Idaho State Police
Forensic Services
Toxicology Section

Section Two
Urine Toxicology

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

### 2.3.1 Amphetamine and Methamphetamine Extraction Employing the Ansys® Diagnostics Spec·Plus™ DAU Column



#### 2.3.1.1 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.

#### 2.3.1.2 PRINCIPLE

This procedure outlines the use of the ANSYS® Diagnostics, Inc SPEC·PLUS™ 3ml DAU column for the extraction of amphetamine and methamphetamine and amine compounds, 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 copolymer sorbent which combines a strong cation exchange phase with a non-polar phase (reversed phase) to interact effectively, physically and chemically, with analytes of interest and minimally with interfering substances in the urine sample. The copolymer binds the analyte primarily with ionic interactions with the anionic sorbent and to a lesser extent, by hydrophobic interactions. The cation exchange component (anionic sorbent) of the phase is effective for recovering amines which are present in the urine sample in a cationic form.

For the extraction of amphetamine, methamphetamine and other phenethylamines of interest, the urine is adjusted with a phosphate buffer and applied to a pre-conditioned SPE column. This pH adjustment maximizes the ionic character of the analyte and the sorbent to take full advantage of the cation exchange mechanism. The conditioning creates an environment which allows for optimal interaction between the sorbent and the analytes of interest. The column is subsequently washed with an

aqueous solvent, to selectively remove matrix components and interfering substances from the column. Next, the column is to dried to remove traces of solvent. When the column is dry, the analytes of interest are recovered from the column by disrupting the ionic bonds with a basic organic solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on the GC/MSD.

### 2.3.1.3 EQUIPMENT AND SUPPLIES

2.3.1.3.1 SPEC·PLUS™ 3ml DAU column (Ansys 532-DAU)

2.3.1.3.2 Drybath (Fisher or equivalent)

2.3.1.3.3 Evaporative concentrator (Zymark TurboVap or equivalent) equipped with nitrogen tank;

2.3.1.3.4 Vacuum Manifold/pump

2.3.1.3.5 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)

2.3.1.3.6 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)

### 2.3.1.4 REAGENTS

*Refer to Manual section 2.6 for solution preparation*

2.3.1.4.1 1.0 M Potassium hydroxide

2.3.1.4.2 0.1M Phosphate Buffer, pH 6.0

2.3.1.4.3 0.1M Acetic acid

2.3.1.4.4 1% Acidic Methanol

2.3.1.4.5 Isooctane (Fisher O-299-1 or equivalent)

2.3.1.4.6 Methanol (Fisher A412-4 or equivalent)

2.3.1.4.7 Ethyl Acetate (Fisher E145-4 or equivalent)

2.3.1.4.8 Ammonium Hydroxide (Fisher A669-500 or equivalent)

2.3.1.4.9 Elution Solvent

80ml ethyl acetate, 20 ml methanol, 2ml of $NH_4OH$ ***Prepare fresh.***

2.3.1.4.10 1M Potassium phosphate dibasic ($K_2HPO_4$)

2.3.1.4.11 Derivatizing Agents - Select from the following:

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

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

**2.3.1.5 CONTROL**

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

**2.3.1.6 STANDARDS**

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

| Standard (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 |



**2.3.1.7 PROCEDURE**

2.3.1.7.1 Initial set-up

Label the test tubes and GC/MS vials with microinserts.

- Negative Control
- Positive Control
- Appropriate Laboratory Numbers

2.3.1.7.2 Manual Extraction Procedure Utilizing the SPEC·PLUS™ •3ml DAU column

2.3.1.7.2.1 Transfer 1mL of urine specimen, negative urine or appropriate control to the properly labeled test tube.

2.3.1.7.2.2 Add 500µL of 0.1M phosphate buffer, pH 6.0, and vortex.

2.3.1.7.2.3 Insert labeled SPEC·PLUS™ 3mL DAU column into vacuum manifold.

2.3.1.7.2.4 Add 200µL of methanol to column and aspirate at approximately 5 in. Hg (17 kPa) for approximately 1 minute.

2.3.1.7.2.5 Pour prepared sample into column and aspirate at approximately 5 in. Hg (17 kPa).

2.3.1.7.2.6 Add 500µL of 0.1M acetic acid and aspirate at approximately 5 in. Hg (17 kPa).

2.3.1.7.2.7 Increase vacuum to 10-20 in. Hg (34-68 kPa) and dry the extraction disc for a minimum of 1 minute.

2.3.1.7.2.8 Add 500µL of Methanol to the column and aspirate at approximately 5 in. Hg (17 kPa).

2.3.1.7.2.9 Increase the vacuum to 10-20 in Hg (34-68 kPa) and dry the disc for a minimum of 1 minute.

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

2.3.1.7.2.11 Add 800µL of elution solvent to column and aspirate slowly, < 3 in. Hg (10kPa).

2.3.1.7.2.12 Increase vacuum to 5 in. Hg (17 kPa) to assist final amount of elution solvent through the disc.

2.3.1.7.2.13 Remove the tapered tip centrifuge tubes containing the collected samples from rack.

2.3.1.7.2.14 Add 50µL of 1% acidic methanol and vortex.

2.3.1.7.2.15 Evaporate to dryness under a gentle stream of nitrogen at approximately 35°C.

2.3.1.7.2.16 In the hood add 50µL of HFAA or PFAA, cap, and vortex.

2.3.1.7.2.17 Heat for 20 minutes at 70°C.

2.3.1.7.2.18 Cool to room temperature.

2.3.1.7.2.19 Add 1 mL of Isooctane and 1mL of 1M $K_2HPO_4$.

2.3.1.7.2.20 Cap and vortex.

2.3.1.7.2.21 Incubate at ~60°C for 15 minutes.

2.3.1.7.2.22 Cool.

2.3.1.7.2.23 Vortex

2.3.1.7.2.24 Centrifuge at 100rpm for 5 minutes to separate the layers

2.3.1.7.2.25 Transfer the isooctane (top) layer to an appropriately labeled ALS vial.

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

2.3.1.7.3.1 Refer to the following attached methods/printouts.

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

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

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

2.3.1.7.5 Detection and Identification Criteria

2.3.1.7.5.1 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.

2.3.1.7.5.2 Acceptable retention time window is ±5%.



## 2.3.1.8 REFERENCES

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

2.3.1.8.2 SPEC·PLUS™·3ML·DAU Drugs of Abuse in Urine Extraction Applications, ANSYS Diagnostics, 1999.

2.3.1.8.3 Instructions for Urine of SPEC·Solid Phase Extraction Columns, SPEC·PLUS™ Solid Phase Extraction Columns with Filter, ANSYS Diagnostics, 1997.

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