TABLE OF CONTENTS

1. TX01 BASIC DRUG SCREEN

1.1 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification and confirmation of select basic drugs present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

1.2 SPECIMENS

1.2.1 Specimens include whole blood, serum, plasma, and urine.

1.2.2 The specimen volume is 2 mL, unless a limited sample quantity dictates otherwise. 1 mL may then be used and documented.

1.2.3 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist determines that less than half of the standard specimen volume should be used or does not wish to complete testing using the standard specimen volume prior to dilution in non-limited specimen cases, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

1.2.4 Analysis of larger specimen volumes must be approved by the FTU Technical Manager or designee and documented prior to analysis.

1.3 REAGENTS AND SOLUTIONS

1.3.1 100 mM Sodium Tetraborate Buffer (pH 9.3)

1.3.2 1-Chlorobutane, Reagent Grade

1.3.3 Bovine, Synthetic, or Human, Drug-Free Blood

1.3.4 Chloroform, Reagent Grade

1.3.5 Concentrated Ammonium Hydroxide (NH_4OH)

1.3.6 Deionized Water (DI water)

1.3.7 Hexanes, Reagent Grade

1.3.8 Hydrocarbon Ladder Containing C10 Through C30, C32, C34, C36

1.3.9 Hydrochloric Acid (HCl), 1.0 N

1.3.10 Methanol, Reagent Grade

1.4 STANDARDS AND CONTROLS

1.4.1 **SKF 525-A:NPA Internal Standard (ISTD)**

Primary Standards: Proadifen (SKF 525-A) and n-Propylamphetamine (NPA) may be available in solution or powder and prepared as needed per manufacturer's instructions. Store refrigerated at 2-8°C. Stability determined by manufacturer.

Working SKF 525-A:NPA ISTD Solution (7 µg/mL:2 µg/mL): Combine 7 mL of a 1.0 mg/mL SKF 525-A and 2 mL of a 1.0 mg/mL N-Propylamphetamine stock standard to a 1 L volumetric flask and dilute to mark with DI water for a final concentration of 7 µg/mL and 2 µg/mL, respectively. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

QC Check: Successful control verification

1.4.2 **TX01 Positive Control (PC)**

Primary Standards (1.0 mg/mL): Phentermine, Phencyclidine (PCP), and Trazodone. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX01 Working Positive Control Solution (5 µg/mL): Combine 50 µL of 1.0 mg/mL of each drug solution (Phentermine, Phencyclidine (PCP), and Trazodone) to a 10 mL volumetric flask and diluting to mark with methanol for a final concentration of 5 µg/mL. Store in freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

1.4.3 **Negative Control (NC)**

Appropriate drug-free matrix.

1.5 SUPPLIES AND EQUIPMENT

- 1.5.1 Autosampler Vials with Inserts and Caps
- 1.5.2 Centrifuge
- 1.5.3 Column: 100% Methyl Silicone or 5% Phenylmethylsilicone
- 1.5.4 Computer
- 1.5.5 Disposable Culture Tubes with Culture Tube Closures
- 1.5.6 Disposable Pasteur Pipettes
- 1.5.7 Five or Ten mL Disposable Conical Centrifuge Tubes with PTFE Lined Screw Caps
- 1.5.8 Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips
- 1.5.9 Gas Chromatograph/Mass Spectrometer (GC/MS)
- 1.5.10 Laminar Flow Hood with HEPA Filter (or Equivalent)
- 1.5.11 pH Paper
- 1.5.12 Repeat Pipette Dispensers
- 1.5.13 Rotary Mixer

1.5.14 Volumetric Flasks

1.5.15 Vortexer

1.6 SAMPLE PREPARATION

1.6.1 Label a clean disposable culture tube, conical centrifuge tube, and autosampler vial for each control and case sample.

1.6.2 Add 2 mL of drug-free matching matrix into each of the positive and negative control culture tubes.

1.6.3 Prepare a 250 ng/mL positive control by adding 100 µL of the working positive control solution to the positive control culture tube(s).

1.6.4 Transfer 2 mL of each case sample to appropriately labeled culture tubes.

1.6.5 Add 200 µL of the working SKF 525-A:NPA ISTD solution to controls and case samples and vortex. Final concentration of NPA:SKF ISTD is 700:200 ng/mL.

1.6.6 Add 1 mL of Sodium Tetraborate Buffer (pH 9.3) to controls and case samples and vortex for approximately 30 seconds.

1.6.7 Add 4 mL of 1-chlorobutane to each culture tube.

1.6.8 Cap the culture tubes and shake vigorously for 2 minutes or rotary mix for 10 minutes.

1.6.9 Centrifuge the culture tubes for approximately 3 minutes at 2800 – 3000 rpm.

NOTE: Centrifuge step may be repeated if insufficient separation occurs and will be documented in the case record.

1.6.10 Transfer the organic (top) layer to appropriately labeled conical centrifuge tubes.

1.6.11 Add 2 mL of 1.0 N hydrochloric acid to each conical.

1.6.12 Cap the conical tubes and rotary mix for 10 minutes.

1.6.13 Centrifuge the conical tubes for approximately 3 minutes at 2800 – 3000 rpm.

NOTE: Centrifuge step may be repeated if insufficient separation occurs and will be documented in the case record.

1.6.14 Aspirate the organic (top) layer to chemical waste.

1.6.15 Add approximately 500 µL of concentrated ammonium hydroxide to each conical and vortex.

NOTE: Solution should be strongly basic (pH of approximately 9). Additional ammonium hydroxide may be added if needed and will be documented in the case record.

1.6.16 Add approximately 100 µL of chloroform to each conical tube.

1.6.17 Cap the conical tubes and shake vigorously for 2 minutes or rotary mix for 10 minutes.

1.6.18 Centrifuge for approximately 3 minutes at 2800 – 3000 rpm.

NOTE: Centrifuge step may be repeated if insufficient separation occurs and will be documented in the case record.

1.6.19 Transfer the chloroform (bottom) layer to appropriately labeled autosampler vials with insert and cap.

1.6.20 Begin each run with the following sequence: positive control, negative control.

1.6.21 Inject 1 – 2 µL of extract onto the GC/MS. The same injection volume must be used for entire sequence.

1.7 INSTRUMENT PARAMETERS

Column:	30 m x 320 µm x 0.25 µm
Carrier:	Helium at 49 cm/sec, measured at 60 °C
Oven:	60 °C for 1 min 60-210 °C at 15 °C/min 210-300 °C at 10 °C/min 300 °C for 5.5 min
Injection:	Pulsed Splitless, 250 °C Injection Pulse Pressure at 20 psi until 1.0 min
Detector:	MSD, 280 °C transfer line Full scan at m/z 40-525 Sample: 1-2 µL
MS Source:	230 °C
MS Quad:	150 °C

1.8 CALCULATIONS

$$Retention\ Index\ (RI) = \left(\left[\frac{\text{Rt(peak of interest)} - \text{Rt(preceding } n\text{–}alkane)}{\text{Rt(following } n\text{–}alkane) - \text{Rt(preceding } n\text{–}alkane)}\right] + C_n\ (\text{preceding } n\text{–}alkane)\right) * 100$$

1.9 DECISION CRITERIA

Refer to "OSBI FTU Quality Manual – Ensuring the Validity of Results" for acceptance criteria.

1.10 NOTES

1.10.1 **Methods Approved for Analysis (GC/MS):**

Methods for toxicological analysis using the Agilent 5975 or 5977 GCMS are available from the FTU Technical Manager. These methods are to be utilized by the Forensic Toxicology Unit. Any requests for new methods/macros or changes to existing methods/macros should be submitted in writing to the FTU Technical Manager for review and approval prior to any changes being made.

The current approved methods are as follows:

TX1010 or TX1010-100: Hydrocarbon retention times method.

TX1030 or TX1030-100: General drug analysis method.

1.10.2 Instrument parameters will be set such that the run time is at a minimum without compromising base line resolution, peak shape, degradation, or other thermal problems.

1.10.3 If the criminalist extracts controls first to check for acceptance, they must extract a negative control along with the case specimens. The instrument parameters should remain the same between the analysis and acceptance of the controls and the analysis of the negative control and case specimens (i.e., the instrument remains set to the TX1030 method used for analysis of the controls until all analysis is complete). This should be utilized for batches that include a limited specimen case(s) only.

1.10.4 A packet containing autotune, tune evaluation, and original data for all controls and standards will be prepared for each analytical run and stored with the batch in the Laboratory Information Management System (LIMS).

1.11 REPORT WORDING

For reporting guidelines refer to “OSBI FTU Quality Manual - Reporting Results”.

1.12 REFERENCES

1.12.1 Forester EH, Mason MF. Preliminary Studies on the Use of n-Butyl Chloride as an Extractant in a Drug Screening Procedure. J. For. Sc., 1974; 19: 155-62.

1.12.2 Forester EH, Hatchett D, Garriott JC. A Rapid, Comprehensive Screening Procedure for Basic Drugs in Blood or Tissue by Gas Chromatography., J. Anal. Toxicol., 1978; 2: 50-5.

1.12.3 Marozzi, E., Ganbaro, V., Saligari, E., Mariani, R., and Lodi, F., Use of the Retention Index in Gas Chromatographic Studies of Drugs, J. Anal. Toxicol., 1982; 6: 185-92.

1.12.4 Perrigo, B. J., Peel, H.W., The Use of Retention Indices and Temperature-Programmed Gas Chromatography in Analytical Toxicology, J. Chromat. Sc., 1981; 19: 219-24.

1.12.5 Oklahoma State Statutes, Title 47: 751-61, Title 63: 4210B, Title 3: 303.

1.12.6 SOFT / AAFS Forensic Toxicology Laboratory Guidelines, 2006.

2. TX04 ELISA DRUG SCREEN

2.1 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the presumptive identification of select drugs and drug groups present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

2.2 SPECIMENS

2.2.1 Specimens include whole blood, serum, plasma, and urine.

2.2.2 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist determines that less than half of the standard specimen volume should be used or does not wish to complete testing using the standard specimen volume prior to dilution, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

2.2.3 Analysis of larger specimen volumes must be approved by the FTU Technical Manager or designee and documented prior to analysis.

2.3 REAGENTS AND SOLUTIONS

2.3.1 Bovine, Synthetic, or Human, Drug-Free Blood

2.3.2 Deionized Water (DI Water)

2.3.3 Dilution Reagent: PBS Buffer (pH= 7.0). This buffer may be purchased from Immunalysis or another approved vendor. Storage conditions and stability determined by manufacturer.

2.3.4 Immunalysis Direct ELISA Kits:

Each kit includes microwell plates, drug-enzyme conjugate solution, substrate reagent, and stop reagent. The substrate and stop reagents are not kit specific and may be interchanged as long as the expiration date has not passed. Storage conditions and stability determined by manufacturer.

Amphetamine	Methadone
Barbiturates	Methamphetamine/MDMA
Benzodiazepines	Morphine Specific
Cannabinoids	Opiates
Carisoprodol	Oxycodone/Oxymorphone
Cocaine/BE	PCP
Fentanyl	Tricyclic Antidepressants
Flunitrazepam	Tramadol
LSD	Zolpidem

2.4 STANDARDS AND CONTROLS

2.4.1 Negative Control (NC)

Drug-free, whole blood

2.4.2 Positive Control (PC) and High Positive Control (HPC)

Lyophilized blood controls purchased from UTAK Laboratories will serve as the positive control (PC) and high positive control (HPC). Reconstitute with five mL of DI water. Storage and stability determined by the manufacturer.

Table 2-1 Analyte concentrations for externally prepared Tecan controls

Analyte	NC (ng/mL)	PC (Cutoff) (ng/mL)	HPC (ng/mL)
7-Amino-Flunitrazepam	0	10	20
Amphetamine	0	20	40
Benzoylecgonine	0	50	100
Carisoprodol	0	500	1000
d-Methamphetamine	0	20	50
Fentanyl	0	0.5	5
Lysergic Acid Diethylamide	0	0.5	1
Methadone	0	50	100
Morphine	0	10	50
Nortriptyline	0	25	50
Oxazepam	0	50	200
Oxycodone	0	10	50
PCP	0	5	10
Secobarbital	0	100	200
THC-COOH	0	10	50
Tramadol	0	50	100
Zolpidem	0	10	20

2.5 SUPPLIES AND EQUIPMENT

2.5.1 12 x 75 mm Polypropylene Round Bottom Culture Tubes

2.5.2 Centrifuge

2.5.3 Computer

2.5.4 Disposable Troughs

2.5.5 Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips

2.5.6 Glass Vials with Caps

2.5.7 Laminar Flow Hood with HEPA Filter (or Equivalent)

2.5.8 Repeat Pipette Dispenser

2.5.9 Tecan Freedom EVO75 – Fully Automated Microplate Processor, Equipped with: Sunrise Spectra Microplate Reader and HydroFlex Microplate Strip Washer

2.5.10 Vortexer

2.6 SAMPLE PREPARATION

2.6.1 **Freedom EVO75 Set Up**

2.6.1.1 The glass vials with caps will be filled with conjugate solution and labeled with the kit lot number. The disposable troughs will be filled with TMB substrate and Stop solution as indicated by the labeling on each trough.

2.6.1.2 Place all reagents and solutions on the Tecan Freedom EVO75 deck in their proper locations.

2.6.1.3 Set up the 96-well micro-plates according to the number of samples listed on the worklist. If the last row contains less than 8 wells, fill the extra spaces with “dummy” or blank wells. Each plate must contain an even number of rows. If there are an odd number of rows, then a row of “dummy” wells must be added. Place the plates on the deck in order, according to the sample input table. Two assays may be run on one plate; however, one assay may not be run across two or more plates.

NOTE: the minimum number of samples for Yeti is eight. If the criminalist is testing less than eight, blanks must be included.

2.6.2 **Tecan Sampling:**

2.6.2.1 Label a clean 12 x 75 mm polypropylene tube for each control and sample.

2.6.2.2 Add 900 µL of dilution reagent to each 12 x 75 mm polypropylene tube.

2.6.2.3 Pipette 100 µL of the controls and case samples into each appropriately labeled 12 x 75 mm polypropylene tube and gently vortex.

2.6.2.4 Check control and sample tubes to ensure that there are no air bubbles present. Vortex and centrifuge as needed.

2.6.2.5 Place the sample tubes into the sample rack(s) and place onto the Tecan Freedom EVO75 deck.

2.6.2.6 Begin each run with the following sequence: positive control, negative control, high positive control.

2.7 INSTRUMENT PARAMETERS

Read plates at the following settings:
Main Filter: 450 nm
Double Beam: 620 nm

2.8 CALCULATIONS

$\%b/b_0 = (b/b_0) \times 100$

2.9 DECISION CRITERIA

2.9.1 Generally speaking, a specimen is considered to give a positive response for a particular drug class if the $\%b/b_0$ for that sample is less than or equal to the positive control (cutoff) for that drug class.

2.9.2 Criminalists may use discretion to determine if the results suggest the need for additional testing. If the specimen is deemed positive for a particular drug class, a separate analysis utilizing a different analytical principle must be performed in order to confirm the positive immunoassay results.

2.9.3 **Control Charting**

For control charting and additional acceptance criteria, refer to "OSBI FTU Quality Manual – Control Charts" and "OSBI FTU Quality Manual – Drug Identification Criteria," respectively.

2.10 NOTES

2.10.1 Remove the appropriate ELISA reagents and controls from the refrigerator and allow the reagents and controls to come to room temperature. Prepare ELISA plates as necessary for analysis.

2.10.2 It is important that the surface of the controls and samples are free of bubbles, or the analyzer will aspirate bubbles instead of the control or sample. Adding buffer to the tubes first and then pipetting the control or sample directly into the buffer will reduce the number of bubbles on the surface of the controls and samples.

2.10.3 Reagents and conjugate solutions should be returned to the refrigerator as soon as possible after completion of run. TMB substrate is light sensitive.

2.10.4 Immunalysis kits will be tracked by kit lot number. The conjugate solutions and plates are lot number specific and must be matched with each other. TMB and stop solutions are not lot number specific. Upon receipt in the laboratory, the kits may be opened and components labeled with the kit lot number and separated for proper storage.

2.11 REPORT WORDING

For reporting guidelines refer to "OSBI FTU Quality Manual - Reporting Results"

2.12 REFERENCES

2.12.1 Levine B. Principles of Forensic Toxicology; USA. American Association for Clinical Chemistry, Inc., 1999; 144-150.

2.12.2 Perrigo BJ, Joynt BP. Use of ELISA for the Detection of Common Drugs of Abuse in Forensic Whole Blood Samples. Can. Soc. Forens. Sci. J., 1995;28:261-269.

2.12.3 Collision IB, *et al*. Setting Cutoff Concentrations for Immunoassay Screening for Postmortem Blood. Journal For. Sc., 1998;43(2):390-394.

2.12.4 Oklahoma State Statutes, Title 47: 751-61, Title 63: 4210B, Title 3: 303.

2.12.5 Logan, BK, *et al.* Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities – 2017 Update. Journal of Ana. Tox., 2018:42:63-68.

2.12.6 Skopp, G, *et al.* Stability of Morphine, Morphine-3-Glucuronide, and Morphine-6-Glucuronide in Fresh Blood and Plasma and Postmortem Blood Samples. Journal of Ana. Tox. 2001; 25:2-7.

2.12.7 Winek, CL, *et al.* Winek's Drug & Chemical Blood-Level Data 2001.

2.12.8 Papoutsis, I., *et al.* Stability of Morphine, Codeine, and 6-Acetylmorphine in Blood at Different Sampling and Storage Conditions. J Forensic Sci. 2014;59(2):550-554.

2.12.9 ANSI/ASB Standard 120. Standard for the Analytical Scope and Sensitivity of Forensic Toxicology Testing of Blood in Impaired Driving Investigations. First Edition 2021

3. TX05 ETHANOL ANALYSIS BY HEADSPACE GAS CHROMATOGRAPHY

3.1 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification and confirmation of ethanol present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

3.2 SPECIMENS

3.2.1 Specimens include whole blood, serum, plasma, and urine.

3.2.2 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist determines that less than half of the standard specimen volume should be used or does not wish to complete testing using the standard specimen volume prior to dilution, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

3.2.3 Analysis of larger specimen volumes must be approved by the FTU Technical Manager or designee and documented prior to analysis.

3.3 REAGENTS AND SOLUTIONS

Deionized Water (DI Water)

3.4 STANDARDS AND CONTROLS

3.4.1 **N-Propanol Internal Standard (ISTD)**

Primary Standard: n-Propanol, Min 99.5% (density = 0.8 g/mL).

Working n-Propanol (0.020 g/100mL) Internal Standard Solution: Transfer 0.500 mL of n-Propanol to a 2 L volumetric flask. Dilute to mark with DI water for a final concentration of 0.020 g/100 mL. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

QC Check: Successful calibration verification

3.4.2 **Ethanol Calibration Solutions**

Standard solutions are purchased from Lipomed or Cerilliant at certified concentrations (0.010, 0.100, 0.200, 0.300, and 0.400 g/100mL). Stability determined by manufacturer. While in storage, Ethanol Calibration Solutions will be stored at room temperature unless otherwise determined by the manufacturer, and will be stored in the refrigerator while in use.

3.4.3 **Negative Control (NC)**

DI water

3.4.4 **Positive Control (PC)**

Purchased from Cerilliant at a certified concentration of 0.080 g/100mL. Store refrigerated at 2-8°C. Stability determined by manufacturer.

3.4.5 **Whole Blood (WB) Volatiles Controls (Levels 1 and 2)**

Purchased commercially from Cliniqa, catalog # 93211 and 93212. Typical assay ranges are 0.068 to 0.083 g/100mL for Level 1 (Low) and 0.175 to 0.214 g/100mL for Level 2 (High), but may vary by manufacturer lot. Store refrigerated at 2-8°C. Stability determined by manufacturer.

3.4.6 **Verification Solution**

NIST Traceable Alcohol Certified Reference Material Solution 0.150 g/100mL (Cerilliant E-041 or equivalent): to verify functionality of instrument throughout batch run. Store refrigerated at 2-8°C. Stability determined by manufacturer.

3.4.7 **Multicomponent Volatile Mix Solution**

Primary Standards: ACS Grade Absolute Ethanol 200 proof – approximately 99.5%, Reagent Grade Acetaldehyde – approximately 99.5%, ACS Grade Acetone – approximately 99.6%, ACS Grade Isopropanol – approximately 99.9%, and ACS Grade Methanol – approximately 99.99%.

Multicomponent Volatile Mix Solution: Transfer approximately 125 µL absolute ethanol, 40 µL acetaldehyde, 40 µL acetone, 75 µL isopropanol, and 125 µL methanol, into a 100 mL volumetric flask containing DI water and dilute to mark with DI water. Store tightly capped volatile mix in a refrigerator at 2-8°C. Total volume may be adjusted as necessary, but beginning concentrations and end ratio must remain the same.

QC Check: When a new volatile mix control is prepared, it will be analyzed and compared to the previous lot number and demonstrate successful separation.

3.5 SUPPLIES AND EQUIPMENT

3.5.1 10- or 20-mL Headspace Vials

3.5.2 20 mm Crimp-on Septa

3.5.3 Agilent Gas Chromatograph (GC)/Flame Ionization Detectors (FID)

3.5.3.1 Back Column: Rtx-BAC PLUS 2 30 m X 0.32 mm X 0.60 µm (or Equivalent)

3.5.3.2 Front Column: Rtx-BAC PLUS 1 30 m X 0.32 mm X 1.8 µm (or Equivalent)

3.5.4 Calibrated Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips

3.5.5 Computer

3.5.6 Crimper

3.5.7 Laminar Flow Hood with HEPA Filter (or Equivalent)

3.5.8 Repeat Pipette Dispenser

3.5.9 Tissue Grinder, Glass Vessel

3.5.10 Volumetric Flasks

3.6 SAMPLE PREPARATION

3.6.1 **Calibration**

3.6.1.1 Label a clean headspace vial for each calibrator, negative and positive control, and low and high whole blood positive controls (WBPC).

3.6.1.2 Add 400 µL of working n-propanol internal standard solution into each headspace vial.

3.6.1.3 Add 100 µL of the appropriate calibrator or control into each headspace vial.

3.6.1.4 Seal the headspace vials by crimping the crimp-on septa.

3.6.1.5 Begin each run with the following sequence: volatile mix, calibrators 0.010 through 0.400, negative control, ethanol positive control, low whole blood control, high blood control.

3.6.1.6 If the criteria for quality control outlined in "DECISION CRITERIA" for the volatile mix solution, calibration solutions, negative/positive controls, and WBPC controls are not satisfied, then the calibration must be repeated with acceptable results prior to ethanol being identified, confirmed, or quantitated.

3.6.1.7 Update the calibration with the calibration solutions.

3.6.2 **Sampling**

3.6.2.1 Label a clean headspace vial for each verifier and case sample, including a position number for case samples. Case samples will be analyzed in duplicate.

3.6.2.2 Ensure that all samples are homogenous by shaking, rotary mixing, and/or vortexing. A tissue grinder can be used to break up any clots.

If a homogenous sample cannot be obtained for any reason, a notation shall be made in the Laboratory Information Management System (LIMS) detailing the condition of the sample and its handling.

3.6.2.3 Add 400 µL of working n-Propanol internal standard solution into each headspace vial.

3.6.2.4 Add 100 µL of case sample into the appropriately labeled headspace vials.

3.6.2.5 Add 100 µL of verification solution into each verifier headspace vial.

3.6.2.6 Seal the headspace vials by crimping with the crimp-on septa.

3.6.2.7 Separate verification solutions will be prepared and analyzed for each of the following positions: at the beginning of each batch run, after every eight injections (4 cases), and at the end of each batch run. The ending verification solution may be in a position which is less than eight injections (4 cases) due to the size of a batch or for batch runs that consist of less than 4 cases in order to bracket the run. If a calibration curve is run in the same sequence with case sample(s) then the calibration curve may be used in place of a verification at the beginning of the run. Verification solutions do not need to be analyzed in duplicate.

3.7 INSTRUMENT PARAMETERS

Temperature settings	Oven Temperature	65°C
	Loop Temperature	65°C
	Transfer Line Temperature	75°C
Transfer line	Transfer Line Type	Fused Silica or Pro Steel
	Transfer Line Diameter	0.32 mm
Timing settings	Vial Equilibration	13 min
	Injection Duration	0.50 min
	GC Cycle Time	5.00
Vial and loop settings	Vial Size	10 or 20 mL
	Loop Size	1 mL
	Fill Mode	Flow to Pressure
	Fill Pressure	9 psi
	Loop Fill Mode	Custom
	Loop Ramp Rate	30 psi/min
	Loop Final Pressure	1.5 psi
	Loop Equilibration Time	0.05 min
	Vial Shaking	OFF
	Vial Pressurization Gas	Nitrogen

Instrument	Agilent 7890B GC
Front Column	BAC-1 30m x 0.32mm x 1.8µm
Back Column	BAC-2 30m x 0.32mm x 0.6µm
Carrier Gas	Nitrogen @ 3.0 mL/min each
Oven Program	45°C Isothermal
Thermal Aux 3 Heater Temp.	85°C
Detector Temperature	300°C

3.8 CALCULATIONS

3.8.1 Calibration is a y = mx + b linear regression model using a five-point curve.

3.8.2 To create the calibration curve by hand:

Ratio of peak area = peak area of ethanol ÷ peak area of ISTD
Plot ratio of the expected concentration as the X-axis versus the ratio of peak area as the Y-axis.

Gradient of the least squares line, *m*

$$m = \frac{\sum_{i=1}^{n} \{(x_i - \bar{x})(y_i - \bar{y})\}}{\sum_{i=1}^{n} (x_i - \bar{x})^2}$$

Calculate b, by entering information into y = mx + b.
Once m and b have been calculated, the concentration of the sample can be calculated by inputting the ratio of the peak area into the equation.

3.9 DECISION CRITERIA

3.9.1 Refer to "OSBI FTU Quality Manual – Ensuring the Validity of Results" for acceptance criteria.

3.9.2 **Control Charting**

For control charting and additional acceptance criteria, refer to "OSBI FTU Quality Manual – Control Charts" and "OSBI FTU Quality Manual – Drug Identification Criteria," respectively.

3.10 NOTES

3.10.1 Once calibrators, controls, verifiers, and samples have been transferred into a headspace vial, they must be run the same day.

3.10.2 A packet containing original data for all calibrators, controls, and standards will be prepared for each analytical run and stored with the batch in the Laboratory Information Management System (LIMS).

3.11 REPORT WORDING

For reporting guidelines refer to "OSBI FTU Quality Manual - Reporting Results"

3.12 REFERENCE

3.12.1 James C. Garriott. Medicolegal Aspects of Alcohol. 5th Ed. (2008).

3.12.2 Musselman J., Solanky A., Arnold W. (2006). Case Study: Increasing Accuracy of Blood-Alcohol Analysis Using Automated Headspace-Gas Chromatography. PerkinElmer, Inc., Shelton, CT.

3.12.3 B.L. Levine, Principles of Forensic Toxicology, American Association for Clinical Chemistry, Inc., 1999, pg. 180.

4. TX09 ACIDS AND NEUTRALS DRUG SCREEN

4.1 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification of select barbiturates and acid/neutral drugs present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

4.2 SPECIMENS

4.2.1 Specimens include whole blood, serum, plasma, and urine.

4.2.2 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist determines that less than half of the standard specimen volume should be used or does not wish to complete testing using the standard specimen volume prior to dilution in non-limited specimen cases, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

4.2.3 Analysis of larger specimen volumes must be approved by the FTU Technical Manager or designee and documented prior to analysis.

4.3 REAGENTS AND SOLUTIONS

4.3.1 1:1 Hexane/Ethyl Acetate: Combine 50 mL of hexanes and 50 mL of ethyl acetate solution into an amber bottle. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

4.3.2 Acetonitrile (ACN), Reagent Grade

4.3.3 Bovine, Synthetic, or Human, Drug-Free Blood

4.3.4 Deionized Water (DI Water)

4.3.5 Ethyl Acetate, Reagent Grade

4.3.6 Hexane, Reagent Grade

4.3.7 Hexanes Saturated with Acetonitrile, Made day of use

4.3.8 Hydrocarbon Ladder Containing C10 through C30, C32, C34, C36

4.3.9 pH 6 Buffer

4.3.10 Reconstitution Solvent, ACN

4.4 STANDARDS AND CONTROLS

4.4.1 **Hexobarbital Internal Standard (ISTD)**

Primary Standard (1.0 mg/mL): Hexobarbital standard, may be available in solution or powder and prepared as needed. Store refrigerated at 2-8°C. Stability determined by manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

Working Hexobarbital ISTD Solution (5.0 µg/mL): Transfer 1.0 mL of a 1.0 mg/mL Hexobarbital stock standard to a 200 mL volumetric flask and dilute to the mark with DI water. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

QC Check: Successful control verification

4.4.2 **TX09 Positive Control (PC)**

Primary Standard (250 µg/mL): Barbiturate Mix-5 reference standard. Stability and storage determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX09 Working Positive Control Solution (10 µg/mL): Transfer 1.0 mL of a 250 µg/mL Barbiturate Mix-5 solution to a 25 mL volumetric flask and dilute to the mark with methanol. Store in freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

4.4.3 **Negative Control (NC)**

Appropriate drug-free matrix

4.5 SUPPLIES AND EQUIPMENT

- 4.5.1 Autosampler Vial with Inserts and Caps
- 4.5.2 Centrifuge
- 4.5.3 Column: 100% Methyl Silicone or 5% Phenylmethylsilicone
- 4.5.4 Computer
- 4.5.5 Disposable Culture Tubes with Culture Tube Closures
- 4.5.6 Disposable Pasteur Pipettes
- 4.5.7 Five or Ten mL Disposable Conical Centrifuge Tubes with PTFE Lined Screw Caps
- 4.5.8 Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips
- 4.5.9 Gas Chromatograph/Mass Spectrometer (GC/MS)
- 4.5.10 Laminar Flow Hood with HEPA Filter (or Equivalent)
- 4.5.11 Nitrogen Evaporator (N-Evap)
- 4.5.12 pH Paper
- 4.5.13 Repeat Pipette Dispenser

4.5.14 Rotary Mixer

4.5.15 Volumetric Flasks

4.5.16 Vortexer

4.6 SAMPLE PREPARATION

4.6.1 Label a clean disposable culture tube, conical tube, and autosampler vial for each control and sample.

4.6.2 Add 1 mL of appropriate drug-free matrix into each of the positive and negative control culture tubes.

4.6.3 Prepare a 1.0 µg/mL positive control by adding 100 µL of the working positive control solution into the positive control culture tube(s). Vortex.

4.6.4 Transfer 1 mL of each case sample into the appropriately labeled culture tubes.

4.6.5 Add 500 µL of the working Hexobarbital ISTD solution into each tube and briefly vortex. Final concentration of the Hexobarbital ISTD is 2.5 µg/mL.

4.6.6 Add 1 mL of pH 6 buffer to each tube and vortex.

4.6.7 Add 5 mL of 1:1 hexane/ethyl acetate solution to each tube.

4.6.8 Cap the tubes and rotary mix for approximately 5 minutes.

4.6.9 Centrifuge the tubes for approximately 5 minutes at 2800 – 3000 rpm.

NOTE: Centrifuge step may be repeated if insufficient separation occurs and will be documented in the case record.

4.6.10 Transfer the solvent (top) layer to appropriately labeled conical tubes.

4.6.11 Evaporate to dryness at approximately 40°C with a steady stream of nitrogen.

4.6.12 Reconstitute with 100 µL of acetonitrile and vortex.

4.6.13 Partition with 0.5 mL of n-hexane, previously saturated with acetonitrile, and vortex. Centrifuge and aspirate n-hexane. This step may be repeated as needed, up to two more times.

4.6.14 Transfer extracts to appropriately labeled autosampler vials with insert and cap.

4.6.15 Begin each run with the following sequence: positive control, negative control.

4.6.16 Inject 1 – 2 µL of extract onto the gas chromatograph. The same injection volume must be used for entire sequence.

4.7 INSTRUMENT PARAMETERS

Column:	30 m x 0.32 mm x 0.25 µm
Carrier:	Helium at 49 cm/sec, measured at 60 °C
Oven:	60 °C for 1 min

	60-210 °C at 15 °C/min 210-300 °C at 10 °C/min 300 °C for 5.5 min
Injection:	Pulsed Splitless, 250 °C Injection Pulse Pressure at 20 psi until 1.0 min
Detector:	MSD, 280 °C transfer line Full scan at m/z 40-525 Sample: 1-2 µL
MS Source:	230 °C
MS Quad:	150 °C

4.8 CALCULATIONS

$$Retention\ Index\ (RI) = \left(\left[\frac{\text{Rt(peak of interest)} - \text{Rt(preceding } n\text{–}alkane)}{\text{Rt(following } n\text{–}alkane) - \text{Rt(preceding } n\text{–}alkane)}\right] + C_n\ (\text{preceding } n\text{–}alkane)\right) * 100$$

4.9 DECISION CRITERIA

Refer to "OSBI FTU Quality Manual –Ensuring the Validity of Results" for acceptance criteria.

4.10 NOTES

4.10.1 **Methods approved for Analysis (GC/MS)**

Methods for toxicological analysis using the Agilent 5975 or 5977 GC/MS are available from the FTU Technical Manager. These methods are to be utilized by the Forensic Toxicology Unit. Any requests for new methods/macros or changes to existing methods/macros should be submitted in writing to the FTU Technical Manager for review and approval prior to any changes being made.

The current approved methods are as follows:

TX1010 or TX1010-100: Hydrocarbon retention times method.

TX1030 or TX1030-100: General drug analysis method.

4.10.2 Instrument parameters will be set such that the run time is at a minimum without compromising base line resolution, peak shape, degradation, or other thermal problems.

4.10.3 If the criminalist extracts controls first to check for acceptance, they must extract a negative control along with the case specimens. The instrument parameters should remain the same between the analysis and acceptance of the controls and the

analysis of the negative control and case specimens (i.e., the instrument remains set to the TX1030 method used for analysis of the controls until all analysis is complete). This should be utilized for batches that include a limited specimen case(s) only.

4.10.4 A packet containing autotune, tune evaluation, and original data for all controls and standards will be prepared for each analytical run and stored with the batch in the Laboratory Information Management System (LIMS).

Table 4-1 Commonly encountered acid and neutral drugs include

Amobarbital	Barbital
Butalbital	Carisoprodol
Guaifenesin	Lamotrigine
Levetiracetam	Meprobamate
Methocarbamol	Pentobarbital
Phenobarbital	Topiramate
Secobarbital	Valproic Acid
Zaleplon	

4.11 REPORT WORDING

For reporting guidelines refer to “OSBI FTU Quality Manual – Reporting Results”.

4.12 REFERENCES

4.12.1 Anderson WH, Fuller DC. A Simplified Procedure for the Isolation, Characterization, and Identification of Weak Acid and Neutral Drugs from Whole Blood. J. Anal. Toxicol 1987; 5: 198-204.

4.12.2 Moffat AC, et al. Clarke’s Isolation and Identification of Drugs. 2nd rev. ed. London: The Pharmaceutical Press, 1986.

4.12.3 Basalt and Cravey. A Compendium of Therapeutic & Toxic Conc. Of toxicology Significant Drugs in Human Biofluids. J. Anal. Toxicol. 1977 March/April 81-97.

4.12.4 Marozzi, E., Ganbaro, V., Saligari, E., Mariani, R., and Lodi, F., Use of the Retention Index in Gas Chromatographic Studies of Drugs, J. Anal. Toxicol., 6: 185-92 (1982).

4.12.5 Perrigo, B. J., Peel, H.W., The Use of Retention Indices and Temperature-Programmed Gas Chromatography in Analytical Toxicology, J. Chromat. Sc., 19: 219-224 (1981).

4.12.6 Oklahoma State Statutes, Title 47: 751-61, Title 63: 4210B, Title 3: 303.

4.12.7 SOFT / AAFS Forensic Toxicology Laboratory Guidelines, 2006.

5. TX14 CANNABINOIDS IN BLOOD BY LC TANDEM MS

5.1 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the confirmation of tetrahydrocannabinols (THC), the primary psychoactive component of marijuana, 11-hydroxy- Δ^9-tetrahydrocannabinol (THC-OH), a major active metabolite of THC, and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH or THCA), a major inactive metabolite of THC present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

5.2 SPECIMENS

5.2.1 Specimens include whole blood.

5.2.2 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist determines that less than half of the standard specimen volume should be used or does not wish to complete testing using the standard specimen volume prior to dilution, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

5.2.3 Analysis of larger specimen volumes must be approved by the FTU Technical Manager or designee and documented prior to analysis.

5.3 REAGENTS AND SOLUTIONS

5.3.1 0.1% Formic Acid in Acetonitrile (Mobile Phase B)

5.3.2 0.1% Formic Acid in Water (Mobile Phase A)

5.3.3 10% Acetic Acid: Add 50 mL of glacial acetic acid to 450 mL DI water. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

5.3.4 88% Formic Acid, Reagent Grade

5.3.5 9:1 Hexane:Ethyl Acetate: Add 900 mL hexane to 100 mL ethyl acetate. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

5.3.6 Acetic Acid, Glacial

5.3.7 Acetonitrile (ACN), LCMS Grade

5.3.8 Bovine, Synthetic, or Human, Drug-Free Blood

5.3.9 Deionized Water (DI Water)

5.3.10 Ethyl Acetate, Reagent Grade

5.3.11 Hexane, Reagent Grade

5.3.12 Methanol, Reagent Grade

5.3.13 Reconstitution Solvent 1:1 DI water:ACN: Add 100 mL DI water to 100 mL acetonitrile. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

5.4 STANDARDS AND CONTROLS

5.4.1 **TX14 Working Internal Standard (ISTD) Solution (delta9-THC-d_3, delta9-THC-OH-d_3, and delta9-THC-COOH-d_3)**

Primary Standards (100 µg/mL): delta9-THC-d3, delta9-THC-OH-d3, and delta9-THC-COOH-d3. Storage conditions and stability determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX14 Secondary delta9-THC-d3, delta9-THC-OH-d3, and delta9-THC-COOH-d3 ISTD Solution (250:250:2500 ng/mL): In a 10 mL volumetric flask, combine 25 µL each of the delta9-THC-d3 and delta9-THC-OH-d3 stock standard. Add 250 µL of delta9-THC-COOH-d3 stock standard. Dilute to mark with methanol. Store in freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

TX14 Working delta9-THC-d3, delta9-THC-OH-d3, and delta9-THC-COOH-d3 ISTD Solution (25:25:250 ng/mL): In a 10 mL volumetric flask, pipette 1 mL of TX14 Secondary Internal Standard Solution and dilute to mark with methanol. Store in freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

QC Check: Successful control verification

5.4.2 **TX14 Working Solution A/High Positive Control (delta9-THC, delta9-THC-OH, and delta9-THC-COOH)**

Primary Standards (1.0 mg/mL and 100 µg/mL): delta9-THC, delta9-THC-COOH and delta9-THC-OH. Storage conditions and stability determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

Delta9-THC Secondary Stock Solution (10 µg/mL): In a 10 mL volumetric flask, add 100 µL of 1 mg/mL delta9-THC Primary Standard and dilute to mark with methanol. Store in freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

TX14 Working Solution A, delta9-THC, delta9-THC-OH, and delta9-THC-COOH (100:100:1000 ng/mL): In a 10 mL volumetric flask, combine 100 µL of delta9-THC secondary stock solution, 10 µL of 1 mg/mL delta9-THC-COOH or 100 µL of 100 µg/mL

delta9-THC-COOH stock standard, and 10 µL of 100 µg/mL delta9-THC-OH stock standard. Dilute to mark with methanol. Store in freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

5.4.3 **TX14 Working Solution B/Low Positive Control (delta9-THC, delta9-THC-OH, and delta9-THC-COOH)**

TX14 Working Solution B, delta9-THC, delta9-THC-OH, and delta9-THC-COOH (10:10:100 ng/mL): In a 10 mL volumetric flask, transfer 1.0 mL of TX14 working solution A and dilute to mark with methanol. Store in freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

5.4.4 **Negative Control (NC)**

Drug-free whole blood

5.5 SUPPLIES AND EQUIPMENT

5.5.1 Autosampler Vials with Inserts and Caps

5.5.2 Centrifuge

5.5.3 Column: Kinetex C18 2.6 µm x 2.1 mm x 75 mm

5.5.4 Computer

5.5.5 Disposable Culture Tubes with Culture Tube Closures

5.5.6 Disposable Pasteur Pipettes

5.5.7 Filter Autosampler Vials

5.5.8 Five or Ten mL Disposable Conical Centrifuge Tubes with PTFE Lined Screw Caps

5.5.9 Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips

5.5.10 Laminar Flow Hood with HEPA Filter (or Equivalent)

5.5.11 Nitrogen Evaporator (N-Evap)

5.5.12 Repeat Pipette Dispenser

5.5.13 Rotary Mixer

5.5.14 Shimadzu 8030 and 8050 LCMS, Argon, Nitrogen Generator

5.5.15 Volumetric Flasks

5.5.16 Vortexer

5.6 SAMPLE PREPARATION

5.6.1 Label a clean, disposable culture tube, conical centrifuge tube, and autosampler vial for each control and case sample.

5.6.2 Add 100 µL of the TX14 working internal standard solution to each control and case sample culture tube.

5.6.3 Prepare the low positive control by adding 100 µL of TX14 Working Solution B to the low positive control culture tube.

5.6.4 Prepare the high positive control by adding 100 µL TX14 Working Solution A to the high positive control culture tube.

5.6.5 Add 2 mL DI water to each culture tube and vortex.

5.6.6 Add 1 mL of drug-free whole blood to control culture tubes and vortex.

5.6.7 Add 1 mL of case specimen to appropriately labeled culture tubes and vortex.

5.6.8 Add 1 mL 10% acetic acid to each culture tube and vortex.

5.6.9 Add 4 mL 9:1 hexanes:ethyl acetate to each culture tube.

5.6.10 Cap the culture tubes and rotary mix for approximately 20 minutes.

5.6.11 Centrifuge for approximately 5 minutes at 2800 – 3000 rpm.

NOTE: Centrifuge step may be repeated if insufficient separation occurs and will be documented in the case record.

5.6.12 Transfer organic (top) layer to a clean, appropriately labeled conical centrifuge tube.

5.6.13 Evaporate to dryness at approximately 40°C with a steady stream of nitrogen.

5.6.14 Add 100 µL of reconstitution solvent to each conical.

5.6.15 Vortex briefly and centrifuge to collect liquid in bottom of conical.

5.6.16 Transfer liquid to appropriately labeled autosampler vials with insert and cap. A filter autosampler vial may be used instead.

5.6.17 Centrifuge at 2800 – 3000 rpm as needed.

5.6.18 Begin each run with the following sequence: low positive control, high positive control, negative control.

5.6.19 Inject 10 µL of sample on Shimadzu 8030 or 5 µL of sample on Shimadzu 8050, injection volume may be adjusted as needed. If a different injection volume is used, it should be documented in the case record. The same injection volume must be used for entire sequence. Utilize “THC.lcm” method.

NOTE: Copies of the method may be renamed and saved with the batch data before or after analysis. The name shall still include “THC” at a minimum.

5.7 INSTRUMENTAL PARAMETERS

Gradient Elution

Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile

Initial Composition	60% A, 40% B, Total Flow 0.7 mL/min
0.10 – 3.50 min	% B increased to 75%
3.50-3.75	% B Increased to 95%, Total Flow 0.9 mL/min
Hold time	0.76 minutes (95% B)
Re-equilibration	0.49 minutes, Total Flow 0.7 mL/min
Column Temp	60°C
Autosampler	
Injection Volume	10 µL on Shimadzu 8030 or 5 µL on Shimadzu 8050, can be adjusted as needed
Sampling Speed	2.0 µL/sec
Cooler Temperature	15°C
Interface DUIS-ESI	
Nebulizing Gas	Nitrogen
Nebulizing Gas Flow	2.0 L/min
Drying Gas	Nitrogen
Drying Gas Flow	20.0 L/min
CID Gas	Argon 230 kPa
DL Temp	300°C
Heat Block Temperature	400°C

5.8 CALCULATIONS

5.8.1 The MRM transition ion ratio ranges will be set to the average of the low and high positive controls. Once set, all cases and controls must be analyzed under those conditions.

5.9 DECISION CRITERIA

5.9.1 Refer to “OSBI FTU Quality Manual – Ensuring the Validity of Results” for acceptance criteria.

5.9.2 Delta8-THC and Delta9-THC are known to co-elute using the current TX14 method. Results for THC are reported as “tetrahydrocannabinol” so a peak that suggests co-elution of delta8- and delta9-THC, can be reported if it meets all other acceptance criteria.

Table 5-1 Precursors and Product Ions for Respective Compounds

Compound	Precursors	MRM Transitions
Delta9-THC-COOH-d3(delta9-THCA-d3)	348.2	330.0, 302.0
Delta9-THC-COOH (delta9-THCA)	345.2	327.3, 299.0
Delta9-THC-d3	318.2	123.3, 196.2
Delta9-THC	315.2	193.0, 122.9
Delta9-THC-OH-d3	334.10	316.30
Delta9-THC-OH	331.10	193.00, 201.10

5.10 NOTES

5.10.1 Evaporating to dryness takes approximately eight minutes. Excessive drying could result in lowered compound response.

5.10.2 Store all standard solutions in amber colored bottles.

5.10.3 If the criminalist extracts controls first to check for acceptance, they must extract a negative control along with the case specimens. The instrument parameters should remain the same between the analysis and acceptance of the controls and the analysis of the negative control and case specimens (i.e., the instrument remains set to the TX14 method used for analysis of the controls until all analysis is complete). This should be utilized for batches that include a limited specimen case(s) only.

5.10.4 A packet containing original data for all controls and standards will be prepared for each analytical run and stored with the batch in the Laboratory Information Management System (LIMS).

5.11 REPORT WORDING

For reporting guidelines refer to “OSBI FTU Quality Manual - Reporting Results”.

5.12 REFERENCES

5.12.1 Texas Department of Public Safety Standard Operating Procedure DRN: TOX-05-19. Extraction for Δ^9-THC and Δ^9-Carboxy-THC in Blood.

5.12.2 Washington State Patrol Toxicology Laboratory Test Method Confirmation Cannabinoids by LC-MSMS with an effective date of 5-8-14. Accessed 12/15/14 http://www.wsp.wa.gov.

6. TX34 DRUG IDENTIFICATION AND CONFIRMATION BY LC TANDEM MS

6.1 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification and confirmation of select drugs present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

6.2 SPECIMENS

6.2.1 Specimens include whole blood.

6.2.2 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist does wish to use a specimen volume besides either specimen volume outlined in this SOP prior to dilution, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

6.2.3 Analysis of larger specimen volumes must be approved by the FTU Technical Manager or designee and documented prior to analysis.

6.3 REAGENTS AND SOLUTIONS

6.3.1 0.1% Formic Acid in Acetonitrile (Mobile Phase B)

6.3.2 0.1% Formic Acid in Water (Mobile Phase A)

6.3.3 88% Formic Acid, Reagent Grade

6.3.4 Acetonitrile (ACN), LCMS Reagent Grade

6.3.5 Bovine, Synthetic, or Human, Drug-Free Blood

6.3.6 Deionized Water (DI Water)

6.3.7 Methanol, LCMS Reagent Grade

6.3.8 Reconstitution Solution, 9:1 DI water: ACN with 0.088% Formic Acid: Combine 5 mL of acetonitrile and 45 mL DI water and 50 µL of formic acid. Stored at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

6.4 STANDARDS AND CONTROLS

6.4.1 **TX34 Internal Standard (ISTD)**

Primary Standards (100 µg/mL): Methamphetamine-d8, PCP-d5, and Prazepam-d5. Storage conditions and stability determined by manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX34 Working Internal Standard Solution (20 ng/mL): Transfer 50 µL of each deuterated primary internal standard into a 250 mL volumetric flask and dilute to mark with acetonitrile. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

Diluted Working Internal Standard Solution (for 100 µL extraction only, day of use): Add an equal amount of Working Internal Standard Solution and Acetonitrile (from the same lot as that used to make the Working Internal Standard Solution if feasible) to a clean vial. If the same lot number of acetonitrile is not available, the lot number used must be recorded in the case record. Prepare enough to add 500 µL to each of your controls and case samples. Total volume can be adjusted as needed, but the final concentration must remain the same.

QC Check: Successful control verification

6.4.2 **TX34 High Positive Control (HPC)**

Primary Standards (1.0 mg/mL): See Table 6-1 for CRM's. Storage conditions and stability determined by manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX34 Working High Positive Control Solution: Transfer designated volume of all primary standards listed in Table 6-1 into a 100 mL volumetric flask. Dilute to the mark with DI water. Store in refrigerator. Total volume may be adjusted as necessary, but the final concentration must remain the same.

6.4.3 **TX34 Low Positive Control (LPC)**

TX34 Working Low Positive Control Solution: Transfer 5 mL of Working High Positive Control Solution to a 10 mL volumetric flask and dilute to the mark with DI water. Store in refrigerator. Total volume may be adjusted as necessary, but the final concentration must remain the same.

6.4.4 **Negative Control (NC)**

Drug-free whole blood

6.5 SUPPLIES AND EQUIPMENT

- 6.5.1 Autosampler Vials with Inserts and Caps
- 6.5.2 Centrifuge
- 6.5.3 Column: Kinetex C18 2.6 µm x 2.1 mm x 75 mm
- 6.5.4 Computer
- 6.5.5 Disposable Microcentrifuge Tubes
- 6.5.6 Disposable Pasteur Pipettes
- 6.5.7 Filter Autosampler Vials

6.5.8 Five or Ten mL Disposable Conical Centrifuge Tubes with PTFE Lined Screw Caps

6.5.9 Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips

6.5.10 Laminar Flow Hood with HEPA Filter (or Equivalent)

6.5.11 Microcentrifuge

6.5.12 Nitrogen Evaporator (N-Evap)

6.5.13 Shimadzu 8030 LCMS, Argon, Nitrogen Generator

6.5.14 Volumetric Flasks

6.5.15 Vortexer

6.6 SAMPLE PREPARATION

6.6.1 **100 µL DILUTION PREPARATION**

Note: The 100 µL DILUTION PREPARATION is for limited case samples in which the available specimen volume does not allow for the completion of two tests using 250 µL. See "6.10.1 NOTES" below.

6.6.1.1 Label a clean disposable microcentrifuge tube, conical centrifuge tube, and autosampler vial for each control and case sample.

6.6.1.2 Prepare the low positive control by adding 10 µL of working low positive control solution to 90 µL of drug-free whole blood in the low positive control microcentrifuge tube and vortex. Add 100 µL of PBS Buffer and vortex.

6.6.1.3 Prepare the high positive control by adding 20 µL of working high positive control solution to 80 µL of drug-free whole blood in the high positive control tube and vortex. Add 100 µL of PBS Buffer and vortex.

6.6.1.4 Add 100 µL of drug-free whole blood and 100 µL of PBS Buffer in the negative control microcentrifuge tube and vortex.

6.6.1.5 Add 100 µL of each case specimen and 100 µL of PBS Buffer to the appropriately labeled microcentrifuge tubes and vortex.

6.6.1.6 Refer to 6.4.1 Diluted Working Internal Standard Solution. Starting at 6.6.2.6, follow the remaining sample preparation steps in 6.6.2.

6.6.2 **250 µL PREPARATION**

6.6.2.1 Label a clean disposable microcentrifuge tube, conical centrifuge tube, and autosampler vial for each control and case sample.

6.6.2.2 Prepare the low positive control by adding 25 µL of TX34 working low positive control solution to 225 µL of drug-free whole blood in the low positive control microcentrifuge tube.

6.6.2.3 Prepare the high positive control by adding 50 µL of the TX34 working high positive control solution to 200 µL of drug-free whole blood in the high positive control microcentrifuge tube.

6.6.2.4 Add 250 µL of drug-free whole blood into the negative control microcentrifuge tube.

6.6.2.5 Transfer 250 µL of each case specimen to the appropriately labeled microcentrifuge tubes.

6.6.2.6 Add 500 µL of working internal standard solution to controls and case samples and vortex for approximately 30 seconds.

6.6.2.7 Centrifuge for 5 minutes at approximately 13,000 rpm.

NOTE: Centrifuge step may be repeated if insufficient separation occurs and will be documented in the case record.

6.6.2.8 Transfer acetonitrile (top) layer to the appropriately labeled conical.

6.6.2.9 Evaporate to dryness at approximately 40°C with a steady stream of nitrogen.

6.6.2.10 Add 100 µL of reconstitution solvent to each conical.

6.6.2.11 Vortex briefly and centrifuge to collect liquid in bottom of conical.

6.6.2.12 Transfer liquid to an appropriately labeled autosampler vial with insert and cap. A filter autosampler vial may be used instead.

6.6.2.13 Begin each run with the following sequence: low positive control, high positive control, negative control.

6.6.2.14 Inject 10 µL of sample, injection volume may be adjusted as needed. If a different injection volume is used, it should be documented in the case record. The same injection volume must be used for entire sequence. Utilize “TX-34.lcm” method.

NOTE: Copies of the method may be renamed and saved with the batch data before or after analysis. The name shall still include “TX-34” at a minimum.

6.7 INSTRUMENTAL PARAMETERS

Gradient Elution

Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Initial Composition	95% A, 5% B, Total Flow 0.50 mL/min
Hold Time	0.50 minutes (5% B)
0.50 -1.5 min	% B increased to 25%
1.50-3.50 min	% B Increased to 75%
3.00 min	Total Flow 0.65 mL/min

3.50 – 4.10 min	% B Increased to 95%
Hold time	0.66 minutes (95% B)
4.75 min	Total flow 0.75 mL/min
Re-equilibration	0.74 minutes
Column Temp	60°C
Autosampler	
Injection Volume	10 µL, can be adjusted as needed
Sampling Speed	10.0 µL/sec
Cooler Temperature	15°C
Interface DUIS-ESI	
Nebulizing Gas	Nitrogen
Nebulizing Gas Flow	3.0 L/min
Drying Gas	Nitrogen
Drying Gas Flow	20.0 L/min
CID Gas	Argon 230 kPa
DL Temp	300°C
Heat Block Temperature	400°C

6.8 CALCULATIONS

None

6.9 DECISION CRITERIA

Refer to "OSBI FTU Quality Manual – Ensure the Validity of Results" for acceptance criteria.

6.10 NOTES

6.10.1 For limited case samples in which the available specimen volume does not allow for the completion of two tests using 250 µL, a substitution of 100 µL of whole blood mixed with 100 µL of PBS buffer can be made (Refer to step 6.6.1 100 µL DILUTION PREPARATION). Zopiclone is not validated for the diluted extraction and cannot be reported. If this substitution is used, a note will be placed in the case record.

6.10.2 If the criminalist extracts controls first to check for acceptance, they must extract a negative control along with the case specimens. The instrument parameters should remain the same between the analysis and acceptance of the controls and the analysis of the negative control and case specimens (i.e., the instrument remains set to the TX34 method used for analysis of the controls until all analysis is complete). This should be utilized for batches that include a limited specimen case(s) only.

6.10.3 A packet containing original data for all controls and standards will be prepared for each analytical run and stored with the batch in the Laboratory Information Management System (LIMS).

6.10.4 Due to the fluctuation and frequency of these compounds, it may not be necessary to include all of the analytes in the control solutions. The exclusion of any analytes

should be based on drug trends, how often they are reported, and when they were last reported.

6.10.5 Caffeine was not validated and cannot be reported from this analysis.

6.11 REPORT WORDING

For reporting guidelines refer to "OSBI FTU Quality Manual - Reporting Results".

6.12 REFERENCES

6.12.1 Applications of LC-MS in Toxicology, ed. Aldo Polettini, (2006).

6.12.2 Analysis of Benzodiazepines in Blood by LC/MS/MS, Agilent Technologies, (2006).

6.12.3 The Mass Spectrometry Primer, Michael P. Balough, (2009).

Table 6-1 Analytical data for each of the compounds in the LC/MS/MS database

	Compound (Primary Stds)	Precursor ion	MRM Transitions	µL of 1° Std to Make 100 mL of 2° Standard	Working High Positive Control Solution Conc. (ng/mL)
1	6-Acetylmorphine	328.1	165,211.2	10	100
2	Alprazolam	309	281,205	20	200
3	Amitriptyline	278.4	105,233.05	50	500
4	Amphetamine	136.2	91.1,65.1,119.1	20	200
5	Benzoylecgonine	290	168,105	100	1000
6	Caffeine	195	138,42,110	100	1000
7	Carisoprodol	261	97.1,158.2	250	2500
8	Chlordiazepoxide	299.9	227.1,283.15	100	1000
9	Clonazepam	316	270,214	20	200
10	Cocaine	304	182.05,81.95,105.1	20	200
11	Codeine	300.1	165.15,198.8	20	200
12	Cyclobenzaprine	276.05	215.1,84.1	20	200
13	Dextromethorphan	272.15	147.05,171.05	40	400
14	Diazepam	285	154,193	40	400
15	Diphenhydramine	256	167.1,165.1,152.1	50	500
16	Ethylone	222.2	173.95,204,146	40	400
17	Flunitrazepam	314	268,239	20	200
18	Flurazepam	387.9	315,288	20	200
19	Hydrocodone	300.1	198.85,171	20	200
20	Lorazepam	321	275,229	20	200
21	MDMA	194	163.1,105.15,77.1	40	400
22	Meprobamate	219.1	158.15,97.15	250	2500
23	Methadone	310.2	265.15,105,57.1	40	400
24	Methamphetamine	150.2	91.1,119.1	20	200
25	Methamphetamine-d8 (IS)	158	92.95,124		

26	Methiopropamine	156.2	96.95,58,125.1	20	200
27	Methylone	208	160.05,132.05,190.05	40	400
28	Midazolam	326	291,244	100	1000
29	N-Desmethyltramadol	250	44	40	400
30	Nordiazepam	270.9	140,165	40	400
31	Nortriptyline	264.15	233.05,91,218	50	500
32	Oxazepam	287	241,269	40	400
33	Oxycodone	316.1	212,174.9	20	200
34	PCP-d5 (IS)	249.3	86.15		
35	Phencyclidine	244.2	86.2,91.15	20	200
36	Phentermine	150.1	91,133.1	40	400
37	Prazepam	325	271.05,140	20	200
38	Prazepam-d5 (IS)	330	276,140		
39	Temazepam	301.2	255,283	40	400
40	Tramadol	264.3	58.15	40	400
41	Trazodone	372.1	176.2,148.05,78.15	50	500
42	Zolpidem	308	235, 263	20	200
43	Zopiclone	389.1	244.9,216.85,139	100	1000

7. TX38 SYNTHETIC CANNABINOIDS BY LC TANDEM MS

7.1 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification and confirmation of select synthetic cannabinoids present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

7.2 SPECIMENS

7.2.1 Specimens include whole blood.

7.2.2 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist determines that less than half of the standard specimen volume should be used or does not wish to complete testing using the standard specimen volume prior to dilution, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

7.2.3 Analysis of larger specimen volumes must be approved by the FTU Technical Manager or designee and documented prior to analysis.

7.3 REAGENTS AND SOLUTIONS

7.3.1 0.1% Formic Acid in Acetonitrile (Mobile Phase B)

7.3.2 0.1% Formic Acid in Water (Mobile Phase A)

7.3.3 88% Formic Acid, Reagent Grade

7.3.4 9:1 Hexanes:Ethyl Acetate: Combine 900mL hexanes and 100 mL of ethyl acetate. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

7.3.5 Acetonitrile (ACN), LCMS Reagent Grade

7.3.6 Aqueous Saturated Sodium Bicarbonate

7.3.7 Aqueous Saturated Sodium Chloride

7.3.8 Bovine, Synthetic, or Human, Drug-Free Blood

7.3.9 Deionized Water (DI Water)

7.3.10 Ethyl Acetate, Reagent Grade

7.3.11 Hexanes, Reagent Grade

7.3.12 Methanol, LCMS Reagent Grade

7.3.13 Reconstitution Solvent, 1:3 DI Water:ACN: Combine 10 mL DI water and 30 mL of ACN. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

7.4 STANDARDS AND CONTROLS

7.4.1 **Spice Internal Standard (ISTD)**

Primary Standards: Refer to Table 7-1. Stability and storage determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

Spice Working Internal Standard Solution (100 ng/mL): Transfer 10 µL of the deuterated primary internal standard (100 µg/mL) into a 100 mL volumetric flask and dilute to mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

QC Check: Successful control verification

7.4.2 **Spice Low Positive Control (LPC)**

Primary Standards: Refer to Table 7-1. Stability and storage determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

Spice Secondary Low Positive Control Solution (1000 ng/mL): Transfer 10 µL of each 1 mg/mL or 100 µL of each 100 µg/mL primary standard into a 10 mL volumetric flask and dilute to mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

Spice Working Low Positive Control Solution (10 ng/mL): Transfer 100 µL of the secondary low positive control solution to a 10 mL volumetric flask and dilute to mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

7.4.3 **Spice High Positive Control (HPC)**

Primary Standards: Refer to Table 7-1. Stability and storage determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

Spice Secondary High Positive Control Solution (2000 ng/mL): Transfer 20 µL of each 1 mg/mL or 200 µL of each 100 µg/mL primary standard into a 10 mL volumetric flask and dilute to mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

Spice Working High Positive Control Solution (200 ng/mL): Transfer 1 mL of secondary high positive control solution to a 10 mL volumetric flask and dilute to

mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

7.4.4 **Negative Control (NC)**

Drug-free whole blood.

7.5 SUPPLIES AND EQUIPMENT

7.5.1 Autosampler Vials with Inserts and Caps

7.5.2 Centrifuge

7.5.3 Column: Kinetex C18 2.6 µm x 2.1 mm x 75 mm

7.5.4 Computer

7.5.5 Disposable Culture Tubes with Culture Tube Closures

7.5.6 Disposable Pasteur Pipettes

7.5.7 Five mL Disposable Conical Centrifuge Tubes with PTFE Lined Screw Caps

7.5.8 Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips

7.5.9 Laminar Flow Hood with HEPA Filter (or Equivalent)

7.5.10 Nitrogen Evaporator (N-Evap)

7.5.11 Rotary Mixer

7.5.12 Shimadzu 8030 LCMS, Argon, Nitrogen Generator

7.5.13 Volumetric Flasks

7.5.14 Vortexer

7.6 SAMPLE PREPARATION

7.6.1 Label two clean disposable culture tubes, one conical centrifuge tube, and one autosampler vial for each control and case sample.

7.6.2 Prepare the low positive control by adding 20 µL of working low positive control solution to 1 mL of drug-free whole blood in the low positive control culture tube.

7.6.3 Prepare the high positive control by adding 20 µL of working high positive control solution to 1 mL of drug-free whole blood in the high positive control culture tube.

7.6.4 Add 1 mL of drug-free whole blood into the negative control culture tube.

7.6.5 Add 1 mL of each case specimen to the appropriately labeled culture tubes.

7.6.6 Add 10 µL of the 100 ng/mL working internal standard solution to each control and case sample and vortex.

7.6.7 Transfer 500 µL of each control and case specimen to clean, appropriately labeled culture tubes.

7.6.8 Add 200 µL of saturated sodium bicarbonate to each culture tube and vortex.

7.6.9 Add 200 µL of saturated sodium chloride to each culture tube and vortex.

7.6.10 Add 3 mL of 9:1 hexanes:ethyl acetate solution to each culture tube.

7.6.11 Cap tubes and rotary mix for 20 minutes.

7.6.12 Centrifuge for approximately 5 minutes at 2800 – 3000 rpm.

NOTE: Centrifuge step may be repeated if insufficient separation occurs and will be documented in the case record.

7.6.13 Transfer organic (top) layer to a clean, appropriately labeled conical centrifuge tubes.

7.6.14 Evaporate to dryness at approximately 40°C with a steady stream of nitrogen.

7.6.15 Add 100 µL of reconstitution solvent to each conical.

7.6.16 Vortex briefly and centrifuge to collect liquid in bottom of conical.

7.6.17 Transfer liquid to appropriately labeled autosampler vials with insert and cap.

7.6.18 Centrifuge at 2800 – 3000 rpm as needed.

7.6.19 Begin each run with the following sequence: low positive control, high positive control, negative control.

7.6.20 Inject 10 to 20 µL of sample, injection volume may be adjusted as needed. If a different injection volume is used, it should be documented in the case record. The same injection volume must be used for entire sequence. Utilize “SPICE.lcm” method.

NOTE: Copies of the method may be renamed and saved with the batch data before or after analysis. The name shall still include “SPICE” at a minimum.

7.7 INSTRUMENTAL PARAMETERS

Gradient Elution	
Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Initial Composition	70% A, 30% B, Total Flow 0.65 mL/min
0.10 – 4.00 min	% B increased to 95%
Hold time	0.75 minutes (95% B)
Re-equilibration	0.74 minutes
Column Temp	60°C
Autosampler	
Injection Volume	10 - 20 µL, can be adjusted as needed
Sampling Speed	1.0 µL/sec
Cooler Temperature	15°C

Interface DUIS-ESI

Nebulizing Gas	Nitrogen
Nebulizing Gas Flow	3.0 L/min
Drying Gas	Nitrogen
Drying Gas Flow	12.5 L/min
CID Gas	Argon 230 kPa
DL Temp	250°C
Heat Block Temperature	400°C

7.8 CALCULATIONS

None

7.9 DECISION CRITERIA

Refer to "OSBI FTU Quality Manual – Ensuring the Validity of Results" for acceptance criteria.

Table 7-1 Precursor & Product Ions for Respective Compounds

Compound	Precursors	MRM Transitions
AM1248	391.25	135.15, 112.10, 98.05
AB-Fubinaca	368.90	109.10, 252.90, 324.10
AB-Pinaca	330.90	215.10, 144.95, 314.50
AB Chminaca	357.20	145.00, 241.05
ADB Pinaca	345.20	215.00,328.25, 299.90
MAB-Chminaca	371.00	354.20, 326.20, 241.00
5-Fluoro-AMB	364.20	304.10, 145.00, 233.00
5-Fluoro-ADB	378.00	318.00,233.40, 212.90
FUB-PB-22	397.10	252.30, 108.95
AM2201	360.30	155.15, 127.15, 232.20
AM2201-d5	365.30	127.10, 155.10
PB-22	358.95	214.10, 144.05, 116.05
MAM2201	374.10	141.20, 169.00, 232.20
JWH-250	336.30	121.15, 91.10, 144.15
JWH-073	328.20	155.05, 127.10, 200.15
XLR11	330.20	144.00,125.15, 232.20
JWH-018	342.30	155.15, 127.05,214.00
JWH-018-d9	351.30	155.05, 127.20
JWH-081	372.10	185.15, 214.15, 157.15
JWH122-d9	365.20	168.90, 141.05
JWH122	356.30	169.05, 141.15,214.00
UR-144	312.20	124.75, 214.10, 144.10
JWH-210	369.90	183.10, 214.10, 155.00

7.10 NOTES

7.10.1 If the criminalist extracts controls first to check for acceptance, they must extract a negative control along with the case specimens. The instrument parameters should remain the same between the analysis and acceptance of the controls and the

analysis of the negative control and case specimens (i.e., the instrument remains set to the TX38 method used for analysis of the controls until all analysis is complete). This should be utilized for batches that include a limited specimen case(s) only.

7.10.2 A packet containing original data for all controls and standards will be prepared for each analytical run and stored with the batch in the Laboratory Information Management System (LIMS).

7.10.3 Due to the fluctuation and frequency of these compounds, it may not be necessary to include all of the analytes in the control solutions. The exclusion of any analytes should be based on drug trends, how often they are reported, and when they were last reported.

7.11 REPORT WORDING

For reporting guidelines refer to “OSBI FTU Quality Manual – Reporting Results”.

7.12 REFERENCES

7.12.1 SOFT / AAFS Forensic Laboratory Guidelines – 2006.

7.12.2 Applications of LC-MS in Toxicology, ed. Aldo Polettini, (2006).

7.12.3 Development and Validation of a Liquid Chromatography-Tandem Mass Spectrometry Method for the Identification and Quantification of JWH-018, JWH-073, JWH-019, and JWH-250 in Human Whole Blood, J Anal Toxicol (2011) 35 (7): 386-393. Doi: 10.1093/anatox/35.7.386.

8. TX39 BENZODIAZEPINES, COCAINE, BE AND ZOLPIDEM BY LC TANDEM MS

8.1 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification and confirmation of select benzodiazepines, cocaine/BE, chlordiazepoxide, and zolpidem present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

8.2 SPECIMENS

8.2.1 Specimens include whole blood.

8.2.2 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist determines that less than half of the standard specimen volume should be used or does not wish to complete testing using the standard specimen volume prior to dilution, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

8.2.3 Analysis of larger specimen volumes must be approved by the FTU Technical Manager or designee and documented prior to analysis.

8.3 REAGENTS AND SOLUTIONS

8.3.1 0.1% Formic Acid in Acetonitrile (Mobile Phase B)

8.3.2 0.1% Formic Acid in Water (Mobile Phase A)

8.3.3 88% Formic Acid, Reagent Grade

8.3.4 Acetonitrile (ACN), LCMS Reagent Grade

8.3.5 Bovine, Synthetic, or Human, Drug-Free Blood

8.3.6 Deionized Water (DI Water)

8.3.7 Methanol, LCMS Reagent Grade

8.3.8 Reconstitution Solvent, 9:1 DI Water: ACN with 0.088% Formic Acid: Combine 5 mL of acetonitrile and 45 mL DI water and 50 µL of formic acid. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

8.4 STANDARDS AND CONTROLS

8.4.1 **TX39 Internal Standard (ISTD)**

Primary Standard (100 µg/mL): Prazepam-d5 reference standard. Stability and storage determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX39 Secondary Internal Standard Solution (1000 ng/mL): Transfer 100 µL of the 100 µg/mL Prazepam-d5 ISTD into a 10 mL volumetric flask and dilute to mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

TX39 Working Internal Standard Solution (10 ng/mL): Transfer 100 µL of the secondary internal standard solution to a 10 mL volumetric flask and dilute to mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

QC Check: Successful control verification

8.4.2 **TX39 Low Positive Control (LPC)**

Primary Standards: Refer to Table 8-1. Stability and storage determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX39 Secondary Low Positive Control Solution (2000 ng/mL): Transfer 20 µL of each 1 mg/mL or 200 µL of each 100 µg/mL primary standard into a 10 mL volumetric flask and dilute to mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

TX39 Working Low Positive Control Solution (50 ng/mL): Transfer 250 µL of the secondary low positive control solution to a 10 mL volumetric flask and dilute to mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

8.4.3 **TX39 High Positive Control (HPC)**

Primary Standards: Refer to Table 8-1. Stability and storage determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX39 Secondary High Positive Control Solution (4000 ng/mL): Transfer 40 µL of each 1 mg/mL or 400 µL of each 100 µg/mL primary standard into a 10 mL volumetric flask and dilute to mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

TX39 Working High Positive Control Solution (500 ng/mL): Transfer 1.250 mL of the secondary high positive control solution to a 10 mL volumetric flask and dilute to mark with methanol. Store in the freezer. Total volume may be adjusted as necessary, but the final concentration must remain the same.

8.4.4 **Negative Control (NC)**

Drug-free whole blood.

8.5 SUPPLIES AND EQUIPMENT

8.5.1 Autosampler Vials with Inserts and Caps

8.5.2 Centrifuge

8.5.3 Column: Kinetex C18 2.6 µm x 2.1 mm x 75 mm

8.5.4 Computer

8.5.5 Disposable Microcentrifuge Tubes

8.5.6 Disposable Pasteur Pipettes

8.5.7 Filter Autosampler Vials

8.5.8 Five or Ten mL Disposable Conical Centrifuge Tubes with PTFE Lined Screw Caps

8.5.9 Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips

8.5.10 Laminar Flow Hood with HEPA Filter (or Equivalent)

8.5.11 Microcentrifuge

8.5.12 Nitrogen Evaporator (N-Evap)

8.5.13 Shimadzu 8030 and 8050 LCMS, Argon, Nitrogen Generator

8.5.14 Volumetric Flasks

8.5.15 Vortexer

8.6 SAMPLE PREPARATION

8.6.1 Label a clean, disposable microcentrifuge tube, conical centrifuge tube, and autosampler vial for each control and case sample.

8.6.2 Prepare the low positive control by adding 25 µL of working low positive control solution to 225 µL of drug-free whole blood in the low positive control microcentrifuge tube.

8.6.3 Prepare the high positive control by adding 25 µL of working high positive control solution to 225 µL of drug-free whole blood in the high positive control microcentrifuge tube.

8.6.4 Add 250 µL of drug-free whole blood into the negative control microcentrifuge tube.

8.6.5 Add 250 µL of each case specimen to the appropriately labeled microcentrifuge tubes.

8.6.6 Add 25 µL of working internal standard solution to each microcentrifuge tube and vortex.

8.6.7 Add 500 µL of acetonitrile to each microcentrifuge tube and vortex for approximately 30 seconds.

8.6.8 Centrifuge for approximately 5 minutes at approximately 13,000 rpm.

NOTE: Centrifuge step may be repeated if insufficient separation occurs and will be documented in the case record.

8.6.9 Transfer organic (top) layer to the appropriately labeled conical centrifuge tube.

8.6.10 Evaporate to dryness at approximately 40°C with a steady stream of nitrogen.

8.6.11 Add 100 µL of reconstitution solvent to each conical.

8.6.12 Vortex briefly and centrifuge to collect liquid in bottom of conical.

8.6.13 Transfer liquid to the appropriately labeled autosampler vial. Filter autosampler vials may be used instead.

8.6.14 Centrifuge at 2800 – 3000 rpm as needed.

8.6.15 Begin each run with the following sequence: low positive control, high positive control, negative control.

8.6.16 Inject 10 µL of sample on Shimadzu 8030 or 5 µL of sample on Shimadzu 8050, injection volume may be adjusted as needed. If a different injection volume is used, it should be documented in the case record. The same injection volume must be used for entire sequence. Utilize "BENZOS.lcm" method.

NOTE: Copies of the method may be renamed and saved with the batch data before or after analysis. The name shall still include "BENZOS" at a minimum.

8.7 INSTRUMENTAL PARAMETERS

Gradient Elution	
Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Initial Composition	90% A, 10% B, Total Flow 0.65 mL/min
0.10 – 2.5 min	% B increased to 75%
2.5 – 2.75 min	% B increased to 95%
Hold time	1 minute (95% B)
Re-equilibration	0.99 minutes
Column Temp	60°C
Autosampler	
Injection Volume	10 µL on Shimadzu 8030 or 5 µL on Shimadzu 8050, can be adjusted as needed
Sampling Speed	5.0 µL/sec
Cooler Temperature	15°C
Measuring Line Purging	3.0 minutes
Interface DUIS-ESI	
Nebulizing Gas	Nitrogen
Nebulizing Gas Flow	1.2 L/min
Drying Gas	Nitrogen

Drying Gas Flow	20.0 L/min
CID Gas	Argon 230 kPa
DL Temp	250°C
Heat Block Temperature	300°C

8.8 CALCULATIONS

None

8.9 DECISION CRITERIA

Refer to "OSBI FTU Quality Manual – Ensuring the Validity of Results" for acceptance criteria.

Table 8-1 Precursor & Product Ions for Respective Compounds

Compound	Precursors	MRM Transitions
Benzoylecgonine	290.00	168.00, 105.00
Cocaine	304.00	182.05, 81.95,105.10
Zolpidem	308.00	235.00, 263.00
Chlordiazepoxide	299.90	227.10, 283.15
Midazolam	326.00	291.00, 244.00
Flurazepam	387.90	315.00, 288.00
Oxazepam	287.00	241.00, 269.00
Clonazepam	316.00	270.00, 214.00
Lorazepam	321.00	275.00, 229.00
Nordiazepam	270.90	140.00, 165.00
Alprazolam	309.00	281.00, 205.00
Flualprazolam	327.00	292.00, 299.00, 223.00
Flunitrazepam	314.00	268.00, 239.00
Temazepam	301.20	255.00, 283.00
Diazepam	285.00	154.00, 193.00
Prazepam	325.00	271.05, 140.00
Prazepam-d5	330.00	276.00, 140.00
Bromazepam	315.90	182.10
Estazolam	295.00	266.80, 205.00
Lormetazepam	335.00	289.10
Nimetazepam	295.90	250.30,221.10,165.10
Nitrazepam	282.10	179.90,236.40
Phenazepam	350.80	205.90,179.10
Triazolam	343.00	307.90,314.90
Delorazepam	304.90	139.90,241.10
Diclazepam	318.90	153.80,226.90,256.00
Flubromazepam	332.90	226.00,183.90,206.00
Etizolam	343.05	313.90,137.95

8.10 NOTES

8.10.1 If the criminalist extracts controls first to check for acceptance, they must extract a negative control along with the case specimens. The instrument parameters should remain the same between the analysis and acceptance of the controls and the analysis of the negative control and case specimens (i.e., the instrument remains set to the TX39 method used for analysis of the controls until all analysis is complete). This should be utilized for batches that include a limited specimen case(s) only.

8.10.2 A packet containing original data for all controls and standards will be prepared for each analytical run and stored with the batch in the Laboratory Information Management System (LIMS).

8.10.3 Due to the fluctuation and frequency of these compounds, it may not be necessary to include all of the analytes in the control solutions. The exclusion of any analytes should be based on drug trends, how often they are reported, and when they were last reported.

8.11 REPORT WORDING

For reporting guidelines refer to “OSBI FTU Quality Manual - Reporting Results”.

8.12 REFERENCES

8.12.1 Applications of LC-MS in Toxicology, ed. Aldo Polettini, (2006).

8.12.2 Analysis of Benzodiazepines in Blood by LC/MS/MS, Agilent Technologies, (2006).

8.12.3 The Mass Spectrometry Primer, Michael P. Balough, (2009).

9. TX40 OPIATES BY LC TANDEM MS

9.1 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification and confirmation of select opiates present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

9.2 SPECIMENS

9.2.1 Specimens include whole blood.

9.2.2 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist determines that less than half of the standard specimen volume should be used or does not wish to complete testing using the standard specimen volume prior to dilution, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

9.2.3 Analysis of larger specimen volumes must be approved by the FTU Technical Manager or designee and documented prior to analysis.

9.3 REAGENTS AND SOLUTIONS

9.3.1 0.1% Formic Acid in Acetonitrile (Mobile Phase B)

9.3.2 0.1% Formic Acid in Water (Mobile Phase A)

9.3.3 88% Formic Acid, Reagent Grade

9.3.4 Acetonitrile (ACN), LCMS Reagent Grade

9.3.5 Bovine, Synthetic, or Human, Drug-Free Blood

9.3.6 Deionized Water (DI Water)

9.3.7 Methanol, LCMS Reagent Grade

9.3.8 Reconstitution Solvent, DI Water

9.4 STANDARDS AND CONTROLS

9.4.1 **TX40 Internal Standard (ISTD)**

Primary Standards: Refer to Table 9-1. Stability and storage determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX40 Working Internal Standard Solution (10 ng/mL): Transfer 25 µL of each 100 µg/mL deuterated primary internal standard into a 250 mL volumetric flask and dilute to mark with ACN. Store in freezer. Total volume may be adjusted as needed, but the final concentration must remain the same.

QC Check: Successful control verification

9.4.2 TX40 Low Positive Control (LPC)

Primary Standards: Refer to Table 9-1. Stability and storage determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX40 Secondary Low Positive Control Solution (1000:250:50 ng/mL): Transfer 100 µL of each 1 mg/mL primary standard for codeine, hydrocodone, morphine, oxycodone, and hydromorphone and 25 µL of each 1 mg/mL primary standard for oxymorphone and 6-acetylmorphine into a 100 mL volumetric flask and dilute to mark with DI water. Store in refrigerator. Total volume may be adjusted as needed, but the final concentration must remain the same.

TX40 Working Low Positive Control Solution (50:12.5:2.5 ng/mL): Transfer 500 µL of the secondary low positive control solution to a 10 mL volumetric flask and dilute to mark with DI water [Note: oxymorphone and 6-MAM will be 12.5 ng/mL]. Store in the refrigerator. Total volume may be adjusted as needed, but the final concentration must remain the same.

9.4.3 TX40 High Positive Control (HPC)

Primary Standards: Refer to table 10-1. Stability and storage determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX40 Secondary High Positive Control Solution (2000:500:100 ng/mL): Transfer 200 µL of each 1 mg/mL primary standard for codeine, hydrocodone, morphine, oxycodone, and hydromorphone, and 50 µL of each 1 mg/mL primary standard for oxymorphone and 6-acetylmorphine into a 100 mL volumetric flask and dilute to mark with DI water. Store in refrigerator. Total volume may be adjusted as needed, but the final concentration must remain the same.

TX40 Working High Positive Control Solution (1000:250:50 ng/mL): Transfer 5.0 mL of the secondary high positive control solution to a 10 mL volumetric flask and dilute to mark with DI water. Store in refrigerator. Total volume may be adjusted as needed, but the final concentration must remain the same.

9.4.4 Negative Control (NC)

Drug-free whole blood.

9.5 SUPPLIES AND EQUIPMENT

9.5.1 Autosampler Vials with Inserts and Caps

9.5.2 Centrifuge

9.5.3 Column: Kinetex C18 2.6 µm x 2.1 mm x 75 mm

9.5.4 Computer

9.5.5 Disposable Culture Tubes with Culture Tube Closures

9.5.6 Disposable Pasteur Pipettes

9.5.7 Filter Autosampler Vials

9.5.8 Five or Ten mL Disposable Conical Centrifuge Tubes with PTFE Lined Screw Caps

9.5.9 Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips

9.5.10 Laminar Flow hood with HEPA Filter (or Equivalent)

9.5.11 Nitrogen Evaporator (N-Evap)

9.5.12 Shimadzu 8030 LCMS, Argon, Nitrogen Generator

9.5.13 Volumetric Flasks

9.5.14 Vortexer

9.6 SAMPLE PREPARATION

9.6.1 Label a clean disposable culture tube, conical centrifuge tube, and autosampler vial for each control and case sample.

9.6.2 Prepare the low positive control by adding 100 µL of working low positive control solution and 400 µL of drug-free whole blood to the low positive control culture tube.

9.6.3 Prepare the high positive control by adding 100 µL of working high positive control solution and 400 µL of drug-free whole blood to the high positive control culture tube.

9.6.4 Add 500 µL of drug-free whole blood to the negative control culture tube.

9.6.5 Add 500 µL of each case specimen to the appropriately labeled culture tubes.

9.6.6 Add 1 mL of cold working internal standard solution and vortex for approximately 30 seconds.

9.6.7 Centrifuge for approximately 5 minutes at 2800 – 3000 rpm.

NOTE: Centrifuge step may be repeated if insufficient separation occurs and will be documented in the case record

9.6.8 Transfer acetonitrile (top) layer to appropriately labeled conical centrifuge tube.

9.6.9 Evaporate to dryness at approximately 40°C with a steady stream of nitrogen.

9.6.10 Add 100 µL of reconstitution solvent to each conical.

9.6.11 Vortex briefly and centrifuge to collect liquid in bottom of conical.

9.6.12 Transfer liquid to appropriately labeled autosampler vials. Filter autosampler vials may be used instead.

9.6.13 Centrifuge at 2800 – 3000 rpm as needed.

9.6.14 Begin each run with the following sequence: low positive control, high positive control, negative control.

9.6.15 Inject 5 µL of sample, injection volume may be adjusted as needed. If a different injection volume is used, it should be documented in the case record. The same injection volume must be used for entire sequence. Utilize "Opiates.lcm" method.

NOTE: Copies of the method may be renamed and saved with the batch data before or after analysis. The name shall still include "Opiates" at a minimum.

9.7 INSTRUMENTAL PARAMETERS

Gradient Elution	
Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Initial Composition	95% A, 5% B, Total Flow 0.35 mL/min
0.10 – 2.5 min	% B increased to 50%
2.5 – 3.25 min	% B increased to 95%
Hold time	2.20 minutes (95% B)
Re-equilibration	1.55 minutes
Column Temp	30°C
Autosampler	
Injection Volume	5 µL, can be adjusted as needed
Sampling Speed	5.0 µL/sec
Cooler Temperature	15°C

Interface DUIS-ESI	
Nebulizing Gas	Nitrogen
Nebulizing Gas Flow	2.0 L/min
Drying Gas	Nitrogen
Drying Gas Flow	15.0 L/min
CID Gas	Argon 230 kPa
DL Temp	250°C
Heat Block Temperature	400°C

9.8 CALCULATIONS

None

9.9 DECISION CRITERIA

Refer to "OSBI FTU Quality Manual – Ensuring the Validity of Results" for acceptance criteria.

Table 9-1 Precursor & Product Ions for Respective Compounds

Compound	Precursors	MRM Transitions
Morphine-d6	292.10	153.00
Morphine	286.10	165.20,153.15
Oxymorphone	302.10	284.15,226.80
Hydromorphone	286.10	184.70,157.15
Codeine-d6	306.10	218.00
Codeine	300.10	165.15,198.80
Oxycodone	316.10	212.00,174.90
6-MAM	328.10	165.00,211.20
Hydrocodone-d6	306.10	202.05
Hydrocodone	300.10	198.85, 171.00
Fentanyl	337.20	188.00,105.00

9.10 NOTES

9.10.1 If the criminalist extracts controls first to check for acceptance, they must extract a negative control along with the case specimens. The instrument parameters should remain the same between the analysis and acceptance of the controls and the analysis of the negative control and case specimens (i.e., the instrument remains set to the TX40 method used for analysis of the controls until all analysis is complete). This should be utilized for batches that include a limited specimen case(s) only.

9.10.2 A packet containing original data for all controls and standards will be prepared for each analytical run and stored with the batch in the Laboratory Information Management System (LIMS).

9.10.3 Due to the fluctuation and frequency of these compounds, it may not be necessary to include all of the analytes in the control solutions. The exclusion of any analytes should be based on drug trends, how often they are reported, and when they were last reported.

9.11 REPORT WORDING

For reporting guidelines refer to "OSBI FTU Quality Manual - Reporting Results".

9.12 REFERENCES

9.12.1 Applications of LC-MS in Toxicology, ed. Aldo Polettini, (2006).

The Mass Spectrometry Primer, Michael P. Balough, (2009).

10. TX42 FENTANYL AND FENTANYL ANALOG ANALYSIS BY LC TANDEM MS

10.1 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification and confirmation of fentanyl and fentanyl analogs present in biological specimens. This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

10.2 SPECIMENS

10.2.1 Specimens include whole blood and urine.

10.2.2 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist determines that less than half of the standard specimen volume should be used or does not wish to complete testing using the standard specimen volume prior to dilution, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

10.2.3 Analysis of larger specimen volumes must be approved by the FTU Technical Manager or designee and documented prior to analysis.

10.3 REAGENTS AND SOLUTIONS

10.3.1 0.1% Formic Acid in Acetonitrile (Mobile Phase B)

10.3.2 0.1% Formic Acid in Deionized (DI) Water (Mobile Phase A)

10.3.3 88% Formic Acid, Reagent Grade

10.3.4 Acetonitrile (ACN), LCMS Reagent Grade

10.3.5 Bovine, Synthetic, or Human, Drug-Free Blood

10.3.6 Concentrated Ammonium Hydroxide (NH_4OH), LCMS Reagent Grade

10.3.7 Elution Solvent – 39:10:1 Ethyl Acetate:ACN:Ammonium Hydroxide. Combine 39 mL of ethyl acetate, 10 mL of acetonitrile, and 1 mL of ammonium hydroxide. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same. Make day of use.

10.3.8 Ethyl Acetate (EA) - approximately 99.9%, LCMS Reagent Grade

10.3.9 Immunalysis Corp. Synthetic Urine – document ELISA kit lot number in reagents

10.3.10 Methanol - >99.99%, LCMS Reagent Grade

10.3.11 Reconstitution Solvent, 50:50 Mobile Phase A: Mobile Phase B

10.4 STANDARDS AND REAGENTS

10.4.1 **TX42 Internal Standard**

Primary Standards (1mg/mL): Beta-hydroxythio-Fentanyl - $^{13}C_6$ and Fentanyl -$^{13}C_6$. Storage conditions and stability determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX42 Secondary Internal Standard Solution (5 µg/mL): Place 2 mL of Mobile Phase A into a twist cap autosampler vial or a culture tube. Remove the amount of liquid that will be added by the addition of the internal standards. For example, if the carbon-13 labeled primary internal standards are at a concentration of 1 mg/mL, remove 20 µL and add 10 µL of each internal standard. If either primary internal standard is in a 100 µg/mL formulation, add 100 µL. If the primary internal standard concentration is 50 µg/mL, add 200 µL. Remove the appropriate amount of liquid, before the addition of either internal standard, so that the final volume is 2 mL. Store in refrigerator. Total volume may be adjusted as needed, but the final concentration must remain the same.

TX42 Working Internal Standard Solution (1 ng/mL): Transfer 10 µL of secondary internal standard solution to a 50 mL volumetric flask and dilute to mark with Mobile Phase A. Store in refrigerator. Total volume may be adjusted as needed, but the final concentration must remain the same.

10.4.2 TX42 High Positive Control (HPC)

Primary Standards (1mg/mL, 100 µg/mL, and 50 µg/mL): See Table 10-2. Storage conditions and stability determined by the manufacturer. Other primary standard concentrations may be used, but the working solution concentration must remain the same.

TX42 Secondary High Positive Control Solution (5:1 µg/mL): Transfer the appropriate amount of each 1 mg/mL primary standard as show in Table 10-2 to a 10 mL volumetric flask and dilute to mark with 50:50 mobile phase A:mobile phase B. Store in refrigerator. Total volume may be adjusted as needed, but the final concentration must remain the same.

TX42 Tertiary High Positive Control Solution (500:100 ng/mL): Transfer 1 mL of secondary high positive control solution, 50 µL of each 100 µg/mL primary standard for sufentanil, alpha-methyl fentanyl, and 4-FIBF, and 100 µL of each 50 µg/mL primary standard for acetyl fentanyl to a 10 mL volumetric flask and dilute to mark with 50:50 mobile phase A:mobile phase B. Store in refrigerator. Total volume may be adjusted as needed, but the final concentration must remain the same.

TX42 Working High Positive Control Solution (50:10 ng/mL): Transfer 1 mL of tertiary high positive control to a 10 mL volumetric flask and dilute to mark with 50:50 mobile phase A:mobile phase B. Store in refrigerator. Total volume may be adjusted as needed, but the final concentration must remain the same.

10.4.3 TX42 Low Positive Control (LPC)

TX42 Working Low Positive Control Solution (5:1 ng/mL): Transfer 1 mL of working high positive control solution to a 10 mL volumetric flask and dilute to mark with 50:50 mobile phase A:mobile phase B. Store in refrigerator.

10.4.4 **Negative Control (NC)**

Appropriate drug-free matrix

10.5 SUPPLIES AND EQUIPMENT

10.5.1 Autosampler Vials with Inserts and Caps

10.5.2 Biotage EVOLUTE EXPRESS CX30 mg/1mL Solid Phase Extraction (SPE) Cartridges

10.5.3 Centrifuge

10.5.4 Column: Restek Raptor Biphenyl 100 x 2.1 mm and 2.7 µm particle size.

10.5.5 Computer

10.5.6 Disposable Culture Tubes with Culture Tube Closures

10.5.7 Disposable Micro-centrifuge Tubes

10.5.8 Disposable Pasteur Pipettes

10.5.9 Five or Ten mL Disposable Conical Centrifuge Tubes with PTFE Lined Screw Caps

10.5.10 Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips

10.5.11 Laminar Flow Hood with HEPA Filter (or Equivalent)

10.5.12 Nitrogen Evaporator (n-Evap)

10.5.13 Positive Pressure Manifold

10.5.14 Shimadzu 8050 LCMS, Argon, Nitrogen Generator

10.5.15 Volumetric Flasks

10.5.16 Vortexer

10.6 SAMPLE PREPARATION

10.6.1 Label a clean, disposable micro-centrifuge tube, culture tube, conical centrifuge tube, and autosampler vial for each control and case sample.

10.6.2 Rotate and thoroughly vortex blood samples before pipetting.

10.6.3 Prepare the low positive control by adding 10 µL of working low positive control solution to 90 µL of appropriate drug-free matrix (whole blood or synthetic urine) in the low positive control micro-centrifuge tube.

10.6.4 Prepare the high positive control by adding 10 µL of working high positive control solution to 90 µL of appropriate drug-free matrix (whole blood or synthetic urine) in the high positive micro-centrifuge tube.

10.6.5 Add 100 µL of the appropriate drug-free matrix (whole blood or synthetic urine) to the negative control micro-centrifuge tube.

10.6.6 Transfer 100 µL of each case specimen to the appropriately labeled micro-centrifuge tubes.

10.6.7 Add 100 µL of working internal standard solution and vortex.

10.6.8 Load each sample onto a separate SPE cartridge previously placed onto the sample plate of the positive pressure manifold.

10.6.9 Apply the necessary pressure to elute the sample into the waste trough, approximately 85 psi for blood (full-flow) and approximately 10-20 psi for urine (regulated-flow).

10.6.10 Wash each cartridge with 1 mL of DI water and elute into the waste trough using the process in 12.6.9.

10.6.11 Wash each cartridge with 1 mL of mobile phase A and elute into the waste trough using the process in 12.6.9.

10.6.12 Wash each cartridge with 1 mL of methanol and elute into the waste trough using the process in 12.6.9.

10.6.13 Dry the cartridges for approximately 1 minute at 20 psi or switch to full-flow.

10.6.14 Replace the waste trough with the sample rack containing appropriately labeled culture tube and elute with 760 µL of elution solvent.

10.6.15 Elute each cartridge again with 760 µL of elution solvent.

10.6.16 Transfer the controls and samples from the culture tubes to clean, appropriately labeled conicals.

10.6.17 Evaporate to dryness at approximately 40°C with a steady stream of nitrogen.

10.6.18 Add 50 µL of reconstitution solvent to each conical.

10.6.19 Vortex briefly and centrifuge to collect the sample in the bottom of the conical.

10.6.20 Transfer the controls and samples to the appropriately labeled autosampler vials.

10.6.21 Centrifuge at 2800-3000 rpm as needed.

10.6.22 Begin each run with the following sequence: low positive control, high positive control, negative control.

10.6.23 Inject 3 µL of sample, injection volume may be adjusted as needed. If a different injection volume is used, it should be documented in the case record. The sample injection volume must be used for the entire sequence. Utilize “TX42.lcm” method.

NOTE: Copies of the method may be renamed and saved with the batch data before or after analysis. The name shall include “TX42” at a minimum.

10.7 INSTRUMENT PARAMETERS

Gradient Elution

Mobile Phase A	0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Initial Composition	95% A, 5% B, Total Flow 0.60 mL/min
0.20 – 3.5 min	% B increased to 50%
3.5 – 4.25 min	% B increased to 95%
Hold time	1.20 minutes (95% B)
5.45 – 6.50 min	% B decreased to 5%
Re-equilibration	
Column Temp	50°C

Autosampler

Injection Volume	3 µL, can be adjusted as needed
Sampling Speed	5.0 µL/sec
Cooler Temperature	15°C

Interface DUIS-ESI

Nebulizing Gas	Nitrogen
Nebulizing Gas Flow	2.0 L/min
Drying Gas	Nitrogen
Drying Gas Flow	15.0 L/min
CID Gas	Argon 230 kPa
DL Temp	250°C
Heat Block Temperature	400°C

10.8 CALCULATIONS

None

10.9 DECISION CRITERIA

10.9.1 See Table 10-1 for minimum requirement for identification for analyte peak area:internal standard peak area ratio comparison to the analyte peak area:internal standard area ratio present in the low positive control. This takes precedence over criteria listed in the OSBI FTU Quality Manual.

10.9.2 Refer to “OSBI FTU Quality Manual – Ensuring the Validity of Results” for additional acceptance criteria.

Table 10-1 Limit of detection for each compound in TX42

Compound	of LPC for Blood	of LPC for Urine
4-ANPP	100%	100%
4-FIBF/PFBF	100%	50%
Acetyl Fentanyl	25%	25%
Acryl Fentanyl	100%	50%
Alpha-Methyl Fentanyl	100%	100%
Butyryl Fentanyl	100%	100%
Cyclopropyl/Crotonyl Fentanyl	25%	25%
Fentanyl	25%	25%
Fluorofentanyl	100%	100%
Furanyl Fentanyl	100%	100%
Methoxy Acetyl Fentanyl	25%	25%
Norfentanyl	100%	100%
Sufentanil	50%	25%
Valeryl Fentanyl	25%	25%

10.10 NOTES

10.10.1 If the criminalist extracts controls only to check for acceptability, they must extract a negative control along with the case samples. The instrument parameters should remain the same between the analysis and acceptance of the controls and the analysis of the negative control and case samples (i.e., the instrument remains set to the TX42 method used for analysis of the controls until all analysis is complete). This should be utilized for batches that include a limited specimen case(s) only.

10.10.2 A packet containing original data for all controls and standards will be prepared for each analytical run and stored with the batch in the Laboratory Information Management System (LIMS).

10.10.3 Due to the fluctuation and frequency of these compounds, it may not be necessary to include all of the analytes in the control solutions. The exclusion of any analytes should be based on drug trends, how often they are reported, and when they were last reported.

10.11 REPORT WORDING

For reporting guidelines, refer to “OSBI FTU Quality Manual – Reporting Results”.

10.12 REFERENCES

10.12.1 Sample Preparation for Fentanyl Analogs in Whole Blood, Biotage, (2019).

Table 10-2 Analytical data for each of the compounds in the LC/MS-MS database

	Compound (Primary Stds)	Precursor ion	MRM Transitions	µL of 1° Std to Make 10 mL of 2° Standard	Working High Positive Control Solution Conc. (ng/mL)
1	Norfentanyl	233.1	84.1, 55.0	50	50
2	4-ANPP	281.0	188.15, 105.2	50	50
3	Acetyl Fentanyl	323.0	105.15, 188.1	--	50
4	Acryl Fentanyl	335.0	188.2, 105.2	10	10
5	Butyryl Fentanyl	351.0	188.1, 105.15	10	10
6	Cyclopropyl*/Crotonyl Fentanyl	349.0	188.15, 105.2	50	50
7	4-FIBF*/PFBF	369.3	188.15, 105.05	--	50
8	Furanyl Fentanyl	375.0	188.1, 105.15	10	10
9	Methoxy Acetyl Fentanyl	353.0	188.1, 105.15	50	50
10	Fluorofentanyl (para- used for std)	355.0	188.15, 105.2	10	10
11	Sufentanil	387.1	238.15, 111.1, 355.05	--	50
12	Valeryl Fentanyl	365.1	188.15, 105.15	50	50
13	Alpha-Methyl Fentanyl	351.3	91.10, 202.0, 119.2	--	50
14	Fentanyl-$^{13}C_6$	343.0	188.2, 105.15	--	--
15	Fentanyl	337.2	188.0, 105.0	50	50
16	Beta-hydroxythio-Fentanyl-$^{13}C_6$	365.0	347.3, 192.05, 110.95	--	--

* Used for standard

11. TX43 VOLATILE ANALYSIS BY HEADSPACE GC-MS

11.1 PURPOSE

This procedure will serve as the laboratory document describing sample preparation, instrumental analysis, data analysis, criteria for acceptance, and reporting of the specified compounds.

11.1.1 The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification and confirmation of ethanol, methanol, and isopropanol in products currently available for sale, new products that are proposed for sale for the Alcoholic Beverage Laws Enforcement (ABLE) Commission, or unknown liquids suspected to contain alcohol.

Most of these cases involve the investigation of minors in possession of alcohol, open intoxicants in vehicles, and illegal sale/distribution of alcohol. These types of cases require the analysis of alcohol content. Any beverage containing greater than 0.5% ethanol by volume is defined as an alcoholic beverage (*O.S. § 37A-1-103*).

11.1.2 The purpose of this standard operating procedure (SOP) is to provide technical direction for the identification and confirmation of select volatile compounds present in biological specimens.

11.2 SPECIMENS

11.2.1 Specimens include whole blood, serum, plasma, urine, and liquids suspected to contain ethanol or other volatile substances.

11.2.2 Dilutions of specimens may be analyzed at the criminalist's discretion and documented; however, this should be done subsequent to testing the standard specimen volume, unless sample quantity dictates otherwise. If the criminalist determines that less than half of the standard specimen volume should be used or does not wish to complete testing using the standard specimen volume prior to dilution, they must receive prior approval from the Forensic Toxicology Unit (FTU) Technical Manager (TM) or designee and document this approval.

11.2.3 Analysis of larger specimen volumes, outside those listed in this SOP, must be approved by the FTU Technical Manager or designee and documented prior to analysis.

11.3 REAGENTS AND SOLUTIONS

11.3.1 Deionized Water (DI Water)

11.3.2 Methanol – approximately 99.99%, ACS Grade

11.4 STANDARDS AND CONTROLS

11.4.1 **N-Propanol Internal Standard (ISTD)**

Primary Standard: n-Propanol, Min 99.5% (density = 0.8 g/mL).

Working n-Propanol (0.020 g/100mL) Internal Standard Solution: Transfer 0.500 mL of n-Propanol to a 2 L volumetric flask. Dilute to mark with DI water for a final concentration of 0.020 g/100 mL. Store at room temperature. Total volume may be adjusted as necessary, but the final concentration must remain the same.

QC Check: Successful calibration verification

11.4.2 Ethanol Calibration Solutions

Standard solutions are purchased from Lipomed or Cerilliant at certified concentrations (0.010, 0.100, 0.200, 0.300, 0.400, and 0.500 g/100mL). Stability determined by manufacturer. While in storage, Ethanol Calibration Solutions will be stored at room temperature unless otherwise determined by the manufacturer, and will be stored in the refrigerator while in use.

11.4.3 Negative Control (NC)

DI water

11.4.4 Ethanol Positive Control (PC)

Purchased from Cerilliant at a certified concentration of 0.080 g/100mL. Store refrigerated at 2-8°C. Stability determined by manufacturer.

11.4.5 Whole Blood (WB) Ethanol Controls (Levels 1 and 2)

Purchased commercially from Cliniqa, catalog # 93211 and 93212. Typical assay ranges are 0.068 to 0.083 g/100mL for Level 1 (Low) and 0.175 to 0.214 g/100mL for Level 2 (High). Store refrigerated at 2-8°C. Stability determined by manufacturer.

11.4.6 Verification Solution

NIST Traceable Ethanol Certified Reference Material Solution 0.150 g/100mL (Cerilliant E-041 or equivalent): to verify functionality of instrument throughout batch run. Store refrigerated at 2-8°C. Stability determined by manufacturer.

11.4.7 Multicomponent Volatile Mix Solution

Primary Standards: ACS Grade Absolute Ethanol 200 proof – approximately 99.5%, Reagent Grade Acetaldehyde – approximately 99.5%, ACS Grade Acetone – approximately 99.6%, ACS Grade Isopropanol – approximately 99.9%, and ACS Grade Methanol – approximately 99.99%.

Multicomponent Volatile Mix Solution: Transfer approximately 125 µL Absolute Ethanol, 40 µL Acetaldehyde, 40 µL Acetone, 75 µL Isopropanol, and 125 µL Methanol, into a 100 mL volumetric flask containing DI water and dilute to mark with DI water. Store tightly capped volatile mix in a refrigerator at 2-8°C. Total volume may be adjusted as necessary, but beginning concentrations and end ratio must remain the same.

The Multicomponent Volatile Mix Solution will be used as the positive control for methanol and isopropanol qualitative identification and does not need to be analyzed a second time if analyzed prior to the calibration curve.

QC Check: When a new volatile mix control is prepared, it will be analyzed and compared to the previous lot number and demonstrate successful separation.

11.4.8 Chloroform Positive Control Solution

Primary Standard: Chloroform – approximately 99.9%, ACS Grade (density = 1.48 g/mL).

Secondary Chloroform Positive Control Solution (740 mg/mL): Combine 100 µL of chloroform and 100 µL of methanol.

Tertiary Chloroform Positive Control Solution (7.4 mg/mL): Transfer 100 µL of Secondary Chloroform Positive Control Solution to a 10 mL volumetric flask and dilute to mark with DI water.

Working Chloroform Positive Control Solution (14.8 µg/mL): Transfer 20 µL of Tertiary Chloroform Positive Control Solution to a 10 mL volumetric flask and dilute to mark with DI water.

Make new for each analytical batch.

11.4.9 Toluene Positive Control Solution

Primary Standard: Toluene – approximately 99.5%, ACS Grade (density = 0.87 g/mL).

Secondary Toluene Positive Control Solution (435 mg/mL): Combine 100 µL of toluene and 100 µL of methanol.

Tertiary Toluene Positive Control Solution (4.35 mg/mL): Transfer 100 µL of Secondary Toluene Positive Control Solution to 10 mL volumetric flask and dilute to mark with DI water.

Working Toluene Positive Control Solution (10.875 µg/mL): Transfer 25 µL of Tertiary Toluene Positive Control Solution to a 10 mL volumetric flask and dilute to mark with DI water.

Make new for each analytical batch.

11.4.10 DFE Positive Control

Primary Standard: 1,1-Difluoroethane, AccuStandard (ALR-CFC-011S-2X) 200 µg/mL (or Equivalent).

Working DFE Positive Control Solution (20 µg/mL): Combine 100 µL of the commercially prepared 1,1-Difluoroethane solution (200 µg/mL) and 900 µL of DI water.

Make new for each analytical batch.

11.5 SUPPLIES AND EQUIPMENT

11.5.1 10- or 20-mL Headspace Vials

11.5.2 20 mm Crimp-on Septa

11.5.3 Agilent Gas Chromatograph (GC)/Flame Ionization Detector (FID)/Mass Spectrometer (MS):

3.5.3.3 FID Column: Agilent 123-9134 DB-ALC1 30 m X 0.32 mm X 1.80 µm (or Equivalent)

3.5.3.4 MS Column: Agilent 122-0733 DB-1701 30 m X 0.25 mm X 1 µm (or Equivalent)

11.5.4 Calibrated Fixed or Adjustable Volume Pipettors with Disposable Pipette Tips

11.5.5 Certified and Calibrated 10 mL Volumetric Flask

11.5.6 Computer

11.5.7 Crimper

11.5.8 Laminar Flow Hood with HEPA Filter (or Equivalent)

11.5.9 Repeat Pipette Dispenser

11.5.10 Tissue Grinder, Glass Vessel

11.5.11 Volumetric Flasks

11.6 SAMPLE PREPARATION

11.6.1 **Calibration and Controls**

11.6.1.1 Quantitation of Ethanol for Alcoholic Beverage Content: Label a clean headspace vial for each calibrator, negative, and positive control.

11.6.1.2 Quantitation of Ethanol for Biological Specimens: Label a clean headspace vial for each calibrator, negative, positive control, low and high whole blood positive controls (WBPC).

11.6.1.3 Qualitative of Volatiles for Alcoholic Beverage and Biological Specimens: Label a clean headspace vial for each positive and negative control.

11.6.1.4 Add 400 µL of working n-propanol internal standard solution into each headspace vial.

11.6.1.5 Add 100 µL of the appropriate calibrator or control into each headspace vial.

11.6.1.6 Seal the headspace vials by crimping the crimp-on septa.

11.6.1.7 Quantitation of Ethanol: Begin each quantitative run with the following sequence: volatile mix, calibrators 0.010 through 0.500, negative control, ethanol positive control, low whole blood control, high whole blood control.

NOTE: Whole blood QC controls can be excluded from alcoholic beverage content QC run.

11.6.1.8 Qualitative of Volatiles: Begin each run with the following: positive control, negative control.

NOTE: If two or more different positive controls need to be analyzed for a single case, the following sequence may be used: Positive control, positive control,...negative control. Each control needs to be assessed for carryover. If analyzing with ethanol quantitation for volatiles other than ethanol, methanol, or isopropanol, include the appropriate positive control at the end of the run. Positive controls should contain only the expected compound and internal standard. The negative control should contain internal standard only.

11.6.2 Sampling Alcoholic Beverage Content and Biological Specimens

11.6.2.1 Label a clean headspace vial for each verifier and case sample, as needed. Including a position number for case samples. Case samples will be analyzed in duplicate.

11.6.2.2 Ensure that all samples are homogenous by shaking, rotary mixing, and/or vortexing. A tissue grinder can be used to break up any clots in biological specimens.

If a homogenous sample cannot be obtained for any reason, a notation shall be made in the Laboratory Information Management System (LIMS) detailing the condition of the sample and its handling.

11.6.2.3 Add 400 µL of working n-Propanol internal standard solution into each headspace vial.

11.6.2.4 Add 100 µL of homogenous case sample into the appropriately labeled headspace vials.

NOTE: Beverage samples are diluted 1:100 with DI water so that most results fall between the lowest and highest calibration points (see “11.10.1 NOTES” below).

11.6.2.5 Add 100 µL of verification solution into each verifier headspace vial.

11.6.2.6 Seal the headspace vials by crimping on the crimp-on septa.

11.6.2.7 Quantitation of Ethanol: Separate verification solutions will be analyzed for each of the following positions: at the beginning of each batch run, after every eight injections (4 cases), and at the end of each batch run. The ending verification solution may be in a position which is less than eight injections (4 cases) due to the size of a batch or for batch runs that consist

of less than 4 cases in order to bracket the run. If a calibration curve is run in the same sequence with case sample(s) then the calibration curve may be used in place of a verification at the beginning of the run. Verification solutions do not need to be analyzed in duplicate.

11.6.2.8 Utilize “TX43” method.

11.7 INSTRUMENT PARAMETERS

Temperature settings	Oven Temperature	50°C
	Loop Temperature	70°C
	Transfer Line Temperature (GC)	90°C
	Transfer Line Temperature (MS)	230°C
Transfer line	Transfer Line Type (GC)	Fused Silica or pro steel
	Transfer Line Diameter (GC)	0.32 mm
Timing settings	Vial Equilibration	10 min
	Injection Duration	0.15 min
	GC Cycle Time	8.00 min
Vial and loop settings	Vial Size	10 or 20 mL
	Loop Size	1 mL
	Fill Mode	Flow to Pressure
	Fill Pressure	10 psi
	Loop Fill Mode	Custom
	Loop Ramp Rate	30 psi/min
	Loop Final Pressure	5 psi
	Loop Equilibration Time	0.05 min
	Vial Shaking	50 shakes/min with acceleration of 180 cm/s^2
	Vial Pressurization Gas	Hydrogen

Instrument	Agilent 7697A headspace sampler, 8890 GC, 5977B GC/MSD
FID Column	DB-ALC1 30m x 0.32mm x 1.8µm
MS Column	DB-1701 30m x 0.25mm x 1µm
Carrier Gas	Helium FID: 5 mL/min MS: 2.2986 mL/min measured at 55°C
Oven Program	Initial: 55°C hold time 2.4 min Then ramp 40°C/min till 100°C then hold for 1.5 min
FID Detector Temperature	300°C
Injection	Split ratio 30:1 flow: 150 mL/min, 120 °C

	Injection Pressure at 20 psi until 1.0 min
MS Detector	Full scan at m/z 45-200
	Sample: 1-2 µL
MS Source	230 °C
MS Quad	150 °C

11.8 CALCULATIONS

11.8.1 Calibration is a y = mx + b linear regression model using a six-point curve.

11.8.2 For alcoholic content cases: Correct the result for the dilution factor used. Divide this corrected value by 0.789, the density of ethanol at 20°C, to obtain the percent volume per volume units.

For example, 0.050 g/100mL (ETOH concentration) x 100 (dilution factor) ÷ 0.789 g/mL (density ETOH) = 6.3 mL/100mL = 6.3% v/v (final ETOH concentration).

11.8.3 To create the calibration curve by hand:

Ratio of peak area = peak area of ethanol ÷ peak area of ISTD

Plot ratio of the expected concentration as the X-axis versus the ratio of peak area as the Y-axis.

Gradient of the least squares line, *m*

$$m = \frac{\sum_{i=1}^{n} \{(x_i - \bar{x})(y_i - \bar{y})\}}{\sum_{i=1}^{n} (x_i - \bar{x})^2}$$

Calculate b, by entering information into y = mx + b.

Once m and b have been calculated, the concentration of the sample can be calculated by inputting the ratio of the peak area into the equation.

11.9 DECISION CRITERIA

11.9.1 Refer to "OSBI FTU Quality Manual – Ensuring the Validity of Results" for acceptance criteria for both headspace GC/FID and GC/MS.

11.9.2 The DFE primary standard is known to contain methanol, and methanol is also the solvent used to prepare the chloroform and toluene secondary standards. Methanol carryover in the negative control is acceptable as long as methanol is not the analyte of interest.

11.9.3 **Control Charting**

For control charting and additional acceptance criteria, refer to "OSBI FTU Quality Manual – Control Charts" and "OSBI FTU Quality Manual – Drug Identification Criteria," respectively.

Table 11-1 Target Analyte by Matrix Type

Analyte	Blood	Urine	Alcoholic Beverage

Ethanol	✓	✓	✓
Methanol	✓	✓	✓
Isopropanol	✓	✓	✓
1,1-Difluoroethane	✓	✓	
Chloroform	✓	✓	
Toluene	✓	✓	

11.10 NOTES

11.10.1 For alcoholic content cases: if results fall outside the lowest or highest calibration point, a different dilution of the sample can be used to obtain results between the lowest and highest calibration point. The acceptable dilution factors for use are: 1:25, 1:100, and 1:200.

11.10.2 For ethanol quantitative analysis: A packet containing autotune, tune evaluation, and original FID data for all calibrators, controls, and standards will be prepared for each analytical run. MS data is also required for ethanol positive controls. This data packet will be stored with the batch in the Laboratory Information Management System (LIMS).

11.10.3 For volatile qualitative analysis: A packet containing autotune, tune evaluation, and the original MS data for all controls will be prepared for each analytical run. FID data will be included in the data packet for toluene and for case samples. This data packet will be stored with the batch in the Laboratory Information Management System (LIMS).

11.10.4 Once calibrators, controls, verifiers, and samples have been transferred into a headspace vial, they must be run the same day.

11.10.5 During the validation of qualitative identification and confirmation of toluene, it was noted that isobutyl nitrite may cause a peak on the MS that matches to toluene. To verify the identification and confirmation of toluene, the peak on the FID must also be within ±2% of the retention time of the toluene positive control. FID data will be included in the data packet listed in 13.10.3 and for case samples.

11.11 REPORT WORDING

For reporting guidelines refer to “OSBI FTU Quality Manual - Reporting Results”.

11.12 REFERENCE

11.12.1 James C. Garriott. Medicolegal Aspects of Alcohol. 5th Ed. (2008).

11.12.2 Musselman J., Solanky A., Arnold W. (2006). Case Study: Increasing Accuracy of Blood-Alcohol Analysis Using Automated Headspace-Gas Chromatography. PerkinElmer, Inc., Shelton, CT.

11.12.3 B.L. Levine, Principles of Forensic Toxicology, American Association for Clinical Chemistry, Inc., 1999, pg. 180.

11.12.4 AOAC 984.14. “Ethanol in Beer: Gas Chromatographic Method.

11.12.5 Ethanol Analysis by Headspace Gas Chromatography (TX05)

11.12.6 Musselman J., Solanky A., Arnold W. (2006). Case Study: Increasing Accuracy of Blood-Alcohol Analysis Using Automated Headspace-Gas Chromatography. Perkin Elmer, Inc., Shelton, CT.

11.12.7 PerkinElmer (2004). Blood Alcohol Analysis Utilizing Headspace Autosampling and Dual-column GC Confirmation. PerkinElmer, Inc., Shelton, CT.

11.12.8 PerkinElmer TurboMatrix HS110 and PerkinElmer Clarus 500 G.C. Instrument Operational Manuals.

11.12.9 Varian CP-3800 Instrument Operational Manuals.

11.12.10 Perrigo, B. J., Peel, H.W., The Use of Retention Indices and Temperature-Programmed Gas Chromatography in Analytical Toxicology, J. Chromat. Sc., 19: 219-224 (1981).

11.12.11 B.L. Levine, Principles of Forensic Toxicology, American Association for Clinical Chemistry, Inc., 1999, pg. 180.

11.12.12 EURACHEM/CITAC Guide C G 4 (1995). Calculating the Combined Uncertainty, in Quantifying Uncertainty in Analytical Measurement (2nd ed.) 2000, pg. 25.

11.12.13 Farrington, Angela. "Uncertainty Calculation." E-mail to Matthew E. Stillwell. 24 July 2007.

11.12.14 B.L. Levine. Principles of Forensic Toxicology, American Association for Clinical Chemistry, Inc., 1999, pg. 180.

11.12.15 T. Hahn, J. Avella, and M. Lehrer. A Motor Vehicle Accident Fatality Involving the Inhalation of 1,1-Difluoroethane. Journal of Analytical Toxicology, Vol. 30, pg 638-642, October 2006.

11.12.16 N. Tiscione, I. Alford, D. Yeatman, X. Shan. Ethanol Analysis by Headspace Gas Chromatography with Simultaneous Flame-Ionization and Mass Spectrometry Detection. Journal of Analytical Toxicology, Vol. 35, pg 501-511, September 2011.

11.12.17 J. Avella, M. Lehrer, S. Zito. A Validated Method for the Quantitation of 1, 1 Difluoroethane Using a Gas in Equilibrium Method of Calibration. Journal of Analytical Toxicology, Vol. 32, pg 680-687, October 2008.

11.12.18 C. Sasaki, et al. A Fatality Due to Inhalation of 1,1-Difluoroethane (HFC-152a) with a Peculiar Device. Forensic Toxicology, Vol. 27, pg 45-48, 2009.

11.12.19 B. Capron, and B. Logan. Toluene-Impaired Drivers: Behavioral Observations, Impairment Assessment, and Toxicological Findings. Journal of Forensic Science, Vol. 54, No. 2, pg 486-489, March 2009.

11.12.20 N.B. Tiscione, et al. Ethanol Analysis by Headspace Gas Chromatography with Simultaneous Flame-Ionization and Mass Spectrometry Detection. Journal of Analytical Toxicology, Vol. 35, pg 501-511, 2011.

11.12.21 N.B. Tiscione, et al. Identification of Volatiles by Headspace Gas Chromatography with Simultaneous Flame Ionization and Mass Spectrometric Detection. Journal of Analytical Toxicology, Vol. 37, pg 573-579, 2013.

11.12.22 B.L. Levine, et al. A Fatality from Sevoflurane Abuse. Journal of Analytical Toxicology, Vol. 31, pg 534-536, 2007.

HISTORY

Issue Date	Revision	Revised by	Document History
06-15-24	6	D. Ross-Carr	Removed history for revision 5. Available in rev05. Correction of grammatical errors. 1.10.4, 4.10.4, 7.10.3, 13.10.2, 13.10.3: added autotune and tune evaluation to required documents in control packet to match current practice. Removed Randox from Section 2. Changed table 2-2 to table 2-1.
11/01/25	7	M. Brous	Corrected grammatical errors. Rescinded TX36 and TX41 procedures from 10-01-25 deviation. Formatting changes. Added 07-03-24 deviation 1.10.1 and 4.10.1 - Change “Agilent 5973 or 5975 GC/MS” to “Agilent 5975 or 5977 GC/MS”. Added 07-09-24 deviation 3.4.2 and 11.4.2 – Change to include Cerilliant as a vendor for ethanol calibrators. Added 09-11-24 deviation 4.5.7, 5.5.8, 6.5.8, 8.5.8, 9.5.8, 10.5.9 – Changed to included ten mL disposable conical centrifuge tubes for use. Added 08-14-25 deviation 1.6.9, 1.6.13, 1.6.18, 4.6.9, 5.6.11, 6.6.7 (changed to 6.6.2.7), 7.6.12, 8.6.8, and 9.6.7 – Added “NOTE: Centrifuge step may be repeated if insufficient separation occurs and will be documented in the case record.”. 6.4.2 – Change “Stock Standards” to “Primary Standards”. Moved 6.6.1- 6.6.14 to 6.6.2.1-6.6.2.14. Moved 6.10.1.1-6.10.1.4 to 6.6.1.2-6.6.1.4. 6.6.1 – Added heading and note to 6.6.1 “100 µL DILUTION PREPARATION Note: The 100 µL DILUTION PREPARATION is for limited case samples in which the available specimen volume does not allow for the completion of two tests using 250 µL. See “6.10.1 NOTES” below.”. Added 6.6.1.1 “Label a clean disposable microcentrifuge tube, conical centrifuge tube, and autosampler vial for each control and case sample.”. 6.6.1.4 – Added “and vortex” at the end. 6.6.1.5 – added “and vortex” at the end. 6.6.1.6 – Added “Refer to 6.4.1 Diluted Working Internal Standard Solution. Starting at 6.6.2.6, follow the remaining sample preparation steps in 6.6.2.”. Added 6.6.2 “250 µL PREPARATION”. 6.6.2.2 – removed “(see “NOTES” for 100 µL extraction steps)”.

			6.10.1 – Added "(refer to step 6.6.1 100 µL DILUTION PREPARATION)" at the end of the first sentence. 7.4.1, 7.4.2, 7.4.3 – Changed "table 8.1" to "Table 7.1". 8.4.2, 8.4.3 – Changed "table 9-1" to "Table 8-1". Removed Fentanyl Stock Solution (was 9.4.2) because Fentanyl moved to TX42. 9.4.2 – Changed "table 10-1" to "Table 9-1". Removed fentanyl from the Secondary and Working Low Positive Control Solutions. 9.4.3 – Changed "table 10-1" to "Table 9-1". Removed fentanyl from the Secondary High Positive Control Solution. 10.4.2 – Changed "table 12-2" to "Table 10-2" in Primary Standards and Secondary High Positive Control. 10.9.1 – Changed "table 12-2" to "Table 10-2". Updated tables in TX42 to "Table 10-1" and "Table 10-2". 11.6.1 – added "and Controls". 11.6.1.1 – changed "For quantitative testing only;" to "Quantitation of Ethanol for Alcoholic Beverage Content:". 11.6.1.2 – changed "For quantitation of biological specimens:" to "Quantitation of Ethanol for Biological Specimens:" and added "each calibrator, negative, positive control,". 11.6.1.3 – changed "For qualitative testing:" to "Qualitative of Volatiles for Alcoholic Beverage and Biological Specimens:". 11.6.1.7 – Added "Quantitation of Ethanol:", changed "If analyzing with ethanol quantitation for volatiles other than ethanol, methanol, or isopropanol, include the appropriate positive control at the end of the run." and moved to 11.6.1.8 NOTE. 11.6.1.8 – Changed "If qualitative identification only," to "Qualitative of Volatiles:". 11.6.2 – Changed "**Sampling Alcoholic Beverage Content (Ethanol, Methanol, Isopropanol)**" to "**Sampling Alcoholic Beverage Content and Biological Specimens**". Moved 11.6.3.2 to 11.6.2.2.

APPROVAL

FTU Technical Manager Melissa Brous Date: 10/15/25

Melissa Brous

CSD Director Date: 10/15/2025

Janice Joslin