Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

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

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

2.3.4.1 BACKGROUND

Refer to manual section 2.4.3.

2.3.4.2 PRINCIPLE

This procedure outlines the use of the 200mg UCT CLEAN SCREEN® DAU Column for the extraction of Benzodiazepines 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 retention mechanisms for the benzodiazepines are hydrophobic interactions and polar adsorption. The nonpolar aspect of the column serves to extract nonpolar compounds from a polar sample matrix.[2] Benzodiazepines form glucuronide conjugates to facilitate their excretion. An enzymatic hydrolysis is therefore required to free them from the glucuronide sugar moiety. For the extraction of benzodiazepines, the hydrolyzed urine is 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 hydrophobic interaction of the functional groups present on both the analyte and the sorbent. The column is subsequently washed to selectively remove matrix components and interfering substances from the column. 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. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

2.3.4.3 EQUIPMENT AND SUPPLIES

2.3.4.3.1 200 mg CLEAN SCREEN® Extraction Column (ZSDAU020 or ZCDAU020 or equivalent)

2.3.4.3.2 Drybath (Fisher or equivalent)

2.3.4.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank.
2.3.4.3.4 Vacuum Manifold/pump
2.3.4.3.5 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)
2.3.4.3.6 pH paper (Fisher 09-876-17 or equivalent)
2.3.4.3.7 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)

2.3.4.4 REAGENTS

Refer to Manual section 2.6 for solution preparation

2.3.4.4.1 ethanol (Fisher A412-4 or equivalent)
2.3.4.4.2 Ethyl Acetate (Fisher E145-4 or equivalent)
2.3.4.4.3 Hexane (Fisher H-292-1 or equivalent)
2.3.4.4.4 Deionized/distilled water
2.3.4.4.5 100 mM Phosphate buffer, pH 6.0
2.3.4.4.6 20 % Acetonitrile in 0.1 M phosphate buffer, pH 6.0
2.3.4.4.7 β-Glucuronidase (Patella vulgata)
2.3.4.4.8 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)

2.3.4.5 CONTROL

2.3.4.5.1 Liquid Urine Control containing a minimum of Oxazepam or Nordiazepam (BioRad 443, Utak 88121 or equivalent)
2.3.4.5.2 Drug Mix (Alltech 601826 {Medazepam, Oxazepam, Lorazepam, Diazepam, Temazepam, and Bromazepam}or similar)
2.3.4.5.3 Oxazepam Glucuronide (Alltech 01541 or equivalent)

2.3.4.6 STANDARDS

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

Standards in Solution	*Potential Vendors*
Alprazolam	Cerilliant A-903, Alltech 01427
α-Hydroxyalprazolam	Cerilliant A-907, Alltech 01545
Bromazepam	Cerilliant B-903, Alltech 6013563
Chlordiazepoxide	Cerilliant C-022
Norchlordiazepoxide	Alltech 6013433
Clonazepam	Cerilliant C-907, Alltech 017943
7-Aminoclonazepam	Cerilliant A-916
Diazepam	Cerilliant D-907, Alltech 017213
Estazolam	Cerilliant E-901, Alltech 601560
Flurazepam	Cerilliant F-003, Alltech 017953
Flunitrazepam	Cerilliant F-907, Alltech 6015123
7-aminoflunitrazepam	Cerilliant A-911
Lorazepam	Cerilliant L-901, Alltech 013583
Medazepam	Alltech 013573
Midazolam	Cerilliant M-908
4-hydroxymidazolam	Cerilliant H-902
Nitrazepam	Cerilliant N-906, Alltech 017933
Nordiazepam	Cerilliant N-905, Alltech 013453
Oxazepam	Cerilliant O-902, Alltech 013703
Temazepam	Cerilliant T-907, Alltech 013833
Triazolam	Cerilliant T-910, Alltech 014283
α-Hydroxytriazolam	Cerilliant T-911

2.3.4.7 PROCEDURE

2.3.4.7.1 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: Oxazepam, Temazepam, Nordiazepam and Lorazepam. Add 10µL of stock solution to labeled tapered bottom centrifuge tube.

2.3.4.7.2 Initial set-up

Label 200mg CLEAN SCREEN® Extraction Column, test tubes, tapered-bottom derivatization tubes and GC/MSD vials with microinserts for the negative control (NC), positive control (PC), Oxazepam Glucuronide control, Standards, and appropriate laboratory numbers without prefix.

2.3.4.7.3 Sample Preparation

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

2.3.4.7.4 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.
- Centrifuge for 10 minutes at 2000 rpm and discard pellet.
- Allow samples to cool.

2.3.4.7.5 Extraction

- Insert labeled 200mg CLEAN SCREEN® DAU column in the vacuum manifold.
- Add 3mL of methanol to the column and aspirate at ≤3 in. Hg (<10 kPa). .
- Add 3mL of deionized water to the column and aspirate at ≤3 in. Hg (<10 kPa). .
- 1mL of 100mM phosphate buffer (pH 6.0) and aspirate at ≤ 3 in. Hg.
- Decant sample into column and aspirate at <3 in. Hg.
- Wash column with 2mL of deionized water and aspirate at ≤ 3 in. Hg.
- Wash column with 2mL 20% acetonitrile in 0.1M phosphate buffer (pH 6.0) and aspirate and aspirate at ≤ 3 in. Hg.
- Increase vacuum to ≥10 in. Hg (≥34 kPa) and dry extraction disc for approximately 5 minutes.
- Wash column with 2mL hexane and aspirate and aspirate at ≤ 3 in. Hg.
- Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled tapered tip centrifuge tubes.
- Add 3mL ethyl acetate to elute the sample from the column at < 3 in. Hg (<10 kPa).
- Evaporate solvent to dryness, under a gentle stream of nitrogen, in TurboVap at ≤ 40°C.

2.3.4.7.6 Derivatization

In fume hood:

- Add 50 µL ethyl acetate.
- Add 50 µL 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/MS ALS vial with microinsert.

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

2.3.4.8.1 Analysis Parameters

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

2.3.4.8.1.2 Analyze sample extract in full scan acquisition.

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

2.3.4.8.2 Detection and Identification Criteria

2.3.4.8.2.1 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%.

2.3.4.9 REFERENCES

2.3.3.9.1 UCT CLEAN SCREEN® Extraction Columns Application Manual

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