 mg CLEAN SCREEN® Extraction Column

2.3.2.7.3.1 Refer to the following attached methods/printouts.

2.3.2.7.4 Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

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

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

2.3.2.7.5 Detection and Identification Criteria

2.3.2.7.5.1 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 that of an authenticated standard.

2.3.2.7.5.2 Acceptable retention time window is ±5%.

2.3.2.8 REFERENCES

2.3.2.8.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

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