MASTER DOCUMENT LIST

1 Quality Assurance

TOX-01-01	Toxicological Services and Examiner Approval	
TOX-01-02	**Instruments and Equipment**	**R**
TOX-01-03	**Drug Standards, Calibrators, and Internal Standards**	**R**
TOX-01-04	**Controls**	**R**
TOX-01-05	**Uncertainty of Measurement**	**R**
TOX-01-06	**Case Documentation**	**R**
TOX-01-06A	Approved Standard Abbreviations	
TOX-01-07	Exam Counting	RES02032014
TOX-01-08	**Report Writing Guidelines**	**R**
TOX-01-09	**Guidelines for Batch Archive Review**	**N**
TOX-01-10	**Guidelines for Administrative and Technical Review**	**N**

2 Examination of Toxicological Evidence

TOX-02-01	**Systematic Examination of Toxicological Specimens**	**R**
TOX-02-02	Disposition of Toxicological Evidence	

3 Immunoassay Screening

TOX-03-01	**Screening of Blood Samples by EMIT**	**R**
TOX-03-02	Screening of Urine and Vitreous Samples by EMIT	

4 Calibrators, Internal Standards and Controls

TOX-04-01	Preparation of Calibrators	RES02032014
TOX-04-02	Preparation of Internal Standards	RES02032014
TOX-04-03	Preparation of Positive Controls	RES02032014

5 Extraction Methods

TOX-05-01	SPE for Cocaine and Cocaine Metabolites	RES02152013
TOX-05-02	SPE for Opiates	RES02152013

5 Extraction Methods

TOX-05-03	SPE for Opiates in Urine	RES02152013
TOX-05-04	SPE for Methadone	RES02152013
TOX-05-05	SPE for 7-Aminoflunitrazepam in Urine	RES02032014
TOX-05-06	Extraction for GCMS Screen	
TOX-05-07	**Extraction for Amines in Urine**	**RES05012019**
TOX-05-08	**Extraction for Amines in Urine with Periodic Acid**	**RES05012019**
TOX-05-09	Extraction for Weak Acids / Neutrals (WANS)	RES02152013
TOX-05-10	Extraction for Benzodiazepines	RES02032014
TOX-05-11	Extraction for Benzodiazepines in Urine	RES02032014
TOX-05-12	Extraction for 9-Carboxy-THC in Urine	
TOX-05-13	Extraction for Chlordiazepoxide	RES02152013
TOX-05-14	Extraction for Valproic Acid	RES02152013
TOX-05-15	Extraction for Zolpidem	RES02152013
TOX-05-16	Extraction for General Neutral/Basic Drugs	RES02152013
TOX-05-17	Extraction for Clonazepam	RES02152013
TOX-05-18	Extraction for Lorazepam	RES02152013
TOX-05-19	SPE for Benzodiazepine Mix with LCMS	
TOX-05-20	Extraction for LCMS Screen	
TOX-05-21	SPE for Carisoprodol and Meprobamate with LCMS	RES02032014
TOX-05-22	SPE for Opiates, Cocaine, and Cocaine Metabolites	
TOX-05-23	Extraction for Barbiturates	
TOX-05-24	LCMS Target Qualitative Analysis (TQUAL)	
TOX-05-25	Extraction for Δ^9-THC and Δ^9-Carboxy-THC in Blood	
TOX-05-26	Extraction for Amines in Blood by SPE	
TOX-05-27	**Extraction for Amines in Urine by SPE**	**N**

6 Data Analysis

TOX-06-01	Screening by GCMS

6 Data Analysis

TOX-06-02	**Target Compound Analysis**	**R**
TOX-06-03	Screening by LCMS	

7 Prepared Solutions

TOX-07-01	Preparation of Borate Buffer	
TOX-07-02	**Preparation of Acidified Ethanol**	**R**
TOX-07-03	**Preparation of 20% Sodium Hydroxide**	**R**
TOX-07-04	**Preparation of 1*M* Potassium Hydroxide**	**R**
TOX-07-05	Ether/Toluene Extraction Solvent	RES02152013
TOX-07-06	**Preparation of 0.1*M* Potassium Phosphate Buffer**	**R**
TOX-07-07	Preparation of Sodium Acetate Buffer	
TOX-07-08	0.1M Sodium Phosphate Buffer	RES02152013
TOX-07-09	Preparation of Syva Buffer	RES04022018
TOX-07-10	Cobas Buffer	RES08192009
TOX-07-11	Preparation of Syva Buffer in Methanol	RES04022018
TOX-07-12	EMIT Amphetamine Reagent A	RES08082007
TOX-07-13	EMIT Amphetamine Reagent B	RES08082007
TOX-07-14	**Preparation of Maleic Acid Solution**	**R**
TOX-07-15	10% Hydroxylamine HCl	RES02152013
TOX-07-16	**Preparation of Dilute Phosphoric Acid**	**R**
TOX-07-17	EMIT Amphetamine Reagents	RES08192009
TOX-07-18	**Verification of EMIT Reagents**	**R**
TOX-07-19	EMIT Opiate Reagents	RES02032014
TOX-07-20	EMIT Barbiturate Reagents	RES02032014
TOX-07-21	EMIT Benzodiazepine Reagents	RES02032014
TOX-07-22	EMIT Phencyclidine Reagents	RES02032014
TOX-07-23	EMIT THC Reagents	RES02032014
TOX-07-24	**Preparation of Ammonium Formate**	**R**

7 Prepared Solutions

TOX-07-25	**Preparation of PPG Dilution Solution**	**R**
TOX-07-26	**Preparation of Potassium Carbonate Buffer**	**R**
TOX-07-27	**Preparation of Mobile Phase for LCMS**	**R**
TOX-07-28	EMIT Amphetamine Reagents	RES02032014
TOX-07-29	**Preparation of 0.1 *M* Hydrochloric Acid**	**R**
TOX-07-30	Preparation of EMIT Reagents for Carisoprodol Class Assay	RES02032014
TOX-07-31	**Preparation Hexane:Ethyl Acetate Extraction Solvent**	**R**
TOX-07-32	Silanization of Glassware	
TOX-07-33	Preparation of 1 *M* Acetic Acid	
TOX-07-34	**Preparation of Acidified Methanol**	**R**
TOX-07-35	**Preparation of Sodium Phosphate Buffer (pH 6.0)**	**R**
TOX-07-36	Preparation of Tris Buffer	
TOX-07-37	Preparation of Tris Buffer in Methanol	

8 Instrument and Equipment Instructions

TOX-INS-01	LCMS Instructions	
TOX-INS-02	GCMS Instructions	
TOX-INS-03	Cerex Pressure Processor Instructions	
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TOX-INS-05	Cerex Evaporator	
TOX-INS-06	Centra MP4 Centrifuge Instructions	
TOX-INS-07	C3I V1 Centrifuge Instructions	RES02152013
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TOX-INS-09	Immunoassay Instructions	
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TOX-INS-12	Top-Loader Balance Operation	
TOX-INS-13	Autoclave Instructions	RES02152013

8 Instrument and Equipment Instructions

TOX-INS-14	Sorvall ST40 Centrifuge Instructions	
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TOX-INS-16	Cerex ALD-III Pressure Processor Instructions	
TOX-INS-17	XcelVap Evaporator System Instructions	
TOX-INS-18	Instructions for pH Meter	

Forms

LAB-TOX-10	Urine Immunoassay Reagent Log	
LAB-TOX-11	Working Solution Log	
LAB-TOX-12	GCMS Data Screen Worksheet	
LAB-TOX-13	LCMS Data Screen Worksheet	
LAB-TOX-14	Toxicology Uncertainty Workbook	
LAB-TOX-15	**Zero Response Calculation**	**R**
LAB-TOX-16	Concentration Corresponding to a Zero Area – High (Zero Response Calculation page)	RES07102017
LAB-TOX-17	Blood Immunoassay Reagent Log	
LAB-TOX-18	Urine Immunoassay Calibrators & Controls Form	
LAB-TOX-19	Urine Immunoassay Reagents Form	
LAB-TOX-20	Urine Immunoassay Control Verification Worksheet	
LAB-TOX-21	Urine Immunoassay Control Log	
LAB-TOX-22	Urine Immunoassay Calibrator Log	
LAB-TOX-23	EMIT Maintenance Log	
LAB-TOX-24	**Reagent Log**	**R**
LAB-TOX-25	Vacuum Pump Oil Level Log	
LAB-TOX-26	Toxicology Matrix Blank Verification Form	
LAB-TOX-27	pH Meter Calibration	
LAB-TOX-28	Instrument Log	
LAB-TOX-29	Control Verification Worksheet	
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Forms

LAB-TOX-31	Post-Calibration Pipette Check Report	
LAB-TOX-32	Pipette Performance Verification Report	
LAB-TOX-33	Unconfirmed EMIT Positives Log	
LAB-TOX-34	Toxicology Pipette Log	
LAB-TOX-35	**Blood EMIT Results Workbook**	**R**
LAB-TOX-35a	**Blood EMIT Individual Results Page (within LAB-TOX-35)**	**N**
LAB-TOX-36	**EMIT Reagent Validation Form**	**R**
LAB-TOX-37	EMIT (Reagent) Verification Checklist - Blood	
LAB-TOX-38	Balance Check Log	

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
…		Previous revision history included in archived documents.
06	06/03/2009	New: TOX-01-08, TOX-05-19, TOX-07-24, TOX-07-25, TOX-07-26, TOX-07-27, TOX-INS-01, TOX-INS-02, TOX-INS-03, TOX-INS-04, TOX-INS-05, TOX-INS-06, TOX-INS-07 Revised: TOX-01-01, TOX-01-02, TOX-01-03, TOX-01-04, TOX-01-06, TOX-01-07, TOX-02-01, TOX-03-01, TOX-04-01, TOX-04-02, TOX-05-01, TOX-05-02, TOX-05-03, TOX-05-04, TOX-05-05, TOX-05-06, TOX-05-07, TOX-05-08, TOX-05-09, TOX-05-10, TOX-05-11, TOX-05-12, TOX-05-13, TOX-05-14, TOX-05-15, TOX-05-16, TOX-05-17, TOX-05-18, TOX-06-01, TOX-06-02, TOX-07-01, TOX-07-06, TOX-07-07, TOX-07-08, TOX-07-09, TOX-07-10, TOX-07-11, TOX-07-17
07	08/19/2009	New: TOX-INS-09, TOX-07-28 Revised: TOX-01-04, TOX-01-08, TOX-03-01, TOX-03-02, TOX-04-01, TOX-05-06, TOX-07-18, TOX-07-19, TOX-07-20, TOX-07-21, TOX-07-22, TOX-07-23, LAB-TOX-10 Rescinded: TOX-07-10, TOX-07-17
08	01/20/2010	New: TOX-05-20, TOX-05-21, TOX-06-03, TOX-07-29, TOX-07-30, TOX-INS-10, TOX-INS-11, TOX-INS-12, TOX-INS-13, LAB-TOX-12, LAB-TOX-13 Revised: TOX-01-01, TOX-01-04, TOX-01-06, TOX-01-08, TOX-02-01, TOX-02-02, TOX-03-01, TOX-03-02, TOX-04-01, TOX-04-02, TOX-05-01, TOX-05-07, TOX-05-19, TOX-06-01, TOX-06-02, TOX-07-27, TOX-07-28
09	01/21/2010	Revised: LAB-TOX-13
10	03/22/2010	New: TOX-01-06A, TOX-05-22, TOX-05-23 Revised: TOX-04-01, TOX-04-02, TOX-05-09, TOX-05-19
11	02/15/2013	New: TOX-05-24 Rescinded: TOX 05-01, TOX05-02, TOX05-03, TOX-05-04, TOX-05-09, TOX-05-13, TOX-05-14,TOX-05-15, TOX-05-16, TOX-05-17, TOX-05-18, TOX-07-05, TOX-07-08, TOX-07-15, TOX-INS-07, TOX-INS-13 Revised: TOX-01-16A, TOX-03-01, TOX-04-01, TOX-05-19, TOX-05-20, TOX-05-22, TOX-05-23

Version #	Effective Date	Brief Description of Change(s)
11a	08/22/2013	Changed from TOC to MDL
12	01/10/2014	Revised: TOX-01-05 New: LAB-TOX-14
13	02/03/2014	Revised: TOX-01-01, TOX-01-02, TOX-01-03, TOX-01-04, TOX-01-06, TOX-01-06A, TOX-01-08, TOX-02-01, TOX-02-02, TOX-03-01, TOX-03-02, TOX-05-06, TOX-05-07, TOX-05-08, TOX-05-12, TOX-05-19, TOX-05-20, TOX-05-22, TOX-05-23, TOX-05-24, TOX-06-03, TOX-07-01, TOX-07-02, TOX-07-03, TOX-07-04, TOX-07-06, TOX-07-07, TOX-07-09, TOX-07-11, TOX-07-14, TOX-07-16, TOX-07-18, TOX-07-24, TOX-07-25, TOX-07-26, TOX-07-27, TOX-07-29, TOX-INS-01, TOX-INS-02, TOX-INS-09, TOX-INS-11, LAB-TOX-11 New: TOX-INS-14 Rescinded: TOX-01-07, TOX-04-01, TOX-04-02, TOX-04-03, TOX-05-05, TOX-05-10, TOX-05-11, TOX-05-21, TOX-07-19, TOX-07-20, TOX-07-21, TOX-07-22, TOX-07-23, TOX-07-28, TOX-07-30
14	04/14/2014	Revised: TOX-03-01, TOX-07-18 New: TOX-05-25, TOX-07-31, TOX-07-32, TOX-INS-15
15	05/19/2014	Revised: TOX-01-03 New: TOX-05-26, TOX-07-33, TOX-07-34, TOX-07-35
16	06/30/2014	Revised: LAB-TOX-11
17	09/19/2014	Revised: TOX-01-08, TOX-05-06, TOX-05-07, TOX-05-08, TOX-05-12, TOX-05-20, TOX-05-22, TOX-05-23, TOX-05-24, TOX-05-25, TOX-05-26 New: TOX-INS-16
18	11/12/2014	Revised: TOX-05-19
19	02/10/2015	Revised: TOX-01-05, LAB-TOX-14
20	04/20/2015	Revised: TOX-01-08, TOX-07-27 New: TOX-INS-17
21	09/02/2015	Revised: TOX-02-02
22	10/19/2015	Revised: LAB-TOX-10 New: LAB-TOX-17, LAB-TOX-18, LAB-TOX-19, LAB-TOX-20, LAB-TOX-21, LAB-TOX-22, LAB-TOX-23, LAB-TOX-24, LAB-TOX-25, LAB-TOX-26, LAB-TOX-27, LAB-TOX-28, LAB-TOX-29

Version #	Effective Date	Brief Description of Change(s)
23	10/26/2015	Revised: TOX-05-19, TOX-05-26, TOX-06-02, TOX-INS-01 New: LAB-TOX-15, LAB-TOX-16
24	01/11/2016	Revised: LAB-TOX-29
25	04/01/2016	Revised: TOX-01-02, TOX-01-03, TOX-05-24, LAB-TOX-11, LAB-TOX-14 New: LAB-TOX-30, LAB-TOX-31, LAB-TOX-32
26	03/15/2017	Revised: TOX-06-01, TOX-06-03, LAB-TOX-13, LAB-TOX-31, LAB-TOX-32 New: LAB-TOX-33, LAB-TOX-34, LAB-TOX-36, LAB-TOX-38 Version numbers removed from MDL
27	07/10/2017	Revised:LAB-TOX-15, LAB-TOX-29, New: LAB-TOX-35, LAB-TOX-37 Rescind: LAB-TOX-16
28	04/02/2018	Revised: TOX-01-02, TOX-01-04, TOX-01-06A, TOX-01-08, TOX-02-02, TOX-03-01, TOX-03-02, TOX-05-12, TOX-05-19, TOX-05-20, TOX-05-22, TOX-05-23, TOX-05-24, TOX-05-25, TOX-05-26, TOX-06-01, TOX-06-02, TOX-07-01, TOX-07-07, TOX-07-18, TOX-07-33, TOX-INS-01, TOX-INS-02 New: TOX-07-36, TOX-07-37, TOX-INS-18 Rescind: TOX-07-09, TOX-07-11
29	07/20/2018	Revised: TOX-06-01
30	05/01/2019	**Revised**: TOX-01-02, TOX-01-03, TOX-01-04, TOX-01-05, TOX-01-06, TOX-01-08, TOX-02-01, TOX-03-01, TOX-06-02, TOX-07-02, TOX-07-03, TOX-07-04, TOX-07-06, TOX-07-14, TOX-07-16, TOX-07-18, TOX-07-24, TOX-07-25, TOX-07-26, TOX-07-27, TOX-07-29, TOX-07-31, TOX-07-34, TOX-07-35, LAB-TOX-15, LAB-TOX-24, LAB-TOX-35, LAB-TOX-36 **New**: TOX-01-09, TOX-01-10, TOX-05-27, LAB-TOX-35a **Rescind**: TOX-05-07, TOX-05-08

TOXICOLOGICAL SERVICES AND EXAMINER APPROVAL

1 Screening for Drugs

Screening for drugs involves two types of testing:

A. Immunoassay analysis will detect if drugs of targeted classes are in a sample. Relative response for each target class is compared to the response for each class calibrator to determine which cases undergo confirmation testing. It is an efficient screen for a large number of abused drugs with minimal sample preparation. It does not give specific identification of a drug.

B. GCMS or LCMS drug screening will detect drugs with specific identification of a drug. It is used when a specific drug is suspected which our immunoassay analysis would not detect. It is also used if observations of the suspect indicate impairment that is not explained by the routine testing. It may also be applied routinely to samples to detect a broader range of drugs or to supplement other methods.

2 Drug Confirmation

A. A drug confirmation by GCMS or LCMS is conducted to confirm presence of a drug as indicated by screening tests or suspicions of the investigator. It involves the specific identification for drugs and can be used to determine the concentration when calibrators are included in the process.

B. Some methods are applicable to a wide range of specimen types and others are more specific to a specimen type or drug class to enhance capabilities of detection.

3 Examiner Approval and Proficiency Requirements

A. Demonstration of competency in the use of Toxicology Standard Operating Procedures is required prior to independent casework. The toxicological examinations that require director approval to allow independent casework by an examiner are:

1. Blood Alcohol
2. Toxicology Screening
   - *a) Immunoassay*
   - *b) GCMS*
   - *c) LCMS*
3. Toxicology Confirmation
   - *a) GCMS*
   - *b) LCMS*

B. Examiners will participate in proficiencies to the extent that they conduct casework.

C. When significantly different technologies or testing procedures are implemented, analysts with approvals for that procedure will be evaluated for continued authorization.

Preparer

Megan Barton Date: 01/27/2014
Advisory Board Chair

Concurrence

Heather Greco Date: 01/27/2014
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
01	07/01/2003	Minor Revision numbering Addition Section 3
02	10/20/2006	Major Revision – restructuring of documents and clarification of text
03	06/03/2009	Major Revision – Sections 1 and 3 Implementation of LCMS and clarification of text
04	01/20/2010	Major Revision – All Sections Advisory Board 01/11/2010
05	02/03/2014	Major Revision – Section 2

INSTRUMENTS AND EQUIPMENT

1 Scope

Analytical instruments are used to produce specific data for determining drug content of specimens. Instruments such as balances and pipettes are used for precise measurements in the preparation of solutions and standards, and in sampling of specimens for sample extraction. Refrigeration equipment is necessary for preservation of samples. Quality assurance guidelines for the maintenance, calibration, and repair of analytical instrumentation, balances, pipettes, and refrigeration equipment are specified. The manufacturer's manuals should be referenced for maintenance instructions and troubleshooting. The Texas DPS Safety Manual and the manufacturer's operation manuals should be followed regarding safety precautions for instruments and equipment.

Additional equipment in processing is important for efficiency. This equipment is evaluated on usage as working or not working. Equipment that may belong in this group includes centrifuges, rotators, vortexers, heaters, incubators, baths, evaporators, SPE manifolds, and sonicators.

2 Maintenance and Calibration of Significant Laboratory Instrumentation

2.1 General Requirements for Analytical Instrumentation

A. All instruments will be maintained in proper working condition and properly calibrated.

B. No instrument is to be used if it is not in proper working order to perform the required task.

C. All instruments will be checked after being moved or if a major repair is performed.

D. If an instrument fails calibration, performance verification check, or a performance problem is detected during routine maintenance, it must be removed from service. The problem must be recorded in a logbook.

E. After the instrument is repaired and before it is returned to service, routine quality control procedures must be performed to ensure it is working properly for its required task. Keep a record of all repairs and the routine maintenance in a logbook or electronic equivalent.

2.2 Gas Chromatograph/Mass Spectrometer (TOX-INS-02)

A. Gas Chromatograph/Mass Spectrometers (GC/MS) used in toxicological analyses shall be tuned on the day of usage for individual samples or on the initial day of usage for a batch of samples. Tunes used for diagnostic purposes and/or tunes applied to the instrument for sample runs will be maintained in the instrument's notebook or electronic equivalent. Tune results will be recorded in the instrument's logbook.

B. The performance of the GC/MS used in toxicological analyses will be demonstrated for each batch with a series of matrix blanks, calibrators, and/or controls as specified in the SOP.

C. GC/MS Spectrometers used in toxicological analyses shall have regular preventative maintenance. Vacuum forepumps will have oil level checked weekly and oil changed at approximate 12 month intervals with allowance to postpone until the pump is shut down for other purposes. If a decrease in sensitivity is noted, autosampler syringes

will be checked for blockage or wear, and injector septa will be inspected. Maintenance is performed as needed when indicated by degradation of system performance. Cleanliness of the ion source is evaluated with tuning results: response for 69 amu attained with suitable multiplier voltage, and higher mass ions 219 and 502 displaying adequate relative response. Some methods require optimum system performance attained by cleaning of the ion source or chromatography system immediately prior to the analysis. A logbook (or electronic equivalent) of all maintenance will be kept with the instrument.

2.3 Liquid Chromatograph/Mass Spectrometer (TOX-INS-01)

A. Liquid Chromatograph/Mass Spectrometers (LC/MS) used in toxicological analyses shall have the tune checked on the day of usage for individual samples or on the initial day of usage for a batch of samples. Tunes used for diagnostic purposes and/or tunes applied to the instrument for sample runs will be maintained in the instrument's notebook or electronic equivalent. Tune results will be recorded in the instrument’s logbook.

B. The performance of the LC/MS used in toxicological analyses will be demonstrated for each batch with a series of matrix blanks, calibrators, and/or controls as specified in the SOP.

C. LC/MS used in toxicological analyses shall have regular preventative maintenance. Vacuum forepumps will have oil level checked weekly and oil changed at approximate 12 month intervals with allowance to postpone until the pump is shut down for other purposes. If a decrease in sensitivity is noted, tubing will be checked for blockage or wear and source probe will be inspected. Maintenance is performed as needed when indicated by degradation of system performance. Performance of the detector is evaluated with tuning results: response for 906 amu, resolution (width) of a peak, and mass shift of the target amu. A logbook (or electronic equivalent) of all maintenance will be kept with the instrument.

2.4 Balances (TOX-INS-10,11,12)

A. Laboratory balances shall be checked for accuracy at least once each quarter of the year.

1. Acceptable specifications for a balance will be documented in the respective balance logbook.
2. Balances will be checked for accuracy whenever they are moved from one location to another.
3. If a balance is found to be out of tolerance at a specific value, that balance may only be used within the range of acceptable values and the acceptable range will be documented on the balance.

B. Balances shall be calibrated and certified by an external vendor once a year.

C. Keep the balances clean and leveled at all times.

D. Maintain a log with the balance specifications, checks, maintenance, and certification.

2.5 Mechanical Pipettes

A. A calibration will be performed by an outside vendor annually on all pipettes used for critical measurements.

B. Conduct a performance verification approximately six months after the vendor calibration or whenever routine maintenance is performed.

1. The gravimetric method will be used to collect four or more data points at three different volumes over the range of the pipette to verify the pipette delivery volume.
2. Compare data points to the manufacturer's specification for the pipette.
3. If the pipette is determined to be out of specification, initiate corrective action and retest.

C. Results of a performance verification (pass/fail) will be recorded and maintained in a logbook.

D. A gravimetric check of a pipette will be performed immediately prior to use for working solution preparation or in a casework batch. This check will be recorded on the batch worklist or Working Solution Log (LAB-TOX-11).

1. For critical measurements, the accuracy must be within +/- 5%.
   a) *Critical measurements include:*
      i. *Preparation of calibrator working solutions, control working solutions, or internal standards for quantitative analytes.*
      ii. *Spiking of calibrator working solutions, control working solutions, or internal standards during quantitative batch analysis.*
      iii. *Aliquotting of samples for quantitative analysis.*
2. For non-critical measurements, the accuracy should be within +/- 5%.

3 Malfunction of a Balance or Pipette

A. If a balance or pipette fails a performance verification or a problem is detected during routine usage, it must be removed from service and the problem recorded in the logbook. Removal from service requires distinctive markings or coverings to avoid inadvertent usage.

B. Perform routine maintenance or have the balance or pipette repaired. Record the repairs made or problem solution in the logbook.

4 Equipment

4.1 Dry Block Heater

A. The temperature should be adjusted to the desired set-point and monitored by the internal thermometer.

B. The surface should be kept clean.

4.2 Water Bath Evaporator (TOX-INS-04 and TOX-INS-17)

A. The temperature should be adjusted to the desired set-point and monitored by an internal thermometer.

B. The water in the bath should be clear and clean with no evidence of bacterial/fungal growth. If the water becomes dirty, discard and clean bath. Replenish with purified water.

C. Evaporators with programming functionality should be verified for each program used.

4.3 Evaporator (TOX-INS-05)

A. The temperature should be adjusted to the desired set-point and monitored by an internal thermometer.

4.4 Refrigeration Equipment

A. Refrigerators/freezers used in the laboratory should be capable of maintaining the optimum temperature range required for storing reagents and samples.

1. A refrigerator maintains optimum temperature with the required range of 2-8°C.
2. The freezer should maintain a temperature below 0°C.
3. Refrigerators/freezers are observed during working hours to ascertain that they are functioning. Weekly records of temperature readings are maintained.

B. The following maintenance procedure should be performed as needed:

1. Each refrigerator/freezer is maintained in working order and repaired or replaced if necessary.
2. Refrigerators/Freezers are kept clean and well organized.
3. The blower system will be monitored for water accumulation. The drain tube can be cleared with pressurized gas.
4. Loss of refrigeration requires immediate action to arrange repair and to preserve refrigerator contents.

4.5 Thermometer

A. All thermometers for critical measurement must be either calibrated or their accuracy must be verified to ensure the temperature is being measured +/-1.0ºC.

B. Thermometers used to monitor set points will be verified with a NIST traceable thermometer annually at the appropriate temperature.

C. If the thermometer varies greater than +/- 1.0ºC, then the amount of deviation will be noted.

D. If the deviation of the thermometer is greater than 2.0ºC, then the thermometer must be replaced.

4.6 Weight Set

A. Laboratory standard weights shall be verified annually and may occur after the annual re-certification of the balance. Acceptable specifications for a weight set will be documented in the respective balance logbook.

B. Laboratory standard weights should be calibrated by an external vendor every 3 years.

C. Documentation for laboratory standard weights will be maintained in a logbook.

4.7 Biological Hood

A. The hood must be certified at least once a year from an external vendor.

B. The hood may be certified after every filter change or maintenance action or at the operator's discretion.

C. Maintenance logs must be kept regarding repairs and/or corrective action.

4.8 Centrifuge (TOX-INS-06, TOX-INS-08, and TOX-INS-14)

A. The relative centrifugal force can be determined as outlined in the manufacturer's instructions or by the following calculation:

1. Ascertain the spinning radius by measuring the distance from the center of the drive shaft to the bottom of the tube when it is in spinning position. The measurement may be provided by the manufacturer for the configuration used in the centrifuge.

2. Calculate the relative centrifugal force (RCF): $g = 1.118 \times 10^{-5} \times r \times n^2$ (r = spinning radius in centimeters; n = revolutions per minute (RPM))

3. The RPM necessary to produce a specified RCF value (i.e., *g* values) can be calculated from this relationship.

B. Interval timing is standardized for all centrifuges to start when the run is started. Deceleration is set for slow to avoid disruption of precipitates or separated layers. Acceleration is set for slow, except for volumes less than 1 mL.

C. Centrifuge housing, rotor chamber, and rotor accessories should be cleaned as necessary. In the event of a spill in the centrifuge, it must be cleaned and disinfected immediately.

D. A bucket seal is required to prevent the release of aerosols from solvents and biological liquids. In the event of tube breakage, stop the centrifuge, move away, and wait 5 minutes before opening the lid to allow aerosol to dissipate.

4.9 pH Meter (TOX-INS-18)

A. The pH meter will be standardized on each day before use.

B. Standardization of the pH meter must bracket the pH for the intended use.

C. Standardization of the pH meter is recorded in the pH meter log. (LAB-TOX-27)

4.10 Pressure Processor (TOX-INS-03)

A. Pressure processors with programming functionality should be verified for each program used.

4.11 Sonicator

A. The water in the bath should be clear and clean with no evidence of bacterial/fungal growth. If the water becomes dirty, discard and clean bath. Replenish with purified water.

4.12 Vacuum Oven (TOX-INS-15)

5 Records

Working Solution Log (LAB-TOX-11)

Vacuum Pump Oil Level Log (LAB-TOX-25)

pH Meter Calibration (LAB-TOX-27)

Instrument Log (LAB-TOX-28)

Post-Calibration Pipette Check Report (LAB-TOX-31)

Pipette Performance Verification Report (LAB-TOX-32)

Toxicology Pipette Log (LAB-TOX-34)

Balance Check Log (LAB-TOX-38)

Temperature Verification Log (AUS-FRM-006)

Calibration Certificates

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
01	07/01/2003	Minor Revision numbering
02	10/20/2006	Major Revision – restructuring of documents and substantive changes to content
03	06/03/2009	Major Revision – Sections 2.2, 2.3, 2.5, and 4.6 Minor Revision – Sections 1, 4.2, and 4.3 Implementation of LCMS, additional procedures and clarification of text
04	02/03/2014	Minor Revision – Sections 2.2 and 2.3 Major Revision – Sections 2.5 and 4.4
05	04/01/2016	Minor Revision - Sections 2.1, 2.3, 2.4
06	04/02/2018	Major Revision – All sections
07	05/01/2019	Revision – All sections

DRUG STANDARDS, CALIBRATORS, AND INTERNAL STANDARDS

1 Scope

To establish guidelines for application and quality of drug reference standards in Toxicology.

Calibrators and internal standards are prepared from drug standards. The guidelines for verification of calibrators and internal standards are included. The preparation of calibrators and internal standards are found in their respective analytical protocol.

2 Safety

A. Universal Bloodborne Pathogen precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

B. A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

C. Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Screening by GCMS (TOX-06-01)

Target Compound Analysis (TOX-06-02)

Screening by LCMS (TOX-06-03)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Appropriate pipette(s)
- Volumetric flasks
- Analytical balance

4.2 Reagents

A. Stock solution (1.0 mg/mL) - Purchased standard or weigh 10.00 mg of standard as the non-salt form or its equivalent weight of salt of a drug into 10 mL volumetric flask and dissolve in organic solvent. Store in freezer.

B. Stock solution (0.10 mg/mL) - Purchased standard, dilution of a purchased standard (e.g. add 1 mL of 1.0 mg/mL stock solution to a 10 mL volumetric flask and dilute to volume), or weigh 10.00 mg of standard as the non-salt form or its equivalent weight of salt of a drug into a 100 mL volumetric flask and dissolve in organic solvent. Store in freezer.

C. Stock solution (25.0 mg/mL) - Purchased standard, dilution of a purchased standard, or weigh 250.00 mg of standard as the non-salt form or its equivalent weight of salt of a drug into 10 mL volumetric flask and dissolve in organic solvent. Store in freezer.

5 Practice

5.1 Application of Drug Standards

A. The application of a standard varies with the analytical requirement:

1. Standards are used to prepare calibrators at designated concentrations in matrix samples, which are then subjected to the same sample processing as case samples to determine the instrumental response at each level in preparation of a quantitative calibration plot.
2. Standards are used to obtain mass spectrum data as a basis for comparison and specific identification of drugs in case samples.
3. Standards are used to obtain chromatographic retention time as a basis for comparison in support of identification of drugs in case samples.
4. Standards are used to prepare calibrators at designated cutoff levels for immunoassay analysis to establish an instrumental response for evaluation of whether case samples are considered negative or have sufficient response to justify confirmation analysis for specific drugs in a class.
5. Standards are used to prepare positive controls as needed to meet assay requirements.

B. To obtain a mass spectrum or chromatographic retention time of a standard without undergoing an extraction process:

1. Prepare a drug standard solution in the appropriate concentration [e.g., transfer 10 µL of a 1 mg/mL drug standard to a 1 mL volumetric and bring to volume with an appropriate solvent (final concentration – 10 mg/L). NOTE: Different volumes of solutions may be prepared in the same proportions specified in the tables.]
2. For non-derivatized standards: Add approximately 30 µL of a standard (or standard mix) in an organic solvent to a microvolume insert in a labeled autosampler vial.
3. For derivatized standards:

   a) Trimethylsilyl (TMS) Procedure

   i. Add 30 µL or an appropriate volume (e.g. 20 µL of a 1 mg/mL solution) of a standard (or standard mix) in an organic solvent to clean, labeled 16X100 mm tubes.

   ii. Evaporate to dryness in an evaporator (e.g. 45°C at 5-20 psi for 2 minutes).

   iii. Add TMS reagent (1:2, BSA:ethyl acetate) using the volume equivalent to the amount used for evaporation, cap tube, and heat for 5 minutes at 60°C.

   iv. Allow tube to cool.

   v. Cap and centrifuge (e.g. 1479 x g for two minutes) to drain the walls.

   vi. Transfer solution to a microvolume insert in a labeled autosampler vial.

b) Heptafluorobutyric Anhydride (HFBA) Procedure

i. Add 50 µL or an appropriate volume (e.g. 20 µL of a 1 mg/mL solution) of a standard (or standard mix) in an organic solvent to clean, labeled 16X100 mm tubes.

ii. Add 200 µL acidified ethanol and evaporate to dryness in an evaporator (e.g. 40°C at 5-20 psi for 2 minutes).

iii. Add 100 µL (1:2, HFBA:ethyl acetate), cap tube, and heat at 70°C for 20 minutes.

iv. Centrifuge (e.g. 1479 x g for two minutes) to drain the walls.

v. Evaporate to dryness in an evaporator (e.g. 35°C at 4 psi for 10-20 minutes).

Note: Maintain low temperature and pressure as HFBA excess is killed. Remove as sample reaches dryness. Check for absence of butyric acid odor.

vi. Add 50 µL ethyl acetate to each tube and vortex briefly to dissolve the analytes.

vii. Cap and centrifuge (e.g. 1479 x g for two minutes) to drain the walls (optional).

viii. Transfer the solution to a microvolume insert in a labeled autosampler vial.

c) Pentafluoropropionic anhydride and pentafluoropropanol (PFPA) Procedure

i. Add 50 µL or an appropriate volume (e.g. 20 µL of a 1 mg/mL solution) of a standard (or standard mix) in an organic solvent to clean, labeled 16X100 mm tubes.

ii. Evaporate to dryness in an evaporator (e.g. 45°C at 5-20 psi for 2 minutes).

iii. Add 50 µL PFPA and 50 µL PFPOH to each tube, cap, vortex briefly to dissolve the analytes, and heat at 60°C for 20 minutes.

iv. Centrifuge (e.g. 1479 x g for two minutes) to drain the walls.

v. Evaporate to dryness in an evaporator (e.g. 40°C at 10 psi for 5 minutes).

vi. Add 50 µL ethyl acetate to each tube and vortex briefly to dissolve the analytes.

vii. Cap and centrifuge (e.g. 1479 x g for two minutes) to drain the walls (optional).

viii. Transfer the solution to a microvolume insert in a labeled autosampler vial.

d) Trifluoroacetic anhydride (TFAA) Procedure

i. Add 50 µL or an appropriate volume (e.g. 20 µL of a 1 mg/mL solution) of a standard (or standard mix) in an organic solvent to clean, labeled 16X100 mm tubes.

ii. Add 100 µL acidified methanol and evaporate to dryness in an evaporator (e.g. 40°C at 5-20 psi for 2 minutes).

iii. Add 50 µL (1:1 TFAA:ethyl acetate) to each tube and vortex briefly. Allow to stand at room temperature, uncapped, for 3 minutes.

iv. Cap tubes and centrifuge (e.g. 1479 x g for two minutes) to drain the walls.

v. Evaporate to dryness in an evaporator (e.g. 35 °C at 4 psi for 2-3 minutes).

vi. Add 50 µL ethyl acetate to each tube and vortex briefly to dissolve the analytes.

vii. Transfer the solution to a microvolume insert in a labeled autosampler vial.

5.2 Quality Control of Drug Standards

A. Drug standards may be prepared by the laboratory or obtained from another source, including purchasing. The laboratory will maintain a certificate of analysis for all purchased standards.

B. Verification of drug standards used in working solutions, control solutions, and internal standard solutions will be completed at the same time as the solution verifications before application in casework.

C. Any standard not used for solution preparations will be verified using the manufacturer provided certificate of analysis.

D. A standard in solution may be applied as a retention time reference standard in qualitative analysis beyond its expiration date.

E. The laboratory will maintain a log of purchased drug standards (LAB-TOX-30).

6 Working Solutions (Calibrators)

6.1 Verification of EMIT Working Solutions

A. Process new EMIT calibrators according to EMIT procedure. If performed within a batch that already contains verified calibrators, run the new calibrators as samples.

B. Evaluate calibrator responses by comparison of the new lot to the current lot of calibrators. There should not be significant changes.

C. Evaluate each assay independently. Results are acceptable if the difference between the positive cutoff and negative meets the criteria for the calibrators in the blood EMIT procedure.

D. A batch archive containing all data used to verify working solutions will be produced and reviewed by the Toxicology Section Supervisor,Toxicology Technical Leader, or designee.

6.2 Verification of Confirmation Calibrator Working Solutions for Quantitative Drugs

A. Verification of confirmation calibrator working solutions can be accomplished by one of the following methods:

1. Comparison of new calibrator working solution(s) to previous working solutions

   a) *Compare samples prepared with the new working solutions to a calibration curve generated from calibrators prepared with the current working solutions. If there is more than one working solution used to prepare a calibration curve for casework, a sample must be prepared from each new working solution.*

   b) *The calculated concentrations of the sample(s) must be within ±20% of the prepared concentration to be acceptable.*

   c) *Casework may be analyzed concurrently with the verification of the new working solution(s).*

2. Comparison of new calibrator working solutions to verified positive controls

   a) *Prepare a calibration curve from the new working solutions.*

   b) *Verify that the concentration of all positive controls are within ±20% of the value acceptable for casework for that lot of the positive control in order to determine the acceptability of the new working solutions.*

   c) *Casework may be analyzed concurrently with the verification of the new working solution(s) but new working solutions will not be used for batch calibration*

B. If previous calibrator working solutions or verified positive controls are not available, the process for verification will be determined by the Toxicology Advisory Board.

C. A batch archive containing all data used to verify working solutions will be produced and reviewed by the Toxicology Section Supervisor, Toxicology Technical Leader, or designee.

7 Internal Standards

7.1 Verification

A. Evaluate area responses for the internal standard compared to previous batches as indication of degradation.

B. All data used to verify the internal standard will be produced and reviewed by the Toxicology Section Supervisor, Toxicology Technical Leader, or designee. Produced data may be stored with a batch archive for which it was run in.

8 Storage of Standards

A. Standards in organic solution are stored in the freezer.

B. Some standards require minimal exposure to light and should be stored in amber bottles, handled expediently, and returned to dark storage.

C. Standards in aqueous solution are stored in the refrigerator.

D. Standards for THC analysis are stored in silanized amber vials.

E. Manufacturer storage recommendations should be considered.

9 Records

Total Ion Chromatogram

Mass spectra

Library match print out

Certificates of analysis

Working Solution Log (LAB-TOX-11)

Drug Standard Log (LAB-TOX-30)

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
01	07/01/2003	Minor Revision – Numbering
02	12/01/2003	Addition Section 2D requiring logbook or equivalent Modification to Section 2C "Purchased standards in solution which have a certificate of analysis may be used ~~without~~ on casework only after verification that the compound is what it was purported to be. ~~If data from the manufacturer demonstrates purity. Other~~ Additional verifications are necessary when standard ~~is performed as~~ solutions are prepared from powder."
03	10/20/2006	Major Revision – Restructuring of documents and substantive changes to content
04	10/01/2007	Addition Section 3.1, C #3, c, i-vi: c. Pentafluoropropionic anhydride and pentafluoropropanol (PFPA) Procedure, i. Add 50 µL PFPA and 50 µL PFPOH to each tube, cap, vortex briefly to dissolve the analytes, and heat at 60°C for 20 minutes., ii. Centrifuge (e.g. 3,000 rpm for one minute) to drain the walls., iii. Evaporate to dryness in an evaporator (e.g. 40°C at 10 psi for 5 minutes)., iv. Add 50 µL ethyl acetate to each tube and vortex briefly to dissolve the analytes., v. Cap and centrifuge (e.g. 3,000 rpm for one minute) to drain the walls (optional)., vi. Transfer the solution to a 100 µL insert in a labeled autosampler vial.
05	06/03/2009	Major Revision – Section 3 Minor Revision – Section 2 Implementation of LCMS and clarification of text
06	02/03/2014	Major Revision- Title, All sections
07	05/19/2014	Major Revision – Sections 5.1, 5.3
08	04/01/2016	Major Revision- Sections 3, 5.2
09	05/01/2019	Revision – Sections 2, 3, 5, 6, 7, 8, and 9

CONTROLS

1 Scope

Guidelines are established for the application and quality of controls for analyses in Toxicology.

A positive control is a sample in a biological matrix used to verify that a procedure is operating within expected parameters. Drug content can be determined by comparison of instrumental response of samples to a calibration plot.

Controls may be commercially available for an analysis with matrix and concentration suitable for an assay. These require minimal laboratory preparation and avoid solution dilutions. If a blood control is unavailable or unacceptable for use, a serum control containing the desired analytes and diluted with blood is acceptable.

Controls may be prepared similarly to calibrator preparation by spiking drug standards into matrix similar to the samples. The drug standard for control preparation should be from a source independent of the calibrator standard.

Target compound quantitative analysis is applied to determine the concentration of analytes in controls using the same method applied to case samples.

Calculated concentrations of controls are compared to target values in evaluating the acceptability of a calibration. The concentrations are recorded for each batch to monitor performance of the method over time.

2 Safety

A. Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

B. A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

C. Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Target Compound Analysis (TOX-06-02)

Screening by GCMS (TOX-06-01)

Screening by LCMS (TOX-06-03)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Appropriate pipette(s)
- Volumetric flasks
- Analytical balance

4.2 Positive Controls

- Whole Blood Control – drug commercially prepared in blood (may be preserved as lyophilized or frozen)
- Urine Control – drug commercially prepared in urine (may be preserved as lyophilized or frozen)
- Serum Control – drug commercially prepared in serum (may be preserved as lyophilized or frozen)
- Serum Spiked Pool Blood Control – blood matrix spiked in bulk with commercial serum control (maximum 10% serum)
- Serum Spiked Blood Control – individual blood matrix spiked with commercial serum control (maximum 10% serum).
- Standard Spiked Pooled Matrix Control –matrix spiked in bulk with working control solution prepared with drug standard from a source independent of the calibrator standard (maximum 10% working control solution). Matrix must match calibrator matrix.
- Standard Spiked Matrix Control – individual matrix sample spiked with working control solution prepared with drug standard from a source independent of the calibrator standard. Matrix must match calibrator matrix. Non-independent standard spiked matrix control or a non-independent standard spiked pooled matrix control from the same reference material as calibrators starting at the first step of laboratory preparation for that material (e.g. sample weighing or dilution of a stock solution). This method may be used if the other materials are unavailable.

4.3 Reagents

- Stock solution for standards purchased independently from material used for calibrators
- Negative matrix – blood, urine
- Stock Solution (1.0 mg/mL) - Purchased standard or weigh 10.00 mg of standard as the non-salt form or its equivalent weight of salt of a drug into 10 mL volumetric flask and dissolve in organic solvent. Store in freezer.
- Stock Solution (0.10 mg/mL) - Purchased standard, dilution of a purchased standard (e.g. add 1 mL of 1.0 mg/mL stock solution to a 10 mL volumetric flask and dilute to volume), or weigh 10.00 mg of standard as the non-salt form or its equivalent weight of salt of a drug into a 100 mL volumetric flask and dissolve in organic solvent. Store in freezer.
- Stock Solution (25.0 mg/mL) - Purchased standard, dilution of a purchased standard, or weigh 250.00 mg of standard as the non-salt form or its equivalent weight of salt of a drug into 10 mL volumetric flask and dissolve in organic solvent. Store in freezer.

5 Practice

5.1 Types and Applications of Controls

A. Controls are subjected to the same data acquisition parameters as case samples.

B. Controls must meet specified criteria for batch acceptance.

C. The composition of a control varies with its application.

D. Negative Controls

1. A matrix blank demonstrates that a negative case sample carried through the sample processing will have no results that would be considered reportable.

   a) *The same matrix material will be used for negative controls, calibrators, internally-prepared positive controls, and sample dilutions.*

      i. *if a batch contains diluted samples of a different matrix than the calibrator matrix, a negative control of the matrix used for dilution will be analyzed in the batch*

      ii. *Matrix material will not contain analytes that would affect calibration or produce a false positive in diluted case samples.*

   b) *Sources for matrix blanks include:*

      i. *Material purchased from commercial sources,*

      ii. *Specimens collected from laboratory personnel.*

   c) *The absence of analytes will be verified in a matrix blank before application in casework.*

2. A solvent blank (GCMS only) demonstrates that the instrument sample injection system has no drug carryover from the previous sample. Solvent used to reconstitute the associated sample is injected prior to that sample. Data is acquired from the injection with the same instrumental parameters used for case samples.

E. Positive Controls

1. Positive controls are prepared in a matrix similar to samples in a batch. If a batch is composed of samples with varied matrices, the matrix of the control is to correspond with the matrix of samples with quantitative results to be reported.

   a) *A positive control in quantitative analysis demonstrates the calibration produces an acceptable quantitative result for a sample prepared with drug standard independent of the drug standard used for calibration.*

   b) *A positive control in qualitative analysis demonstrates the sample processing provides adequate detection of analytes of interest.*

2. Sources for positive controls include:

   a) *Blood, urine, or serum material purchased from commercial sources:*

      i. *in a lyophilized state to extend shelf life.*

      ii. *in a liquid state but stabilized with preservatives to extend shelf life.*

      iii. *in a frozen state to extend shelf life and allow thawing for use.*

   b) *Controls prepared within the laboratory:*

      i. *Purchase a drug standard from a source independent from the reference material used for calibrator. Standards are considered to*

be from an independent source despite coming from the same manufacturer if the certificate indicates an independent verification.

ii. Prepare a spiked pooled control using larger amounts of matrix and serum control or drug standard in multiples of volumes used for individual spiked controls. This method allows sampling of the control to match sampling of case samples.

iii. Prepare a standard spiked matrix control or a standard spiked pooled matrix control from the same reference material as calibrators starting at the first step of laboratory preparation for that material (e.g. sample weighing or dilution of a stock solution).

F. Other Positive Controls

1. A reference control in qualitative analysis provides data for reference in identification (e.g. retention time and/or mass spectrum).
2. A conversion control in analysis of amines in urine demonstrates that no conversion from one amine to another occurs in the analytical process up to its concentration level. (e.g. ephedrine to methamphetamine, phenylpropanolamine to amphetamine).

5.2 Concentration of controls

A. Concentration of controls should not be equivalent to a calibration point on the curve.

B. Preparation within the laboratory or purchase of custom controls enables choice of concentrations to demonstrate suitability of the calibration at levels typically detected or at a mid-range level.

5.3 Selection of controls

A. Each batch for qualitative or quantitative analysis must include a matrix blank.

B. Each batch for qualitative analysis must include a positive control to validate detection by the extraction and data analysis method. It may be analyzed quantitatively to verify a calibration. This positive control may be a calibrator.

C. Each batch for quantitative analysis must include a positive control to validate detection by the extraction and data analysis method. It is analyzed quantitatively to verify a calibration before quantitative values can be reported.

D. Specific controls are included in analytical methods.

E. Additional controls may be prepared or purchased for the purposes of:

1. Study of new controls.
2. Verification of new controls.
3. Producing overlapping data for transition from a specified control.
4. Establishing control for a calibration if sufficient data supports its application.

F. Additional controls selected should be evaluated on the basis of:

1. Availability – The market demand determines which drugs and concentrations in blood, urine, and serum controls are manufactured.

2. Suitability – A control in a form that can be directly sampled in the same manner as case samples is preferable. This avoids individually spiked controls having a duplication of any problem in calibrator preparation.
3. Reliability – Degradation in storage or manufacturing variations may lead to a false indication of poor method reliability.

6 Preparation of Positive Controls

6.1 Serum Spiked Pooled Blood Control

A. Blood calibrator matrix spiked in bulk with commercial serum control (maximum 10% serum).

B. To prepare a 10 mL control with a concentration 10% of the serum control, add 1 mL of serum control to 9 mL of negative blood matrix.

C. Different volumes of control may be prepared in the same proportions as specified.

D. A lower volume of serum and an appropriately higher volume of blood may be mixed to produce lower concentrations.

6.2 Serum Spiked Blood Control

A. Individual blood calibrator matrix spiked with commercial serum control (maximum 10% serum).

B. To prepare a 1 mL control with a concentration 10% of the serum control, add 100 µL of serum control to 900 µL of negative blood matrix.

C. Different volumes of solution control may be prepared in the same proportions as specified.

D. A lower volume of serum and an appropriately higher volume of blood may be mixed to produce lower concentrations.

6.3 Standard Spiked Pooled Matrix Control

A. Calibrator matrix spiked in bulk with working control solution prepared with drug standard from a source independent of the calibrator standard (maximum 10% working control solution)

B. To prepare a 10 mL control with a concentration 10% of the working control solution, add 1 mL of working control solution to 9 mL of negative calibrator matrix.

C. Different volumes of control may be prepared in the same proportions as specified.

D. A lower volume of working control solution and an appropriately higher volume of negative calibrator matrix may be mixed to produce lower concentrations.

6.4 Standard Spiked Matrix Control

A. Individual negative calibrator matrix spiked with working control solution prepared with drug standard from a source independent of the calibrator standard (maximum 10% working control solution).

B. Method 1 to produce controls prepared similarly to calibrators: Choose appropriate stock solutions and working solutions to prepare the concentration needed.

C. Method 2 to produce controls similarly to serum spiked controls:

1. To prepare a 1 mL control with a concentration 10% of the working control solution, add 100 µL of working control solution to 900 µL of negative calibrator matrix.
2. Different volumes of control may be prepared in the same proportions as specified.
3. A lower volume of working control solution and an appropriately higher volume of negative calibrator matrix may be mixed to produce lower concentrations.

6.5 Non-Independent Standard Spiked (Pooled) Matrix Control

A. Use the method for standard spiked matrix control or standard spiked pooled matrix control

B. Prepare from the same reference material as calibrators

C. Start at the first step of laboratory preparation for that material (e.g. sample weighing or dilution of a stock solution).

D. This method may be used if the other materials are unavailable.

7 Verification of Controls

7.1 Verification of Controls for Quantitative Analysis

A. Controls for quantitative analysis will have the identification of drugs and their concentration verified with current calibrators for each new lot before application in casework. Verification analyses may be included with a casework batch if an established control is included in the batch.

B. A batch archive containing all data used to verify controls and Control Verification Worksheet will be produced and reviewed by the Toxicology Section Supervisor, Toxicology Technical Leader, or designee.

C. Each new lot of control will have a concentration established by either:

1. Confirmation of manufacturer's verified value: Analytical confirmation of the new control is done by processing three prepared samples. The new control may be applied in casework with the manufacturer's verified value if its identification is correct, the calibration is acceptable, and the new control's mean calculated concentration is within 10% of the manufacturer's verified value.
2. Confirmation of lab prepared value: Analytical confirmation of the new control is done by processing three prepared samples. The new control may be applied in casework with the lab prepared value if its identification is correct, the calibration is acceptable, and the new control's mean calculated concentration is within 5% of the lab's prepared value.
3. Determination of lab established value: If the value of the new control did not initially meet acceptance criteria by confirmation of manufacturer's value or lab prepared value, the new control may be verified by the determination of a lab established value. Analytical determination of the new control is done by processing at least three batches while including the new control as three

prepared samples in each batch. The new control may be applied in casework with the lab established value if its identification is correct and the calibration is acceptable. The lab established value is the mean of at least nine concentrations from acceptable batches. However, if the final lab established value meets criteria of the manufacturer's verified value or the lab prepared value, then the manufacturer's verified value or lab prepared value may be applied in casework in lieu of the lab established value. If the new control's mean calculated concentration is greater than 20% different from the target value, the new control data must be reviewed by the Toxicology Advisory Board for approval of use in casework.

7.2 Verification of Controls for Qualitative Confirmation Analysis

A. Controls for qualitative analysis will have the identification of drugs and their concentration verified with current calibrators for each new lot before application in casework. Verification analyses may be included with a casework batch if an established control is included in the batch.

B. A batch archive containing all data used to verify controls and Control Verification Worksheet will be produced and reviewed by the Toxicology Section Supervisor, Toxicology Technical Leader, or designee..

C. Analytical determination of the new control is done by processing the new control as three prepared samples.

   The new control may be applied in casework with the lab established value if its identification is correct. The lab established value is the mean of at least three concentrations from acceptable batches.

D. Controls for qualitative analysis require verification by mass spectrum match to an electronic library, literature reference, or in-house printed reference of drug standard before application in casework.

7.3 Verification of GCMS and LCMS Screen Control

A. Controls for qualitative analysis by GCMS or LCMS screening procedures require verification by:

   1. Mass spectrum match to an electronic library, literature reference or an in-house printed reference of a drug standard.
   2. Detection of analytes is determined by a signal to noise ratio of at least 3:1 for the base peak in GCMS or XIC of the MRM in LCMS.
   3. Retention time must be ± 0.2 minutes for GCMS or ± 0.4 minutes for LCMS of a known reference standard based on the plot of a major ion. Relative retention time may be used in lieu of running a concurrent known reference standard.

B. For each analyte in the control, plot a major ion/transition and determine its area and ratio to the area of a major ion/transition in the reference analyte (e.g., methadone). This initial value should be used for monitoring consistency of performance in subsequent batches.

C. A batch archive containing all data used to verify controls will be produced and reviewed by the Toxicology Section Supervisor, Toxicology Technical Leader, or designee.

7.4 Verification of Controls for Urine Immunoassay Analysis

A. Controls for immunoassay analysis can be verified in a similar manner as lab established values for a quantitative control. Each batch must include a new calibration. Calibration is acceptable if comparable to previous calibration.

B. The lab established value (concentration for each drug class) will be determined by processing at least three batches while including the new control as three prepared samples in each batch. The concentrations produced by each sampling of the control will be averaged and that average is the lab established value.

C. A batch archive containing all data used to verify controls will be produced and reviewed by the Toxicology Section Supervisor, Toxicology Technical Leader, or designee.

8 Documentation

A. The laboratory will maintain a logbook (LAB-TOX-11) or equivalent that includes lot number, source, date of preparation, date of expiration, initials of preparer, and certificate of analysis (if available) for quantitative and qualitative controls.

B. The laboratory will maintain control plots of results of quantitative controls in batches for detection of trends such as calibrator or control decline.

1. Plot for each quantitative analyte specified by method, even if qualitative for batch.
2. Begin new plot when new lot number is implemented.
3. Control plot data summary should include for each data point: batch date, measured concentration, preparation date of calibrator solutions, and % difference from verified value.
4. Control plot data summary should include a cumulative average, standard deviation, % Relative Standard Deviation (%RSD), target value, and verified value.
5. Control plots will be reviewed Technical Leader (or designee) at least annually and documentation of the review will be maintained with the control plot data.

C. Control Verification Archive

1. Verification archives will be maintained the same as case batch archives.
2. Reference case batch archives associated with this control verification on the archive cover sheet.
3. Include a data summary table of results (LAB-TOX-29). Designate values used and rejected.
4. Control verification archives will be reviewed prior to implementation. The control archives will be maintained in the Toxicology Section and may be electronically archived in a similar manner as case records.

9 Storage of Controls

A. Controls in organic solution are stored in the freezer.

B. Some standards require minimal exposure to light and controls containing these drugs should be stored in amber bottles, handled expediently, and returned to dark storage.

C. Drug standards for laboratory preparation of controls in aqueous solution are stored in the refrigerator.

D. Drug standards for THC analysis are stored in silanized amber vials.

Apply manufacturer guidelines for storage and handling of purchased controls.

10 Limitations

A. To equalize extraction conditions, controls prepared similarly to calibrators with solution addition should have a total volume of solvent added to match the solvent in the largest volume of added calibrator solution. The total volume of solvent includes solvent in control working solution added and/or compensating solvent added

B. Avoid weighing solid materials less than 10 milligrams.

11 Records

Working Solution Log (LAB-TOX-11)

Control Verification Worksheet (LAB-TOX-29)

Drug Standard Log (LAB-TOX-30)

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	10/20/2006	Original Issue
01	06/03/2009	Major Revision – Section 3 Minor Revision – Sections 2, 4, and 6 Implementation of LCMS and clarification of text
02	08/19/2009	Major Revision – Section 3.3 Implementation of procedures used with MGC240 EMIT Immunoassay instrument
03	01/20/2010	Major Revision – Sections 2, 3.3, 3.4, 3.6, 3.7, and 3.8 Minor Revision – Sections 3.2 and 3.5 Advisory Board 01/11/2010
04	02/03/2014	Major Revision – All sections
05	04/02/2018	Major Revision – Sections 5, 6, 7 8, and 11
06	05/01/2019	Revision – Sections 1, 3, 4, 5, 6, 7, 8, 9, and 11

UNCERTAINTY OF MEASUREMENT

1 Scope

Estimated measurement uncertainty values for the Toxicology discipline will be based on the following practices.

2 Practice

A. The estimated measurement uncertainty, which conveys approximately 95% confidence level (k=2), is determined from historical laboratory data. The estimated measurement uncertainty for quantitated drugs is included in the case record, such that it can be applied to the concentration reported.

B. The estimated measurement uncertainty calculations for drug quantitation in blood samples can be found in the uncertainty budget for that category of testing from the year of testing.

C. To determine a measurement uncertainty estimate, the reported concentration of a drug is multiplied by the measurement uncertainty percentage (k=2) of that drug. The calculated measurement uncertainty estimate is rounded up to the appropriate number of digits for the drug value. It is expressed as the reported concentration plus or minus the associated uncertainty in units consistent with the reported drug.

D. The Toxicology Report Uncertainty Calculation Sheet (LAB-TOX-14) may be used to calculate the measurement uncertainty of a reported drug value and documented in case record.

E. For example, a reported cocaine concentration of 0.05 milligrams per liter and an uncertainty percentage of 9.0% would be calculated and expressed as follows:

(0.05) x 9.0% = 0.0045, where 0.0045 is rounded up to 2 digits in this example = 0.01

0.05 (+/- 0.01) milligrams per liters

[Reported concentration] (+/- [calculated uncertainty estimate]) [units] (95% Confidence Level)

F. Methods with quantitative values issued in reports include:

- LCMS Opiates-Cocaine
- GCMS Barbiturates
- GCMS Amphetamines
- LCMS Benzodiazepine Mix
- LCMS THC

G. The measurement uncertainty is based on:

- Examiner variation
- Method extractions
- Instrumentation

H. When reported measurement uncertainty is requested, a supplemental report may be issued. The original quantitative test result will be included and the relevant measurement uncertainty estimate, applicable units, and confidence level will be clarified on the report.

3 Records

Toxicology Report Uncertainty Calculation Sheet (LAB-TOX-14)

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	10/20/2006	Original Issue
01	01/01/2014	Major Revision – All sections
02	02/10/2015	Major Revision – All sections
03	05/01/2019	Removed Section 2 – Related Documents Added Section 3 – Records

CASE DOCUMENTATION

1 Scope

These policies are established as minimum requirements for case documentation and record-keeping required for Toxicology cases.

2 Contents of Case Record (may be located in physical case folder or LIMS)

A. Laboratory Report

B. Laboratory Submission Form (LAB-203/LAB-201)

C. Specimen Record Form

D. Toxicology Report Uncertainty Calculation Sheet (LAB-TOX-14, when applicable) (optional)

E. Blood and/or Urine Drug Screen and Confirmation Data – all analyses are recorded with information to show examiner, date of sampling, drug(s) detected, and/or quantitative results.

F. Administrative documentation – such as notes concerning case status inquiries, special analysis requests, special evidence instructions, interpretative information, disposition notices, and court inquiries.

G. Analytical data - Charts, spectra, and notes associated with samples in the case will be maintained. Each page of the data will include case number and handwritten initials or, if uploaded to LIMS, secure electronic initials/signature of examiner. Date of testing and sample identifier will be indicated on each set of multiple page data. Additional charts, spectra, and notes associated with batch processing of the case sample are archived separately from the case record. An appropriate reference to the batch archive will be maintained in the case notes.

3 Batch Archives

A. Each batch of samples will include an archive of printed data relevant to the batch.

If re-injection of samples, calibrators, and/or controls is needed within the same instrument run or a batch is re-started, the batch archive will include data related to the original injection. A note will be included on the batch archive and should indicate the reason for re-injection and notes about the original data.

B. Batch archives will include as available:

1. A cover sheet that includes the batch identifier and additional notes
2. Toxicology Measurement Uncertainty Values summary
3. Batch worklist showing samples, calibrators, and controls analyzed in batch
4. Instrument sequence file
5. Quantitative/Qualitative response plots with all data points
6. Quantitative/Qualitative response plots which are used for calculations
7. Data Acquisition method

8. Data Processing method, if any changes are made besides ion ratio, retention time, and response factors
9. Standardized printout (e.g. spectra, total ion chromatogram, ion plots, area ratios, or quantitative report) for target analysis of all injections of calibrators, blanks, and controls.

C. Batch archives will be reviewed prior to reporting any cases from that batch. The batch archives will be maintained in the Toxicology Section and may be electronically archived in a similar manner as Case Records.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	07/01/2003	Original Issue
01	10/20/2006	Major Revision – restructuring of documents and clarification of text
02	06/03/2009	Minor Revision – Sections 2 and 3 Implementation of LCMS and clarification of text
03	02/03/2014	Major Revision – Sections 2 and 3
04	05/01/2019	Revision – Sections 2 and 3

APPROVED STANDARD ABBREVIATIONS

1 Scope

Standard abbreviations used in the Toxicology examination documentation. Some abbreviations are necessary due to limited space at the point of use. Abbreviations should not be used when clarity of meaning is necessary.

2 Abbreviations

--	Indications – used for analyte result LIMS entry
-	used for “No drug detected” or “Inconclusive” result in LIMS for report formatting
-1	Possible – used for analyte result LIMS entry
0	used for “Detected” result LIMS entry
ALR	Administrative License Revocation
%CV	Percent Coefficient of Variation
F	Fail
P	Pass
∆A	Change in Absorbance
AAFS	American Academy of Forensic Sciences
ACS	American Chemical Society
ALP, Alpr	Alprazolam
Amph	Amphetamine
amu	Atomic mass unit
Barb	Barbiturate
BCR	Blood Collection Report
BENZ, Benzo	Benzodiazepine
BK	Blood Kit
BL	Blood
BNM	Benzodiazepine mix
BSA	N,O-bis(trimethylsilyl)acetamide
BSB	Biological Specimen Box
BTM	Blood Tube Mailer
BZE	Benzoylecgonine
Calib, Cals, Cal	Calibrator
Ctrls, Ctrl	Controls
CE	Cocaethylene
CFTAF	Clinical Forensic Toxicology Assessment Form

COA , CofA	Certificate of Analysis
COC	Cocaine
CofC	Chain of Custody, Chain of Possession
Coef of Det	Coefficient of Determination
CONC	Concentration
CONF	Confirmation
CPS	Counts Per Second
Cyl	Plastic cylinder, as used in blood kit
DAU	Drugs of Abuse – Urine
Det	Detected
Diaz	Diazepam
Dil	Dilution, diluted
DNR	Do Not Report
DNT	Do Not Test
DOA	Drugs of Abuse - Blood
Elev	Elevated
EMIT	Enzyme Multiplied Immunoassay Technique
Eph	Ephedrine
EtAc	Ethyl Acetate
Exp	Expected
Ext	Extraction
FN	First Name
FID	Flame Ionization Detector
Flunit	Flunitrazepam
GBI	Georgia Bureau of Investigation
GC	Gas Chromatography, Gas Chromatograph
GT, GTT	Gray Top Tube
HCT	Hematocrit
HFBA	Heptafluorobutyric Anhydride
Hi Cal	High Calibrator
Hi Neg, HN	High Negative
HOX	Hydroxylamine
Ind	Indications
Inj	Injection

IP	Inner packaging
IPA	Isopropyl Alcohol
ISTD, IS	Internal Standard
JT, JTrax	Justice Trax
LC	Liquid chromatography, liquid chomatograph
LN	Last Name
Low Cal	Low Calibrator
LV	Low Volume
Meth	Methamphetamine
MN	Middle Name
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry, Mass Spectrometer
Mtb	Metabolite
N/B	Neutral/Basic
ND	Not detected
Neg	Negative
Nordiaz	Nordiazepam
n-prop	n-Propanol
NS	Not Sealed
OP	Outer Packaging
OPI	Opiate
PD	Police Department
PE	Pseudoephedrine
PFPA	Pentafluoropropionic Anydride
PFPOH	Pentafluoropropanol
Phent	Phentermine
Pos, +	Positive
POR	Police Officer Report
PPA	Phenylpropanolamine
PT, PTT	Purple Top Tube
PW	Paperwork
QlAC	Qualitative Administrative Cutoff
QtAC	Quantitative Administrative Cutoff
Qual	Qualify/Qualitative/Qualification

Quant	Quantify/Quantitate/Quantitation
rs	Resealed
S	Sealed
RSD	Relative Standard Deviation
RRT	Relative Retention Time
RT	Retention Time
SAK	Sexual Assault Kit
SAT	Southwestern Association of Toxicologists
SCF	Signed Consent Form
SD	Standard Deviation
SIM	Selected Ion Monitoring
SO	Sheriff's Office
SOFT	Society of Forensic Toxicologists
SPE	Solid Phase Extraction
THC-COOH	9-Carboxy-tetrahydrocannabinol
TMS	Trimethylsilyl
STD	Standard
TQual	Target Qualitative
TT	Test Tube Taped
UCF	Unsigned Consent Form
UM	Urine Mailer
Unk	Unknown
UR	Urine
USC	Urine Specimen Container
WAN	Weak Acid Neutral
WBC	Whole Blood Control
WC	Working Control
WS	Working Solution
ZPLK	Ziplock Bag

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	03/22/2010	Original Issue
01	02/15/2013	Major Revision – Additions to approved abbreviations
02	02/03/2014	Major Revision – Additions to approved abbreviations
03	04/02/2018	Major Revision – Additions to approved abbreviations

REPORT WRITING GUIDELINES

1 Scope

To establish guidelines for writing toxicology reports

2 Report Terminology

[additional analysis]	description of additional testing (e.g. “Additional mass spectral screening”, “Quantitative/Qualitative analysis of [drug name]”, “Additional analysis”, “Further analysis”)
[adjusted concentration and units]	concentration and units of drug adjusted for dilution
[concentration and units]	concentration and units of drug
[description]	description of non-standard sample container
[drug name]	drug name[s], drug class name[s], trade name[s]
[GCMS/LCMS]	depending on instrument used
[laboratory name]	name of laboratory
[method]	method of instrumental analysis (i.e., immunoassay screening, gas chromatography/mass spectrometry (GCMS), liquid chromatography/mass spectrometry (LCMS), headspace gas chromatography or a combination of one or more of these methods)
[parent drug name]	parent drug name[s]
[evidence description]	description of evidence packaging
[submission type]	e.g. U.S. Mail, Certified Mail #, in person
[units]	milligrams per liter or nanograms per milliliter

3 Screening by Immunoassay

A. The following interpretations will be specified for each specimen type undergoing immunoassay testing, if more than one is provided on a case.

B. Reporting results

Immunoassay results are reported directly on the final report. If the immunoassay result indicates the need for confirmation testing, the report will note “**Further Analysis**” for that drug class.

C. If the volume of sample submitted is low, the immunoassay screen may not have been done if investigative information supported using the sample for a specific analysis. The report will include: "**No immunoassay screen was performed due to insufficient amount of sample**."

4 Screening by GC/MS and/or LC/MS

A. Report results of GC/MS and/or LC/MS screening as specified below unless other testing performed has results that override its results (e.g., drugs detected by other methods eliminate the negative report, drugs quantitated in other methods eliminate the no quantitation report; analytes below their administrative cut-off in target analysis will eliminate reporting these analytes from screens).

1. If GC/MS and/or LC/MS screening is negative, report: "**No drug detected**".
2. Reportable drugs from GC/MS and/or LC/MS screening, for any specimen type, will be reported as "**[Drug name] Detected**".
3. Instrument type(s) will be on the final report.

B. If the volume of sample submitted was initially evaluated to be insufficient to allow for confirmation of any possible analytes, the immunoassay screen may not have been done if investigative information supported using the sample for a screening analysis with more specific identification. The report will include: "**Mass spectral screening was performed for [the] suspected drug[s] [drug name]. No immunoassay screen was performed due to insufficient amount of sample**." The word "drug" may be excluded or changed to category when referring to a class of drugs.

C. If the EMIT indicates the presence of a class of drugs due to a high concentration of a documented cross-reactive drug but could not be confirmed, the report will include: **"[Drug name] was not detected by [GC/MS/LC/MS]. A high concentration of [drug name] is known to produce an immunoassay response for [drug name].**"

D. If results were reported with a lowered objective, the report will include a note: "**[Drug name] was not excluded by the analysis.**"

E. If detection sensitivity is impaired for the batch, the report will include a note for each case with a negative result for that drug unless another testing method has excluded that drug: "**The analytical methodology applied in this case did not exclude [drug name].**"

5 Confirmation by GCMS and/or LCMS

A. Cut-offs

1. Quantitative administrative cut-off is the value of the lowest calibrator used for the batch calibration plot.
2. Qualitative administrative cut-off is the administrative cutoff listed in the analytical method unless the calibration range for the batch is adjusted. (Typically, 50% of the lowest calibrator used for the batch calibration plot.)

B. Reporting of analytes in blood from quantitative calibration plots:

1. For concentrations that are within the calibration range, report as "**[Drug name] [concentration and units]**"
2. Reported drug concentrations are stated as milligrams per liter (mg/L) or nanograms per milliliter (ng/mL), as specified in the analytical method.
3. Reported concentrations and calibration points are truncated (not rounded).
   a) *Concentrations greater than or equal to 0.10 mg/L are reported with two significant digits (e.g. 33 mg/L, 3.3 mg/L, 0.33 mg/L)*

b) *Concentrations between 0.01 mg/L and 0.10 mg/L are reported with one significant digit unless calibrators extend below 0.01 mg/L in which case two significant digits will be used*

c) *Concentrations less than 0.01 mg/L are expressed with one significant digit (e.g. 0.003 mg/L)*

d) *Concentrations greater than or equal to 100 ng/mL are reported with three significant digits (e.g. 255 ng/mL)*

e) *Concentrations less than 100 ng/mL are reported with two significant digits (e.g. 9.9 ng/mL, 99 ng/mL)*

4. For concentrations after truncation that are greater than the high calibration point (xxx), the analyte detected may be reported as "**[Drug name] Greater than xxx [units]**", where xxx is the concentration of the high calibration point.

5. For concentrations that are less than the quantitative administrative cut-off and greater than or equal to the qualitative administrative cut-off, the analyte detected is reported as "**[Drug name] Less than yyy [units]**", where yyy is the concentration of the quantitative administrative cutoff.

6. For concentrations that are less than the qualitative administrative cut-off, the drug is not reported.

7. Concentration values of analytes detected from blood samples whose volume is less than the normal extraction volume (e.g. dilutions due to expected high concentration of an analyte, dilutions due to limited sample availability)

   a) *If the unadjusted concentration of the analyte determined from a diluted sample is within the calibration range, the measured value is adjusted for sample dilution and the analyte detected is reported as "**[Drug name] [adjusted concentration and units]**"*

   b) *If the unadjusted concentration after truncation of the analyte determined from a diluted sample is greater than the high calibration point (xxx), the analyte detected may be reported as "**[Drug name] Greater than zzz [units]**" where zzz is the concentration of the high calibration point (xxx) adjusted for the dilution.*

   c) *If the unadjusted concentration of the analyte determined from a diluted sample is less than the calibration range:*

      i. *If both the unadjusted concentration and the adjusted concentration are greater than or equal to the qualitative administrative cut-off (yyy) and less than the quantitative administrative cut-off, the sample may be reported as **"[Drug name] Less than yyy [units]**" where yyy is the quantitative administrative cutoff.*

      ii. *If the unadjusted concentration is greater than or equal the qualitative administrative cutoff (yyy) and less than the quantitative administrative cutoff and the adjusted concentration is greater than the quantitative administrative cutoff, it may be reported as "**[Drug name] Less than zzz [units]**" where zzz is the quantitative administrative cutoff (yyy) adjusted for the dilution.*

iii. *If the unadjusted concentration is less than the qualitative administrative cutoff (yyy), the drug is not reported unless results from a test of the sample with less dilution meet reporting criteria.*

d) *Adjustment for concentration and administrative cutoff is calculated:*

$$\frac{normal\ volume}{used\ volume} \times unadjusted\ concentration = adjusted\ concentration$$

C. For reporting of analytes in blood from qualitative calibration plots, a drug may be reported as "[Drug name] Detected" if the concentration is equal to or greater than the qualitative administrative cutoff.

D. For reporting of analytes in other biological fluids (e.g. urine, serum), a drug may be reported as "[Drug name] Detected" if the concentration is equal to or greater than the qualitative administrative cutoff.

E. For reporting of analytes in a diluted sample of any biological fluid from qualitative calibration plots, a drug may be reported as "[Drug name] Detected" if the unadjusted concentration is equal to or greater than the qualitative administrative cutoff.

F. If overall analysis is negative, include on the report "No drug detected".

G. If a drug's concentration is below the method qualitative administrative cutoff, it will not be reported from qualitative identification by other methods.

6 Supplemental Reports

A. If a report has been previously issued, the results for additional analysis will be reported.

1. The report for additional analysis will be issued as a supplemental report.
2. An additional note to explain the supplemental report: "**Additional testing was conducted utilizing [method]. The previous report dated mm/dd/yyyy was based on [method].**"
3. Only results of the additional testing not previously reported will be included in the Supplemental Report.
4. The word "different" may be added to specify the additional testing procedure when the instrumental method is the same for both tests.
5. If drugs were reported in the previous report and no drugs were detected during the additional testing, then report "**No additional drugs detected**"

7 Additional Reporting Statements

In all statements in this section, the phrase **[Additional analysis]** may be adjusted to reflect a specific analysis, such as quantitation of an analyte qualitatively found, screening for a suspected drug, or any analysis that would normally be performed had the analysis not been suspended.

A. When a drug exists in multiple isomers with no analytical distinction made, the name of the drug included in the report will be in a general form that will include any possible isomers.

B. All reports for samples tested by GCMS and/or LCMS will indicate the instrument type used for each reported result.

If there is a reportable drug with one instrument, but a second instrument was used with no reportable result, a note will be included: "**[LCMS/GCMS] analysis was also performed on the specimen with no additional drug detected.**"

C. If multiple specimen types are submitted, but not all are tested, a note will be included: "**No analysis performed on exhibit xx due to other exhibits in the case.**"

D. If multiple specimen types are submitted, but not all additional testing is performed, a note will be included: "**[Additional analysis] was not performed on exhibit xx due to other exhibits in the case.**"

E. If a suspected drug cannot be detected by methodology: "**The DPS Crime Laboratory does not test for the suspected drug[s] [drug name].**" The word "drug" may be excluded when referring to a class of drugs.

F. If a suspected drug cannot be detected at some therapeutic levels: "**The analytical methodology applied in this case could not identify the suspected drug[s] [drug name]; however, the concentration of drug may have been below the detection limit of the method.**" The word "drug" may be excluded when referring to a class of drugs.

G. If a suspected drug can be detected by methodology that is not applied because the drug is not considered to be significantly impairing: "**The analytical methodology applied in this case did not test for the suspected drug[s] [drug name].**" The word "drug" may be excluded or changed to category when referring to a class of drugs. Wording may also be changed to indicate not all drugs from a category can be identified.

H. If any/additional analysis is declined by the officer or a representative of the court: "**[Additional analysis] will not be performed [on this item/exhibit] as per customer request.**"

I. If additional analysis will be performed by another laboratory: "**[Additional analysis] for [drug name] will be completed by [laboratory name]**."

J. When the volume of case sample is insufficient for further testing: **"[Additional analysis] was not completed due to insufficient amount of sample**."

K. When a sample is not submitted to laboratory: **"[Additional analysis] was not performed because a sample was not submitted to the DPS Austin Laboratory.**"

L. When the condition of case sample prevents further testing: **"[Additional analysis] was not completed due to condition of the sample.**"

M. When the sample has previously been returned to the agency then re-submitted for additional testing: "**The evidence was returned to the submitting agency on mm/dd/yy and resubmitted to the DPS Austin Laboratory on mm/dd/yy in [evidence description] by [official name] via [submission type].**" (e.g., 6x9 yellow envelope by Jane Doe via in person, 6x9 yellow envelope via U.S. mail) ("in [evidence description]" may be excluded if [evidence description] is same as original submission, "by [official name]" may be excluded if submission was not in person,)

N. When evidence has been separated from original packaging (e.g. sexual assault kit) and forwarded to DPS Austin Laboratory for analysis: "**The [evidence type] specimen[s] was/were forwarded from the DPS [laboratory name] Laboratory in [evidence description].**"

O. When evidence has been separated from original packaging (e.g. sexual assault kit) by the DPS Austin Laboratory: "**The [evidence type] specimen[s] was/were repackaged in [evidence description].**"

P. If the entire sample leaked out of the proximal container and into a nonabsorbent outer container (e.g., a ziplock plastic bag) and is sampled out of the outer container, the report will include the statement: "**Specimen leaked prior to analysis, therefore sampling was not from original container.**"

May add "**and specimen was transferred to a leak proof container.**"

Q. If the evidence is submitted in a container that does not appear to be a standard biological evidence container (e.g., a beverage bottle), the report will include the statement: "**The specimen was submitted in a non-standard container: [description].**"

R. When separate reports are issued for different subjects under the same case number, the report will include the statement: "**Reports are independently issued for relevant subjects under this case number.**"

S. Minor adjustment of wording of a statement for clarification may be applied in special cases with approval documented by Toxicology Section Supervisor, Toxicology Technical Leader, or designee.

8 Report Signatures

A reporting analyst will sign the report containing the reported results. A secondary analyst is one who has worked on the case but has no reported results.

8.1 Closed Before Analysis

When the toxicology analysis has been waived by an official, the analyst that processed the authorization to close case without analysis will sign the report. If there is no such analyst then the Toxicology Section Supervisor will assign an analyst to sign the report. A "Closed Without Analysis" report will be issued.

8.2 Positive Findings

A. For each drug reported as a result of confirmation testing and/or GCMS/LCMS screening: an analyst participating in those tests will be a reporting analyst. If one analyst performs both qualitative and quantitative analysis of the drug, then a secondary analyst performing only qualitative analysis for that drug will not be a reporting analyst. When more than one quantitative value is obtained by multiple analysts and the requirements of reporting quantitative values are met, one of the participating analysts will be a reporting analyst and report the appropriate value (the reporting analyst does not have to be the person whose value is reported).

B. When possible, one reporting analyst will be designated to minimize the number of reporting analysts for each laboratory report. However, only reporting analysts who have been authorized to take ownership of data based on instrumentation and/or methodology may assume responsibility for results.

8.3 Negative Findings

A. If the immunoassay is negative and no additional analysis is performed, the immunoassay analyst will be the reporting analyst.

B. If additional analysis is performed and the chromatographic screen and/or confirmation is negative, the chromatographic screen or confirmation analyst will be the reporting analyst.

C. When possible, one reporting analyst will be designated to minimize the number of reporting analysts for each laboratory report. However, only reporting analysts who have been authorized to take ownership of data based on instrumentation and/or methodology may assume responsibility for results.

8.4 Signatures for Casework Done by Analysts on Leave or No Longer Employed by DPS

If casework was performed by an analyst who is on leave or is no longer employed by DPS, the report will be signed by either an analyst who performed other work on the case or an analyst who is qualified to perform the analysis that was originally performed by the absent analyst.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue
01	08/19/2009	Major Revision – Sections 2.1, 2.2, and 4.4 Integration of report writing guidelines from applicable documents and clarification of text
02	01/20/2010	Major Revision – Sections 2 through 7 Advisory Board 01/11/2010
03	02/03/2014	Major Revision – All Sections
04	09/19/2014	Minor Revision – Section 2 Major Revision – Section 5
05	04/20/2015	Minor Revision – Sections 3, 5, and 7.3 Advisory Board recommendations
06	04/02/2018	Major Revision – Sections 2, 3, 4, 5, 6, and 8
07	05/01/2019	Revised – Sections 2, 3, 4, 5, 6 (now 7), and 7 (now 8) Added – Section 6 Supplemental Reports Removed – Section 8 Case Review

GUIDELINES FOR BATCH ARCHIVE REVIEW

1 Scope

The review of a batch archive is completed to ensure quality control acceptability relevant to a batch.

The individual conducting the review shall be an employee of the Texas DPS Crime Laboratory. They must be a current or previously qualified analyst in the methodologies being reviewed.

2 Related Documents

LOG-03-03 Case Review

TOX-01-06 Case Documentation

3 Practice

3.1 Substance of Batch Archive Review

The following should be examined during review of the batch archive:

A. Required components of batch archive

1. For Immunoassay batches:
   - *a) Batch Archive Summary Sheet*
   - *b) Batch Worklist*
   - *c) Results summary table (LAB-TOX-35)*
   - *d) Log results for initial injections of calibrators*
2. For LCMS/GCMS screening batches
   - *a) Batch Archive Summary Sheet*
   - *b) Batch Worklist*
   - *c) Sequence*
   - *d) Instrument method used*
   - *e) Necessary worksheets and required data for each matrix blank and control injection.*
3. For LCMS/GCMS confirmation batches
   - *a) Batch Archive Summary Sheet*
   - *b) Batch Worklist*
   - *c) Sequence*
   - *d) Instrument method used*
   - *e) Toxicology Measurement Uncertainty Value Sheet, as applicable*
   - *f) Calibration curves*
   - *g) Zero Response sheets (LAB-TOX-15)*
   - *h) Drug reports for matrix blank, calibrators, and controls*

B. Review of Batch Archive

1. The batch archive and required components must have a documented review prior to the reporting of any case samples in the batch.
2. Every page in the batch archive must be initialed by the examiner excluding the Toxicology Measurement Uncertainty Values Sheet.
3. Every batch archive shall contain a batch archive summary sheet that includes:
   - *a) The correct dates for batch ID and all relevant data files.*
   - *b) Relevant lot numbers for blank matrix, internal standard, calibrator working solutions, and control(s), as applicable.*
   - *c) All case numbers examined in the batch, including those that may not have been analyzed to completion.*
   - *d) Instrument, method, column, and processing computer used, as applicable.*
   - *e) Evaluation of quality control for case and/or calibration acceptance.*
   - *f) A list of relevant archive components.*
   - *g) Any additional comments relevant to the batch, including steps taken to achieve acceptable results. Additional comments may need to be acknowledged by the Toxicology Section Supervisor, Toxicology Technical Leader, or designee.*
   - *h) For screens analysis, a table listing the detectability of drugs in each injection of the positive control.*
   - *i) For target analysis, a table including:*
     - *i. Analyte with respective ISTD*
     - *ii. For quantitative analytes, the calibration range with the batch QIAC*
     - *iii. For qualitative analytes, the low calibration point with the batch QIAC*
     - *iv. Evaluation of zero response*
4. Batch review is documented by signature of reviewer and date on the batch archive summary sheet.

3.2 Identification of Potential Technical Inconsistencies or Administrative Issues

A. Any corrections made will be documented in the batch archive. In the event that additional data is generated as a result of review, the original data will also be retained in the batch record.

B. Any corrections made after a completed review of the batch archive must be initialed and dated by the analyst and batch archive reviewer. If corrections to the batch archive summary sheet could potentially affect the case review, the corrected batch archive summary sheet will be replaced in the case folder or electronic equivalent.

C. When the reviewer does not concur with the information in the batch record, the case review resolution process must be followed.

4 Records

A copy of the batch archive summary sheet will be retained in each case folder or electronic equivalent.

All reviewed batch archives are retained electronically.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	05/01/2019	Original Issue

GUIDELINES FOR ADMINISTRATIVE AND TECHNICAL REVIEW

1 Scope

The case review is a thorough technical and administrative review of the case record that is performed in order to ensure compliance with laboratory policy, to verify administrative accuracy, and to assess all analytical data produced in the course of testing.

The individual conducting the administrative and technical reviews shall be an employee of the Texas DPS Crime Laboratory. They must be a current or previously qualified analyst in the methodologies being reviewed.

Technical and administrative reviews may be performed simultaneously or separately. One reviewer will perform both the technical and administrative review of a case record. For an amended report, technical review will be performed by a qualified analyst and administrative review will be performed by the quality manager. The reviewer must be an individual who has not performed any analysis on the case request.

2 Related Documents

LOG-03-03 Case Review

LOG-04-01 Case Record

LOG-04-02 Laboratory Case Reports

TOX-01-06 Case Documentation

TOX-01-08 Report Writing Guidelines

3 Practice

3.1 Substance of Administrative Review

The following should be examined during administrative review:

A. Case Record Documentation

1. All case notes and data in the record generated by an examiner must be labeled with the case number, the handwritten initials of the examiner (or electronic equivalent), and the date generated.
2. Information contained on the laboratory submission form shall be accurately reflected in LIMS for agency information, offense type, subject information, and type of request. Any deviation from the information contained on the laboratory submission form will be supported with documentation in the case record.
3. All information contained in case activities shall be reviewed. Any additional individuals requesting copies of the final report should be included in the LIMS CC list, or will have a copy of the final report emailed to them by the reviewer.
4. Evidence itemization, descriptions, and location shall be reviewed for correctness in LIMS.
5. The LIMS Additional Data for Evidence Screen shall be reviewed for completeness. The reviewer shall ensure that the sample volume, analyst initials, and batch date are entered for the first and the last batch of testing (if more than one analysis is performed).

6. The reviewer shall ensure that the request contains the appropriate reporting statements. This may include but is not limited to: exclusion notes, disposition notes, resubmission notes, additional testing notes, multiple exhibit notes, instrumentation notes, and/or closed without further analysis notes.
7. The reviewer shall view the draft report and ensure the text of the report contains logical and complete statements, the results are consistent with data in the case record, and review for spelling and grammatical accuracy.

3.2 Substance of Technical Review

The following points should be examined during technical review:

A. The reviewer shall ascertain that sufficient and relevant testing was correctly performed based on typical workflows and submitted case information.

B. All data shall be labeled with the case number, the handwritten initials of the examiner (or electronic equivalent), and the date generated.

C. All results shall be entered correctly into LIMS with the batch date entered into the "completed on" column, the instrument used for confirmation tests, and with appropriate units and significant figures, if applicable.

D. Results shall be evaluated based on the type of analysis performed.

1. EMIT Data

   a) *The reviewer will ensure that confirmation testing was conducted on the categories indicating the need for further analysis. If confirmation testing was conducted, the analyst(s) signing the report will take ownership of the EMIT data.*

   b) *The reviewer will ensure that the lower end of the concentration range is listed, that the correct radial button is selected, and that high negative and elevated results are adequately recorded through the use of 50 and 25 respectively, with 80 being added to categories that will be confirmed.*

2. Target Analysis Data

   a) *GCMS- The reviewer will ensure that all data meets criteria for reporting by evaluating concentrations, ion ratios, calibration date/time stamps, retention times, mass spectra, peak integrations, carryover, and the batch archive review summary sheet.*

   b) *LCMS- The reviewer will ensure that all data meets criteria for reporting by evaluating concentrations, MRM ratios, retention times, peak integrations, and the batch archive review summary sheet.*

3. Screens Data

   a) *GCMS-The reviewer will ensure that all data meets criteria for reporting by evaluating ion peaks, mass spectra, co-eluters (if applicable), carryover, library match, retention time, and the batch archive review summary sheet.*

   b) *LCMS- The reviewer will ensure that all data meets criteria for reporting by evaluating ion peaks, mass spectra, fit/reverse fit, retention time, and the batch archive review summary sheet.*

E. The reviewer shall evaluate the batch archive review summary sheets for any information relevant to the technical review of the case. This may include, but is not limited to: cutoffs, control data, qualitative and/or quantitative analytes, calibration ranges, and any notes regarding the batch.

F. The reviewer shall verify all manual calculations. This may include, but is not limited to: dilution adjustments, ion/MRM ratio comparisons, and percent difference between multiple results.

G. The reviewer shall view the draft report and ensure the results on the report reflect the results of the data contained in the case record and that all reporting analysts are on the report.

H. Once technical and administrative review has been completed, the reviewer will change both milestones in LIMS.

3.3 Identification of Potential Technical Inconsistencies or Administrative Issues

A. Any corrections made will be documented in the case record. In the event that additional data is generated as a result of review, the original data will also be retained in the case record.

B. When the reviewer does not concur with the laboratory case report and/or information in the case record, the case review resolution process must be followed.

4 Records

Technical and administrative reviews will be documented in LIMS.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	05/01/2019	Original Issue

SYSTEMATIC EXAMINATION OF TOXICOLOGICAL SPECIMENS

1 Scope

To describe a basic analytical scheme, utilizing screening tests, extraction techniques, and instrumental analytical procedures for the identification and/or quantitation of compounds of toxicological significance.

2 Safety

A. Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

B. A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

C. Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

D. Use caution and proper technique when using sharp instruments to cut into evidence packaging.

3 Quality Assurance

A. Case Sample Identification

1. Evidence is assigned a laboratory case number and an evidence exhibit identifier
2. Multiple evidence containers may be collectively considered as a single evidence exhibit if:
   - *a) Samples are from the same individual,*
   - *b) Samples were collected within 30 minutes of each other,*
   - *c) Samples are of the same type, and*
   - *d) samples are in the same type of blood tube or container.*
3. Laboratory case number and associated name identification (or other unique information) are matched to the sample container when aliquots of sample are drawn.
4. The laboratory case number and evidence exhibit identifier (if needed to distinguish exhibits) are used in labeling sample containers during processing, to identify case documents and reports, and to match sample containers to instrument sequence lists..

B. Protection of samples from cross contamination

1. The laboratory case number is used to match tubes when transfers are made from one tube to another during batch extraction steps.
2. Extraction tubes are handled carefully to avoid splashes or drips from one tube to another.

3. Disposables are used for sample processing.

   a) *Tubes (16X100 with cap or screw cap) referred to in the analytical methods are for single use at one stage of processing of one sample. Later references in an extraction to a clean tube refer to a new tube rather than a tube that has been washed. This assures a clean tube with no chemical interferences and eliminates drug cross contamination from any previous usage.*

   b) *Different pipette tips are used for each case during sampling.*

   c) *The same pipette tip may be used for multiple dispensing of a solution to samples being processed without contact with the sample.*

   d) *Solid Phase Extraction (SPE) columns are for single use for extraction of one sample.*

C. Specific identification: All reported drugs are identified by GCMS and/or LCMS. Non-confirmed immunoassay screen results will include a qualifying statement on the report.

D. Glassware (e.g., large volumetric flasks, volumetric pipettes, and graduated cylinders) used for preparing and storage of chemical solutions are used only for delivery to a sample and are not used as containers for samples or drug standards where they could be contaminated with drugs.

4 Procedure

4.1 Retrieval and Initial Examination of the Evidence

1. Prepare and review a batch worklist that includes information for reference at the biological hood when drawing aliquots of samples.

Note: The case folder will not be available in the sampling area nor is it handled with gloves used in handling samples.

2. Retrieve evidence containers from evidence storage or evidence custodian following laboratory policy concerning barcodes and the transfer of the items to the examiner.

3. Select the evidence container(s) to sample with evaluation of the types of sample available, the time of collection, the type of tube or container (preservative) available, and the amount of sample in each different container.

4. If multiple containers with sufficient sample for required analysis are present in an evidence package, apply the following criteria:

   a) *For general toxicological testing, prioritize by type of sample (listed in decreasing priority):*

      i. *Peripheral Blood*

      ii. *Heart Blood*

      iii. *Urine*

      iv. *Tissue with biological fluid (if no blood or urine samples submitted: biological fluid in the container is screened by immunoassay,*

GCMS, and/or LCMS, then referred for quantitative analysis if requested)

v. *Gastric contents (screened by immunoassay, GCMS, and/or LCMS, then referred for quantitative analysis if requested)*

b) *Analysis for cases with both blood and urine submitted:*

i. *For sexual assault cases, urine is the preferred specimen. If both blood and urine specimens are submitted, perform analysis on the urine only.*

ii. *For driving impairment cases, blood is the preferred specimen. After immunoassay screen on the blood is completed, evaluate the need for sampling urine on a case by case basis. If urine is not tested, a note will be included on the report.*

iii. *For all other types of cases (e.g., homicides, questioned deaths, suicides, accidental deaths), determine appropriate sample type to be tested on a case by case basis. The Toxicology Section Supervisor or Toxicology Technical Leader may assist with sample selection.*

c) *Once the sample type to be tested is determined, priorities for container selection in decreasing order may vary depending on the time of collection and collection container. The factors in decreasing order of preference include:*

i. *For sample containers with various times of collection indicated, select the container(s) with the shortest elapsed time after the incident unless specifically noted by requestor to test a different tube.*

- *Make a note on the batch worklist of the time from the container.*
- *Samples in separate containers that are collected within 30 minutes of one another are collectively considered as the same time for the evaluation of the best tube type available.*

ii. *Types of blood tubes (or equivalently preserved sample in other containers):*

- *Gray top tubes with sodium fluoride and potassium oxalate preservative (particularly important for cocaine analysis)*
- *Purple top tubes with EDTA anticoagulant*
- *Red top tubes with no preservative added*
- *Tubes of other color tops for various preservatives*
- *Serum separator tubes*

5. If multiple containers (e.g. blood tubes) are present in an evidence package with insufficient sample in the preferred container for all required analysis, select the container for each type of analysis using priorities above as much as possible. Possible compromises to provide as much information as possible include:

   a) *If a container is available with a lower preferred preservative or type of sample, preserve sufficient sample from gray top tube for cocaine confirmation (unless ruled out by immunoassay).*

   b) *If urine is available, preserve sufficient blood for 2 confirmation analyses (minimum 2 mL) until urine immunoassay is complete and assess options.*

   c) *If choosing a container from various collection times, preserve the one with the shortest elapsed time after the incident for confirmation testing until immunoassay screening is completed on another container collected within the hour after the initial container is collected.*

6. If limited sample is available to complete all anticipated analysis, adjust volume of sample aliquot with consideration of possible compromises to provide as much information as possible:

   a) *Divide sample equally between types of analysis involved.*

   b) *Divide sample proportional to normal aliquots for each type of analysis involved.*

   c) *Decrease the proportion allotted to the type of analysis with the range of immunoassay response highest compared to the lower limit of quantitation for its confirmation.*

7. If below evidence is submitted, testing will not be performed but noted and referred to a different laboratory, if requested.

   a) *Tissue - liver, kidney, brain (not tested if biological fluid is not present, but noted and referred if requested)*

   b) *Hair*

   c) *Blood or gastric residues*

   d) *Vitreous fluid*

8. Perform the confirmation that will provide the most significant information regarding impairment and allow qualitative testing for other drugs (e.g. prioritize the confirmation of a depressant at a high level over a low level stimulant, prioritize the confirmation of the type of drug with relatively equivalent significance to another type by choosing the analytical method that allows extraction of both drug types, quantitative analysis of the one type, and qualitative analysis of data for the other).

9. Compare the information on the batch worklist to the physical evidence to verify that:

   a) *Sufficient information (e.g. name, agency number) is available to uniquely identify the specimen exclusive of the lab case number or a*

note is made of other information to be used for comparison to the case folder.

b) Information is consistent regarding the sample type and case number, or a note is made of the inconsistency.

10. Mark the specimen container(s) sampled with the laboratory case number (unless previously marked), examiner's handwritten initials, evidence exhibit identifier (if needed to distinguish exhibits), and the date sampled for analysis.
11. Work in a biological hood while preparing aliquots of samples with the worklist as a reference.
12. Assure uniformity of blood samples by completely inverting specimen container several times before sampling.
13. Make an entry on the batch worklist noting the volume of sample aliquot, unusual characteristics of the specimen (e.g. clotted, decomposed), and unusual processing steps (e.g. used tissue grinder).
14. Add a note to the batch worklist for later documentation in the case file to describe changes (e.g. leakage, transfer to new container, evidence tube breakage).
15. Add a note to the batch worklist for later documentation in the case file to describe unique information to differentiate multiple containers so that the report can include an unambiguous identification (e.g. evidence exhibit identifier).
16. Following sample preparation and analysis, specimen container(s) should be re-packaged in the original container as soon as practical.
17. Store the evidence in a secure refrigerator until its final disposition.
18. Transfer notes from the batch worklist to the case record.

4.2 Evidence Sampling

1. Allow specimens, controls, and reagents to equilibrate to room temperature.
2. Setup and label tubes for samples, calibrators, blanks, and controls as listed on the batch worklist.
3. A volume of the sample appropriate to the type of analysis is withdrawn in order as listed on the batch worklist:

   a) If clotted blood is present in a container, the entire contents should be ground in a clean glass tissue grinder to a homogeneous mixture.

   b) If there is insufficient specimen or dilution ofsamples is necessary, lower volumes of sample may be used with an addition of an appropriate and valid matrix blank, appropriate buffer, or without addition of a matrix or buffer (however method validation dictates). The analysis results may be adjusted for the requisite dilution.

4.3 Basic Analytical Scheme

The basic analytical scheme for the analysis of toxicological specimens consists of extraction of drugs from biological matrix for instrumental analysis.

A. Samples may be extracted to recover drugs of concern and eliminate interferences from the matrix. Recovery and "cleanup" varies according to the drugs and samples analyzed. Extraction methods for each drug or group of drugs are specified in methods. Any deviation (e.g. spillage, breakage of tubes, sample issues, equipment malfunction) for a sample or control during extraction is documented on the batch worklist for consideration when processing data.

B. Analytical methods

1. Drugs in biological specimens are typically screened using the following types of testing:

   a) *Immunoassay analysis will detect if certain drugs of targeted classes are in a sample. Results are evaluated relative to cutoff levels and relative response of potential drugs in a case. These methods are efficient to screen for a large number of abused drugs, classes of drugs, and samples.*

   b) *GCMS and/or LCMS drug screening is used for general detection of drugs which immunoassay analysis would not detect. The extraction process for GCMS and/or LCMS screening is designed to recover as many types of drugs as possible. While screening by GCMS and/or LCMS gives identification of a specific drug, it may be followed by GCMS or LCMS confirmation testing to determine the quantity of drug that is present.*

2. Drugs in biological specimens are confirmed by GCMS and/or LCMS (target compound analysis) when screening detects particular drugs or classes of drugs. Confirmation testing may include quantitative calculations. Drugs passing the identification criteria are reported.

C. Variation from Basic Analytical Scheme

1. Judgment of the examiner and consultation with the submitting official is needed for some cases with limited samples. Emphasis is on providing as much information as possible with careful selection of tests.

2. Samples with insufficient volume for normal testing may be:

   a) *Screened for drug classes by immunoassay with positive drug classes indicated with an appropriate disclaimer.*

   b) *Screened by GCMS and/or LCMS for a large group of drugs with only identification reported.*

   c) *Tested with target testing for one group of analytes to give specific identification and quantitation of drugs within that group. If the sample preparation is appropriate for general screening, the prepared extract should be re-injected with data collection to allow a GCMS and/or LCMS screen.*

3. Samples with variations from the scheme will have documentation to explain the variation and its choice.

   a) *A sample from a deceased individual may be best utilized to screen by immunoassay to exclude or indicate drugs from the largest group possible with the one test.*

b) *A sample from a living suspect with external information indicating a limited group of drugs may be best utilized with a target test and possible GCMS and/or LCMS screening to confirm the most information. The testing would be "blind" to all other drugs.*

c) *A sample from a living suspect with external non-specific information indicating drug impairment may be best utilized with GCMS and/or LCMS screening to identify a large pool of drugs.*

4. Unusual samples or tests are referred to another laboratory with methods set up for that analysis to avoid analytical delay for the case and to maximize laboratory efficiency in serving the needs of the most customers.

4.4 Concluding Examination and Disposition of the Evidence

A. All original exhibits should be re-packaged in the original container if possible. The evidence should be re-sealed in a manner that would detect tampering. If samples are taken from an exhibit for preservation, such as in bulk cases, the samples will be packaged appropriately and sealed in a manner that would detect tampering.

B. The evidence should be transferred to the evidence custodian or filed in the refrigerated laboratory vault until its final disposition.

5 Record

Batch Worklist

Laboratory Submission Form (Form LAB-201 and/or LAB-203)

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	07/01/2003	Original Issue
01	10/20/2006	Major Revision – restructuring of documents and substantive changes to content
02	06/03/2009	Major Revision – Sections 3, 4, 6.1, and 6.3 Minor Revision – Sections 1 and 5 Implementation of LCMS, evidence exhibit identification practices and clarification of text
03	01/20/2010	Major Revision – Sections 6.1, 6.3, and 6.4 Minor Revision – Sections 3 and 7 Advisory Board 01/11/2010
04	02/03/2014	Major Revision – Sections 3, 5, 6.1, 6.3, and 7
05	05/01/2019	Removed – Sections 4 Calibration and 5 Controls Revision – Sections 2, 3, 6 (now 4), and 7 (now 5)

DISPOSITION OF TOXICOLOGICAL EVIDENCE

1 Scope

The following practices are to direct the disposition of evidence or the forwarding of evidence to a non-DPS laboratory for analysis. At this time, the DPS Austin Laboratory is the only DPS laboratory that has the capability to conduct drug toxicology.

2 Related Documents

Disposition of Toxicological Evidence (BA-01-03)

3 Practices

3.1 Disposition of Toxicological Evidence

A. Toxicological evidence will be preserved in a refrigerated environment while in DPS custody.

B. For evidence with alcohol and drug results, disposition will be as follows:

1. Non-DPS Evidence will be returned to the original submitting agency.
2. DPS evidence will be retained until court authorization for destruction is received.

C. Alternate arrangements with submitting agencies or laboratories may always be made and will supersede previously defined dispositions.

3.2 Submission to Non-DPS Toxicology Laboratory

A. Unusual samples or tests, in consultation with the customer, may be referred to another laboratory after determining it has methods validated for that analysis.

1. Fees for forensic testing for referred cases are the responsibility of the submitting agency, prosecutor's office, or the requester. Communication between the referral laboratory and any agency will be directed to that responsible individual rather than through the DPS Laboratory.
2. Evidence referred for testing for criminal cases must be forwarded to forensic laboratories accredited by the Texas Forensic Science Commission.
3. Arrangements should be made to forward only a portion of the sample if possible. Shipping arrangements for samples forwarded for referred testing may be at DPS Laboratory expense.

B. Samples with a court order to be released for re-analysis in conjunction with criminal cases will be released to the designated entity.

C. Samples with a court order to be released for re-analysis in conjunction with civil cases will be released to the designated entity after ensuring all criminal proceedings have been finalized.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	10/20/2006	Original Issue
01	01/20/2010	Major Revision – All sections Advisory Board 01/11/2010
02	02/03/2014	Major Revision – Sections 2.1, 2.3
03	09/02/2015	Major Revision – All sections
04	04/02/2018	Major Revision – Section 3

SCREENING OF BLOOD SAMPLES BY EMIT

1 Scope

Classes of drugs in blood, serum/plasma and gastric content samples can be detected by Enzyme Multiplied Immunoassay Technique (EMIT) immunoassay analysis. Extraction and purification of analytes in blood specimens is accomplished with a protein precipitate extraction. Target classes for screening include amphetamines, barbiturates, benzodiazepines, cocaine and its metabolites, opiates, phencyclidine, carisoprodol/meprobamate, THC and its metabolite, oxycodone, and zolpidem. Semi-quantitative measurement is obtained by comparison to calibrators with increasing concentrations of a selected drug from each class.The cutoff level is based on significant concentrations of drugs in each class and confirmation capabilities.

2 Safety

A. Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

B. A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

C. Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Preparation of Tris Buffer (TOX-07-36)

Preparation of Tris Buffer in Methanol (TOX-07-37)

Preparation of Acidified Ethanol (TOX-07-02)

Verification of EMIT Reagents (TOX-07-18)

Immunoassay Instructions (TOX-INS-09)

XcelVap Evaporator System Instructions (TOX-INS-17)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Appropriate sized pipette with disposable tips
- Repeater pipette with appropriate sized tips
- Vortexer
- Centrifuge
- Sample evaporator
- Immunoassay analyzer
- Bottletop Dispenser

4.2 Materials

- Test tubes and caps
- EMIT sample cups and tubes
- EMIT reagent containers

4.3 Chemicals

- H_2O (purified water)
- Hydrochloric acid (HCl): ACS grade
- Ethanol (190-200 proof)
- Acetone (high purity solvent suitable for trace analysis)
- Methanol (MeOH) (high purity solvent suitable for trace analysis)
- 1.0 mg/mL benzoylecgonine stock solution
- 1.0 mg/mL morphine stock solution
- 1.0 mg/mL amphetamine stock solution
- 1.0 mg/mL butalbital stock solution
- 1.0 mg/mL phencyclidine stock solution
- 1.0 mg/mL alprazolam stock solution
- 1.0 mg/mL hydrocodone stock solution
- 1.0 mg/mL meprobamate stock solution
- 1.0 mg/mL oxycodone stock solution
- 1.0 mg/mL zolpidem stock solution
- 100 µg/mL (-)-11-nor-9-Carboxy-Δ9-THC stock solution
- Microgenics acid wash solution
- Microgenics base wash solution

4.4 Solutions - Different volumes may be prepared in the same proportions as specified.

1. Tris buffer
2. Tris buffer: MeOH
3. Acidified Ethanol
4. 2% alkaline wash solution

   Mix 20 mL Microgenics Base Wash solution in 1000 mL H_2O.
5. 1% acid wash solution

   Mix 10 mL Microgenics Acid Wash solution in 1000 mL H_2O.
6. 100 mg/L phencyclidine solution

   Dilute 500 µL phencyclidine stock solution to 5 mL with MeOH in volumetric flask.
7. 100 mg/L alprazolam and oxycodone solution

   Dilute 500 µL alprazolam stock solution and 500 µL oxycodone stock solution to 5 mL with MeOH in volumetric flask.

8. 100 mg/L zolpidem solution

   Dilute 500 µL zolpidem stock solution to 5 mL with MeOH in volumetric flask.

4.5 Immunoassay Reagents

1. Amines: DRI reagents A and E (purchased prepared)
2. Carisoprodol: Immunalysis reagents A and E (purchased prepared)
3. Cocaine: DRI reagents A and E (purchased prepared)
4. Opiates: DRI reagents A and E (purchased prepared)
5. Barbiturates: DRI reagents A and E (purchased prepared)
6. Benzodiazepines: DRI reagents A and E (purchased prepared)
7. Phencyclidine: DRI reagents A and E (purchased prepared)
8. THC: DRI reagents A and E (purchased prepared)
9. Zolpidem: Immunalysis reagents A and E (purchased prepared)
10. Oxycodone: DRI reagents A and E (purchased prepared)

5 Standards, Controls, and Calibration

A. Calibrators:

1. Drug Standards: benzoylecgonine, morphine, amphetamine, butalbital, alprazolam, phencyclidine, meprobamate,carboxy-THC, oxycodone, and zolpidem.
2. Calibrator series is 0.05, 0.1, 0.3, and 0.9 mg/L for benzoylecgonine and morphine.
3. Calibrator series is 0.50, 1.0, 2.0, and 6.0 mg/L for butalbital.
4. Calibrator series is 0.02, 0.04, 0.05, and 0.15 mg/L for phencyclidine.
5. Calibrator series is 0.01, 0.02, 0.1, and 0.3 mg/L for alprazolam.
6. Calibrator series is 0.05, 0.1, 0.4, and 1.2 mg/L for amphetamine.
7. Calibrator series is 1.0, 2.0, 4.0, and 12.0 mg/L for meprobamate.
8. Calibrator series is 7.5, 15, 25, and 75 ng/mL for carboxy-THC.
9. Calibrator series is 0.01, 0.02, 0.1, and 0.3 mg/L for oxycodone.
10. Calibrator series is 0.01, 0.02, 0.06, and 0.18 mg/L for zolpidem.
11. Sample volume is 1 mL.
12. Blood matrix blank
13. Low multi-drug working solution (2 mg/L benzoylecgonine, morphine, and amphetamine, 20 mg/L butalbital, 0.8 mg/L phencyclidine, 0.4 mg/L alprazolam, oxycodone, and zolpidem, 40 mg/L meprobamate, 0.3 mg/L carboxy-THC)

    Dilute 20 µL benzoylecgonine stock solution, 20 µL morphine stock solution, 200 µL butalbital stock solution, 20 µL amphetamine stock solution, 80 µL 100 mg/L phencyclidine solution, 400 µL meprobamate stock solution, 30 µL

carboxy-THC stock solution, 40 µL 100 mg/L alprazolam and oxycodone solution, and 40 µL 100 mg/L zolpidem solution to 10 mL with MeOH in volumetric flask.

14. High multi-drug working solution (12 mg/L benzoylecgonine and morphine, 80 mg/L butalbital, 16 mg/L amphetamine, 2 mg/L phencyclidine, 4 mg/L alprazolam and oxycodone, 160 mg/L meprobamate, 1.0 mg/L carboxy-THC, 2.4 mg/L zolpidem)

    Dilute 120 µL benzoylecgonine stock solution, 120 µL morphine stock solution, 800 µL butalbital stock solution, 160 µL amphetamine stock solution, 200 µL 100 mg/L phencyclidine solution, 1.6 mL meprobamate stock solution, 100 µL carboxy-THC stock solution,400 µL 100 mg/L alprazolam and oxycodone solution, and 240 µL 100 mg/L zolpidem solution to 10 mL with MeOH in volumetric flask.

15. Hydrocodone cutoff solution (1 mg/L hydrocodone)

    Dilute 25 µL hydrocodone stock solution to 25 mL with MeOH in volumetric flask.

NOTE: Different volumes of working solutions may be prepared in the same proportions as specified.

B. Calibration

1. Process (extract and acquire data) calibrators and cutoff control under the same conditions as samples.
2. Each assay class is evaluated independently. Batch results are acceptable if:
   a) *The results of the calibrator re-injection (usually level 5) are within 15% of the first injection.*
   b) *The difference between the cutoff and the negative is greater than or equal to the minimum acceptable difference as established through historical data or as established for the current lot number.*
3. For any unacceptable class results, evaluate cause and document steps taken to achieve acceptable results in the batch archive. If QC criteria is still not met, the entire batch will be repeated.

6 Procedure

6.1 Extraction

1. Allow specimens, matrix blank, working solutions, and hydrocodone cutoff solution to equilibrate to room temperature.
2. Add a 1 mL aliquot of the specimen to clean, labeled tubes.
3. Prepare calibrator samples by adding the specified amounts of working standard solution to 1 mL blank blood in clean, labeled 16X100 mm tubes.
   a) *Negative: Add 1 mL blank blood.*
   b) *High negative: Add 25 µL low multi-drug working solution to 1 mL blank blood*
   c) *Cutoff: Add 50 µL low multi-drug working solution to 1 mL blank blood*

d) *Level 4: Add 25 µL high multi-drug working solution to 1 mL blank blood*

e) *Level 5: Add 75 µL high multi-drug working solution to 1 mL blank blood*

f) *Additional Level 5: Add 75 µL high multi-drug working solution to 1 mL blank blood*

g) *Hydrocodone cutoff: Add 20 µL hydrocodone cutoff solution to 1 mL blank blood*

4. Vortex each calibrator sample and allow to equilibrate for approximately 10 minutes.
5. Add 3 mL acetone, cap, and shake and/or vortex.
6. Vortex all samples for approximately 30 seconds.
7. Centrifuge to pack solids (e.g. 2629 x *g* for twelve minutes).
8. Decant acetone supernatant into a clean, labeled tube.
9. Add 50 µL of acidified ethanol.
10. Evaporate to dryness in an evaporator (e.g. 55ºC for 30 minutes at 5-20 psi).
11. Add 0.5 mL Tris buffer: MeOH to each sample and cap.
12. Vortex for approximately 30 seconds to dissolve residue.
13. Combine the two Level 5 calibrator samples into one test tube and vortex.
14. Centrifuge all samples to pack solids (e.g. 2629 x *g* for twelve minutes)
15. Decant the supernatant into sample cups in the barcoded tubes on the sample trays.

6.2 MGC240 Analysis

1. Ready the instrument after the lamp has warmed up for approximately 30 minutes.
2. Set the current round to blood and check the number of tests remaining per assay as estimated by the software. Fill the assay reagents if needed.
3. Check the reagents lot numbers in the reagent tray and their expiration dates. The reagents must have satisfied the QC requirements for the lot when received. (Note: The EMIT reagents are provided as matched sets. They should not be interchanged with components of kits with different lot numbers.)
4. Order tests for the unknowns, including a second sampling of the level 5 calibrator (if sample volume for the level 5 calibrator is limited, it is acceptable to re-test another calibrator in its place). Group all unknowns as a batch.
5. Process the negative, calibrators, and hydrocodone cutoff with addition of reagents and readings of changes in optical density of solutions.
6. Process unknown samples by addition of reagents and readings of changes in optical density of solutions.
7. Prepare the batch archive with the worklist, EMIT Results page (LAB-TOX-35), and the log data from MGC 240.

8. Acceptability of the batch shall be reviewed by a second qualified analyst. The batch reviewer will sign and date the batch archive review sheet to document batch acceptability.

9. Prepare the individual EMIT Results pages and evaluate data from cases for reporting. The individual EMIT Results page (LAB-TOX-35a) and batch archive review sheet will be retained in the case record.

7 Interpretation

A. Evaluate response results for each case sample to determine the range in which it fits in each drug class.

1. Negative: Responses ranging from the response for the negative calibrator (level 1) up to the response for the high negative calibrator (level 2). Within this response range, a response for a sample is considered elevated if there is a response distinguishable above the negative calibrator.

2. Elevated:

   a) *For Opiate category, responses ranging from the response for the Hydrocodone cut-off up to the response for the high negative calibrator (level 2).*

   b) *For PCP category, responses ranging from the calculated PCP cut-off up to the response for the high negative calibrator (level 2).*

$$Calculated\ PCP\ cutoff = ((Level\ 3\ response - Level\ 1\ response) * 50\%) + Level\ 1\ response$$

   c) *For other categories, *elevated responses are considered those close to the high negative response and will be confirmed.*

3. High Negative: Responses ranging from the high negative calibrator (level 2) up to the cutoff calibrator (level 3).

4. Positive: Responses ranging from the cutoff calibrator (level 3) and above.

B. Identify all cases that require further analysis by category and range to designate these cases for GCMS and/or LCMS confirmation.

C. Identify all cases that are negative for all classes.

D. Evaluate all cases with additional information provided regarding suspected drugs or type of offense. Designate cases requiring further analysis for GCMS and/or LCMS confirmation and screening.

E. Record information for each case in the case record and/or on the pending analysis list.

8 Limitations

8.1 Specificity of Immunoassay Analysis

Immunoassay tests detect multiple drugs within a class of similar drugs. There is a variation in the amount of response to specific drugs. Quantitative estimates are based on one drug from a class and may be significantly different for other drugs within that class. Interpretation is subject to evaluation of any drugs specifically suspected and their relative response to the calibrator drug.

9 Literature and Supporting Documentation

Lewellen LJ, and McCurdy HH. A Novel Procedure for the Analysis of Drugs in Whole Blood by Homogeneous Enzyme Immunoassay (EMIT). J Anal Tox. 1988. 12, pp. 260-267.

Microgenics DRI reagent information

Immunalysis reagent information

10 Records

Blood EMIT Results Page (LAB-TOX-35)

Individual Blood EMIT Results Page (LAB-TOX-35a)

Batch Archive Review Sheet

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
…		Previous revision history in archived copy of documents.
06	01/20/2010	Major Revision – Sections 1, 3 - 7, and 9 Minor Revision – Section 8 Advisory Board 01/11/2010
07	02/15/2013	Major Revision- Section 5, 6
08	02/03/2014	Major Revision- Sections 3-6
09	04/14/2014	Major Revision – Sections 1, 4.3, 4.4, 4.5, and 7 Minor Revision – Section 7
10	04/02/2018	Major Revision – Sections 1, 3, 4, 5, 6, and 7
11	05/01/2019	Revision – 1, 3, 4, 5, 6, 7, and 8 Added – Section 10 Records

SCREENING OF URINE SAMPLES BY EMIT

1 Scope

Classes of drugs in urine samples can be detected by EMIT immunoassay analysis. Target classes for screening include amphetamines, barbiturates, benzodiazepines, cocaine and its metabolites, opiates, phencyclidine, carisoprodol/meprobamate, and THC/metabolite. Semi-quantitative measurement is obtained by comparison with multiple levels of a selected drug from each class. Detection is based on the response of the sample compared to the cutoff level response. The cutoff level is based on significant concentrations of drugs in each class and confirmation capabilities.

2 Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Controls (TOX-01-04)

Verification of EMIT Reagents (TOX-07-18)

4 Equipment, Materials, and Reagents

Equipment

- Appropriate sized pipette with disposable tips
- Repeater pipette with appropriate sized tips
- Centrifuge
- Immunoassay analyzer

Materials

- Test tubes and caps
- EMIT sample cups and tubes
- EMIT reagent containers

Chemicals

- H_2O (purified water)
- Microgenics Acid Wash solution
- Microgenics Base Wash solution
- 1.0 mg/mL meprobamate stock solution
- 1.0 mg/mL d-methamphetamine stock solution

Solutions

- 2% alkaline wash solution

  Mix 20 mL Microgenics Base Wash solution in 1000 mL H_2O.
- 1% acid wash solution

  Mix 10 mL Microgenics Acid Wash solution in 1000 mL H_2O.
- Different volumes may be prepared in the same proportions as specified.

Immunoassay Reagents

- Amines: DRI reagents A and E (purchased prepared)
- Carisoprodol: Immunalysis reagents A and E (purchased prepared)
- Cocaine: DRI reagents A and E (purchased prepared)
- Opiates: DRI reagents A and E (purchased prepared)
- Barbiturates: DRI reagents A and E (purchased prepared)
- Benzodiazepines: DRI reagents A and E (purchased prepared)
- Phencyclidine: DRI reagents A and E (purchased prepared)
- THC: DRI reagents A and E (purchased prepared)

5 Standards, Controls, and Calibration

A. Calibrators

1. Drug standards: meprobamate and d-methamphetamine
2. High meprobamate/methamphetamine working solution (200 mg/L meprobamate, 24 mg/L d-methamphetamine)

   Dilute 1.0 mL meprobamate stock solution and 120 µL d-methamphetamine stock solution to 5 mL with H_2O in volumetric flask. Different volumes may be prepared in the same proportions as specified.
3. Low meprobamate/methamphetamine working solution (40 mg/L meprobamate, 6 mg/L d-methamphetamine)

   Dilute 200 µL meprobamate stock solution and 30 µL d-methamphetamine stock solution to 5 mL with H_2O in volumetric flask. Different volumes may be prepared in the same proportions as specified.
4. Urine matrix blank: DRI Negative Urine Calibrator or other negative urine matrix blank
5. Meprobamate/methamphetamine EMIT urine calibrator level 2 containing:

   a) Meprobamate: 1000 ng/mL

   b) d-Methamphetamine: 150 ng/mL

   c) Dilute 250 µL low meprobamate/methamphetamine working solution to 10 mL with blank urine in volumetric flask. Different volumes may be prepared in the same proportions as specified.
6. Meprobamate/methamphetamine EMIT urine calibrator level 3 containing:

a) *Meprobamate: 2000 ng/mL*

b) *d-Methamphetamine: 300 ng/mL*

c) *Dilute 500 µL low meprobamate/methamphetamine working solution to 10 mL with blank urine in volumetric flask. Different volumes may be prepared in the same proportions as specified.*

7. Meprobamate/methamphetamine EMIT urine calibrator level 4 containing:

   a) *Meprobamate: 5000 ng/mL*

   b) *d-Methamphetamine: 600 ng/mL*

   c) *Dilute 250 µL high meprobamate/methamphetamine working solution to 10 mL with blank urine in volumetric flask. Different volumes may be prepared in the same proportions as specified.*

8. Meprobamate/methamphetamine EMIT urine calibrator level 5 containing:

   a) *Meprobamate: 10000 ng/mL*

   b) *d-Methamphetamine: 1200 ng/mL*

   c) *Dilute 500 µL high meprobamate/methamphetamine working solution to 10 mL with blank urine in volumetric flask. Different volumes may be prepared in the same proportions as specified.*

9. DRI Multi-Drug Urine Calibrator 1, 2, 3, and 4 containing:

   a) *Secobarbital: 100, 200, 500, and 1000 ng/mL*

   b) *Oxazepam: 100, 200, 500, and 1000 ng/mL*

   c) *Benzoylecgonine: 150, 300, 500, and 1000 ng/mL*

   d) *Phencyclidine: 12.5, 25, 50, and 100 ng/mL*

10. DRI Opiate Urine Calibrator 1 and Calibrator 3

    Morphine: 150 and 500 ng/mL

11. DRI Drugs of Abuse Urine Calibrators Low and High

    Morphine: 300 and 1000 ng/mL

12. DRI 20 ng/mL THC Urine Calibrator

    11-nor-Δ^9-THC-9-carboxylic acid: 20 ng/mL

13. DRI 50 ng/mL THC Urine Calibrator

    11-nor-Δ^9-THC-9-carboxylic acid: 50 ng/mL

14. DRI 100 ng/mL THC Urine Calibrator

    11-nor-Δ^9-THC-9-carboxylic acid: 100 ng/mL

15. Urine calibrator series

Urine Calibrators (ng/mL)

	Level 1 (NEGATIVE)	Level 2	**Level 3 (CUTOFF)**	Level 4	Level 5
Amphetamines	0	150	**300**	600	1200
Barbiturates	0	100	**200**	500	1000
Benzodiazepines	0	100	**200**	500	1000
Cocaine	0	150	**300**	500	1000
Opiates	0	150	**300**	500	1000
Phencyclidine	0	12.5	**25**	50	100
Carisoprodol	0	1000	**2000**	5000	10000
THC	0	-	**20**	50	100

B. Controls

1. Drug standards: meprobamate and d-methamphetamine
2. Meprobamate/methamphetamine control working solution (50 mg/L meprobamate, 7.5 mg/L d-methamphetamine)

   Dilute 250 µL meprobamate stock solution and 37.5 µL d-methamphetamine stock solution to 5 mL with H_2O in volumetric flask. Different volumes may be prepared in the same proportions as specified. **NOTE: The stock standard solutions must be from a different source than those used in the working solutions.**
3. Meprobamate/methamphetamine low control containing:
   a) *Meprobamate: 1500 ng/mL*
   b) *d-Methamphetamine: 225 ng/mL*
   c) *Dilute 300 µL meprobamate/methamphetamine control working solution to 10 mL with blank urine in volumetric flask. Different volumes may be prepared in the same proportions as specified.*
4. Meprobamate/methamphetamine high control containing:
   a) *Meprobamate: 2500 ng/mL*
   b) *d-Methamphetamine: 375 ng/mL*
   c) *Dilute 500 µL meprobamate/methamphetamine control working solution to 10 mL with blank urine in volumetric flask. Different volumes may be prepared in the same proportions as specified.*
5. Purchased MGC Primary DAU Control Set (Low and High) containing:
   a) *Secobarbital: 150 and 250 ng/mL*
   b) *Oxazepam: 150 and 250 ng/mL*
   c) *Benzoylecgonine: 225 and 375 ng/mL*
   d) *Phencyclidine: 19 and 31 ng/mL*
6. Purchased MGC Clinical DAU Control Set (Low and High)

   Morphine: 225 and 375 ng/mL

7. DRI 40 ng/mL THC Urine Control

   11-nor-Δ^9-THC-9-carboxylic acid: 40 ng/mL

8. DRI Negative Urine Calibrator used as THC Low Control

   11-nor-Δ^9-THC-9-carboxylic acid: 0 ng/mL

C. Calibration

1. Process (acquire data) calibrators under the same conditions as controls and samples.
2. Evaluate the negative, calibrator, and control responses by comparison to previous batch data. There should not be significant changes from batch to batch. Each assay class is evaluated independently. Results for the batch are acceptable if:
   a) *The low and high controls are within acceptable ranges established for the current lot. (±30% of the lab established value).*
   b) *The calculated concentration of the calibrator reinjection (usually level 4) is within 30% of the target concentration or absorbance is within 15% of the first injection.*
3. For any unacceptable class results, evaluate cause, take appropriate corrective action, and rerun the calibrators, controls, and samples for the affected class assay. Document steps taken to achieve acceptable results in the batch archive.

6 Procedure

6.1 Sample Preparation

1. Add 1 mL aliquot of the respective samples to clean tubes. If sample is limited, less volume may be used without dilution (minimum of 0.5 mL).
2. Centrifuge to concentrate any particles in the sample to the bottom of the tube. (e.g. 2629 x *g* for twelve minutes)
3. Aliquot calibrators to appropriate sample cups on the calibrator tray. (See Appendix A)
4. Aliquot controls into sample cups in the barcoded tubes on the sample tray. (See Appendix A)
5. Decant approximately 0.5 mL of sample into sample cups in the barcoded tubes on the sample trays.

6.2 MGC240 Analysis

1. Ready the instrument after the lamp has warmed up for approximately 30 minutes.
2. Set the current round to urine and check the number of tests remaining per assay as estimated by the software.
3. Check the reagents in the reagent tray. The reagents must have satisfied the QC requirements for the lot when received. (Note: The EMIT reagents are provided as matched sets. They should not be interchanged with components of kits with different lot numbers.)

4. Order tests for the negative and calibrators. Process the negative, calibrators, and controls with addition of reagents and readings of changes in optical density of solutions.
5. Order tests for the unknowns including a second sampling of the level 4 calibrator for all assays (if sample volume for the level 4 calibrator is limited, it is acceptable to re-inject another calibrator in its place). Group all unknowns as a batch.
6. Process unknown samples with addition of reagents and readings of changes in optical density of solutions.
7. Prepare calibration report for the batch with responses for the negative and calibrators. Prepare quality control summary report for the batch with calculated concentrations for control samples, acceptable concentration range, and the outcome of the control.
8. Evaluate data from cases for reporting. Prepare batch results summary report and individual reports for the results of each case.
9. Prepare the batch archive, including the worklist, batch results summary report, quality control summary report, and the calibration report.
10. Acceptability of the batch shall be reviewed by a second qualified analyst.

7 Interpretation

A. Evaluate response results for each case sample.

1. Negative: Responses ranging from the response for the negative calibrator (level 1) up to the response for the high negative calibrator (level 2).
2. Elevated: Responses that fall between three-fourths the concentration of the high negative calibrator (level 2) and the high negative calibrator (level 2).
3. High Negative: Responses that fall between a concentration at or above half the concentration of the cutoff calibrator (level 3) and the cutoff calibrator (level 3).
4. Positive: Responses at or above the cutoff calibrator (level 3).

B. Identify all cases that require further analysis by category and range to designate these cases for GCMS and/or LCMS confirmation.

C. Identify all cases that are negative for all classes.

D. Evaluate all cases with additional information provided regarding suspected drugs or type of offense. Designate cases requiring further analysis for GCMS and/or LCMS confirmation.

E. Record information for each case in the case record and/or on the pending analysis list.

8 Limitations

Specificity of Immunoassay analysis:

Immunoassay tests detect multiple drugs within a class of similar drugs. There is a variation in the amount of response to specific drugs. Quantitative estimates are based on one drug from a class and may be significantly different for other drugs within that class.

Interpretation of positive/negative is subject to evaluation of any drugs specifically suspected and their relative response to the calibrator drug.

9 Literature and Supporting Documentation

Microgenics DRI reagent information

Immunanalysis reagent information

APPENDIX A: CALIBRATOR/CONTROL TABLES

The following tables provide information on how to fill sample cups for Calibrators and Controls.

Calibrator Table

Assay(s)	ID	Calibrator Level	Position
AMPH, BARB, BENZ, COC, OPI, PCP, THC, SOMA	Negative Urine Cal	1	B1
BARB, BENZ, COC, PCP	Multi Drug Cal 1	2	S1
BARB, BENZ, COC, PCP	Multi Drug Cal 2	3	S2
BARB, BENZ, COC, PCP	Multi Drug Cal 3	4	S3
BARB, BENZ, COC, PCP	Multi Drug Cal 4	5	S4
THC	THC Urine 20 Cal	3	S5
THC	THC Urine 50 Cal	4	S6
THC	THC Urine 100 Cal	5	S7
OPI	Opiate Cal 1	2	S8
OPI	Low Urine Cal	3	S9
OPI	Opiate Cal 3	4	S10
OPI	High Urine Cal	5	S11
AMPH, SOMA	Mep/Meth Level 2 Cal	2	S12
AMPH, SOMA	Mep/Meth Level 3 Cal	3	S13
AMPH, SOMA	Mep/Meth Level 4 Cal	4	S14
AMPH, SOMA	Mep/Meth Level 5 Cal	5	S15

Control Tables

Assay(s)	ID	Specimen ID #
BARB, BENZ, COC, PCP	Primary Low	1
BARB, BENZ, COC, PCP	Primary High	2
OPI	Clinical Low	3
OPI	Clinical High	4
THC	Negative Urine Cal	5
THC	THC 40 Control	6
AMPH, SOMA	Mep/Meth Low Control	13
AMPH, SOMA	Mep/Meth High Control	14

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue;
01	07/01/2003	Major Revision and renumbering
02	10/20/2006	Major Revision: restructuring of documents and substantive changes to content
03	08/10/2007	Major Revision: Addition, clarification and quality control of Immunoassay reagents
04	08/19/2009	Major Revision – Sections 3 - 7, and 9 Implementation of procedures used with MGC240 EMIT Immunoassay instrument and clarification of text
05	01/20/2010	Major Revision – Sections 1, 3 through 7, and 9 Advisory Board 01/11/2010
06	02/03/2014	Major Revision – Sections 3 and 4
07	04/02/2018	Major Revision – Title, Sections 1, 2, 6, 7, and Appendix

EXTRACTION FOR GCMS SCREEN

1 Scope

Extraction and purification of analytes is accomplished with liquid-liquid extraction. Specimens are analyzed using a gas chromatograph/mass spectrometer (GCMS).

Detection of drugs is by evaluation of a library search report. Qualitative identification is based on comparison of case sample and standard mass spectra supported by chromatographic retention time. In addition, ion plots of suspected drugs detect or eliminate drugs mentioned in case information.

2 Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the MSDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Controls (TOX-01-04)

Screening by GCMS (TOX-06-01)

Borate Buffer (pH 11) (TOX-07-01)

Acidified Ethanol (TOX-07-02)

4 Equipment / Materials / Reagents

Equipment

- Pipette (0.1 - 1.0 mL) with disposable tips
- Micropipette with disposable tips
- Repeater pipette with appropriate size tip
- Vortexer
- Sample Rotator, platform type
- Centrifuge
- Sample Evaporator

Materials

- 16X100 mm tubes
- Polyethylene plug caps
- Autosampler vials with microvolume inserts (100 µL)
- Pasteur pipettes and bulbs

Chemicals

- H_2O (purified water)
- Ethanol (190-200 proof)
- n-Butyl chloride (1-Chlorobutane) (High Purity Solvent suitable for trace analysis)
- Methanol (High Purity Solvent suitable for trace analysis)
- Concentrated Sulfuric acid: ACS grade
- 1.0 mg/mL stock solution Carisoprodol Standard
- 1.0 mg/mL stock solution Phencyclidine Standard
- 1.0 mg/mL stock solution Methadone Standard
- 1.0 mg/mL stock solution Nortriptyline Standard
- 1.0 mg/mL stock solution Codeine Standard
- 1.0 mg/mL stock solution Zolpidem Standard
- SKF-525 (Proadifen)

Solutions - Different volumes may be prepared in the same proportions as specified.

- Borate Buffer (pH 11)
- Acidified Ethanol
- 0.2 *N* H_2SO_4 (if needed)

Into a 250 mL volumetric flask (approximately ¾ filled with H_2O), add (slowly) 1.4 mL concentrated sulfuric acid. Allow solution to cool to room temperature. Dilute to total volume with H_2O. (Prepare fresh.)

- SKF-525 (1.0 mg/mL)

Weigh 10.00 mg of standard as the non-salt form or its equivalent weight of salt of SKF-525 per 10 mL volumetric flask and dissolve in methanol. Note: SKF-525 (Proadifen): 11.03 mg of proadifen HCl is equivalent to 10.0 mg proadifen.

- SKF-525 ISTD (40 mg/L)

Add 4.0 mL of 1 mg/mL stock solution per 100 mL in a volumetric flask and dilute to volume with H_2O

- 100 mg/L Phencyclidine.

Dilute 500 µL phencyclidine stock solution to 5 mL with methanol in volumetric flask.

- Screens Control Mix (10 mg/L carisoprodol, 0.8 mg/L phencyclidine, 3 mg/L methadone, 8 mg/L nortriptyline, 2 mg/L codeine, and 2 mg/L zolpidem)

Dilute 100 µL carisoprodol stock solution, 80 µL phencyclidine 100 mg/L solution, 30 µL methadone stock solution, 80 µL nortriptyline stock solution, 20 µL codeine stock solution, and 20 µL zolpidem stock solution to 10 mL with H_2O in volumetric flask.

5 Standards, Controls, and Calibration

A. Internal Standard:

1. Internal Standard: SKF 525
2. Working solution: 40 mg/L SKF 525

3. 0.4 mg/L final concentration in samples

B. Blanks

1. Matrix blank
2. H_2O blank (optional)

C. Standard Spiked Positive Control (Use for blood or urine qualitative batch)

1. Analytes: carisoprodol, phencyclidine, methadone, nortriptyline, codeine, and zolpidem
2. Screens Control Mix: 10 mg/L carisoprodol, 0.8 mg/L phencyclidine, 3 mg/L methadone, 8 mg/L nortriptyline, 2 mg/L codeine, and 2 mg/L zolpidem
3. Target Concentration: 0.5 mg/L carisoprodol, 0.04 mg/L phencyclidine, 0.15 mg/L methadone, 0.4 mg/L nortriptyline, 0.1 mg/L codeine, and 0.1 mg/L zolpidem

6 Procedure

1. Allow specimens, controls, and reagents to equilibrate to room temperature.
2. Add a 1 mL aliquot of the respective samples, blood blank, and/or urine blank to clean, labeled 16X100 mm tubes.
3. Prepare a Standard Spiked Positive Control by adding 50 µL Screens Control Mix to 1 mL blank blood and/or blank urine in a clean, labeled 16 x 100 mm tube.
4. Add 50 µL H_2O to each sample and all blanks. Add 10 µL of 40 mg/L SKF-525 ISTD to each tube. Vortex briefly, and allow mixture to equilibrate for approximately 15 minutes.
5. Add 5 mL n-butyl chloride, cap, and mix on rotator for at least 15 minutes.
6. Centrifuge to separate layers (e.g. 2629 x *g* for six minutes).
7. Transfer organic layer to a clean labeled tube and save.
8. To the aqueous (blood or urine) layer of the previous extraction step, add 1 mL Borate Buffer (pH 11) and 5 mL n-butyl chloride. Mix on rotator for at least 15 minutes.
9. Centrifuge to separate layers (e.g. 2629 x *g* for six minutes).
10. Transfer organic layer to combine in tube with previously transferred organic layer.
11. Use this step only if other tissues are analyzed or special cleanup is needed.

Note: Basic drugs will be retained and neutral drugs will be lost with this extraction step.

a) Evaporate to half volume in an evaporator (e.g. 45°C at 5-20 psi for time necessary).

b) Add 4 mL 0.2N H_2SO_4

c) Vortex briefly to extract and centrifuge to separate layers (e.g. 2629 x g for six minutes).

d) Draw off and discard n-butyl chloride with Pasteur pipette.

e) Add 2 mL Borate Buffer (pH 11) and 5 mL n-butyl chloride to the aqueous layer, vortex briefly to extract, centrifuge to separate layers (e.g. 2629 x g for six minutes), and transfer organic layer to a clean labeled tube.

12. Add 100 µL acidified Ethanol to each tube.
13. Evaporate to dryness in an evaporator (e.g. 45ºC at 5-20 psi for 20 minutes).
14. Add 50 µL methanol and vortex briefly to dissolve the analytes.
15. Cap and centrifuge (e.g. 1479 x g for two minutes) to drain the walls (optional).
16. Transfer solution to a microvolume insert in a labeled autosampler vial.
17. If data analysis indicates a need for derivatization of the extracted sample, the following procedures should be followed. Each derivatization should include a negative and positive control.

a) HFBA Derivatization

i. Allow case sample in autosampler vial to evaporate to dryness. For the negative control, allow 50 µL MeOH and 100 µL acidified Ethanol to evaporate to dryness in a test tube. For the positive control, allow 50 µL of appropriate diluted standard and 100 µL acidifed Ethanol to evaporate to dryness in a test tube.

ii. Add 100 µL (1:2, HFBA:EtAC) to autosampler vial for the case sample and to the test tubes for the negative and positive controls. Transfer the case sample to test tube, cap all tubes, and heat at 70 ºC for 20 minutes.

iii. Centrifuge (e.g. 1479 x g for two minutes) to drain the walls.

iv. Evaporate to dryness in an evaporator (e.g. 35 ºC at 4 psi for 10 minutes)

Note: Maintain low temperature and pressure as HFBA excess is killed. Remove as sample reaches dryness. Check for absence of butyric acid odor.

v. Add 50 µL ethyl acetate to each tube and vortex briefly to dissolve the analytes.

vi. Cap and centrifuge (e.g. 1479 x g for two minutes) to drain the walls (optional).

vii. Transfer the solution to a microvolume insert in a labeled autosampler vial.

b) TMS Derivatization

i. Allow sample in autosampler vial to evaporate to dryness. For the negative control, allow 50 µL MeOH to evaporate to dryness in an autosampler vial. For the positive control, allow 50 µL of the appropriate diluted standard to evaporate to dryness in an autosampler vial.

ii. *Add 50 µL TMS reagent (1:2, BSA: EtAC) to each autosampler vial and vortex briefly to dissolve the analytes.*

c) *PFPA/PFPOH Derivatization*

i. *Allow case sample in autosampler vial to evaporate to dryness. For the negative control, allow 50 µL MeOH to evaporate to dryness in a test tube. For the positive control, allow 50 µL of appropriate diluted standard to evaporate to dryness in a test tube.*

ii. *Add 50 µL PFPA and 50 µL PFPOH to the autosampler vial for the case sample and to the test tubes for the negative and positive controls. Transfer the case sample to test tube, cap all tubes, and heat at 60 °C for 20 minutes.*

iii. *Centrifuge (e.g. 1479 x g for two minutes) to drain the walls.*

iv. *Evaporate to dryness in an evaporator (e.g. 40 °C at 10 psi for 5 minutes).*

v. *Add 75 µL ethyl acetate to each tube, cap, and vortex briefly to dissolve the analytes.*

vi. *Transfer the solution to a microvolume insert in a labeled autosampler vial.*

7 Interpretation

Proceed to “Screening by GCMS”.

8 Literature / Supporting Documentation

Baselt RC. Disposition of Toxic Drugs and Chemicals in Man, 7th edition. 2004.

Pierce WO, Lamoreaux TC, Urry FM, Kopjak L, and Finkle BS. A New, Rapid Gas Chromatography Method for the Detection of Basic Drugs in Postmortem Blood, Using a Nitrogen Phosphorus Detector. Part I. Qualitative Analysis. J Anal Tox. 1978. 2, pp. 26-31.

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Foerster EH, and Mason MF. Preliminary Studies on the Use of *n*-Butyl Chloride as an Extractant in a Drug Screening Procedure. J For Sci. 1974. 19(1), pp. 155-162.

Foerster EH, Hatchett D, and Garriott JC. A Rapid, Comprehensive Screening Procedure for Basic Drugs in Blood or Tissues by Gas Chromatography J Anal Tox. 1978. 2, pp. 50-55.

Preparer

Megan Barton Date: 09/08/2014
Advisory Board Chair

Concurrence

Heather Greco Date: 09/08/2014
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue;
01	07/01/2003	Major Revision and renumbering
02	10/20/2006	Major Revision: restructuring of documents and substantive changes to content
03	06/03/2009	Major Revision – Sections 4, 5, and 6 Minor Revision – Section 8 Implementation additional procedures and clarification of text
04	08/19/2009	Major Revision – Section 4 Clarification of text
05	02/03/2014	Major Revision – Sections 3 and 4 Minor Revision – Section 6
06	09/19/2014	Minor Revision – Section 4

EXTRACTION FOR 9-CARBOXY-THC IN URINE

1 Scope

The presence of 9-carboxy-tetrahydrocannabinol (9-Carboxy-THC), a THC metabolite, can be determined by analyzing urine specimens.

Hydrolysis of the sample improves recovery of the analyte. Extraction and purification of the analyte is accomplished with liquid-liquid extraction. Chromatographic performance is improved with trimethylsilylation (TMS derivatization). Specimens are analyzed using a gas chromatograph/mass spectrometer (GCMS) in selected ion monitoring (SIM) mode.

Quantitative calculation is with an internal standard method based on a multipoint calibration. Quantitative results are not included in reported findings.

Qualitative identification is based on comparison of case sample and standard mass spectra supported by chromatographic retention time.

2 Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Controls (TOX-01-04)

Target Compound Analysis (TOX-06-02)

Preparation of Maleic Acid Solution (TOX-07-14)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Pipette with disposable tips
- Repeater pipette with appropriate size tip
- Vortexer
- Sample Rotator, platform type
- Centrifuge
- Sample Evaporator

4.2 Materials

- 16X100 mm tubes (silanized)
- 16X100 mm screw cap tubes (silanized)
- Screw caps

- Polyethylene plug caps
- Autosampler vials with microvolume inserts (silanized)
- Pasteur pipettes and bulbs

4.3 Chemicals

- H_2O (purified water)
- Potassium Hydroxide (KOH) (ACS reagent grade)
- Maleic Acid (Assay 99% min.)
- Hexane (High Purity Solvent suitable for trace analysis)
- Methanol (High Purity Solvent suitable for trace analysis)
- Ethyl Acetate (High Purity Solvent suitable for trace analysis)
- N,O-bis(trimethylsilyl)acetamide (BSA)
- 0.1 mg/mL 9-carboxy-THC (9-carboxy-11-nor-Δ^9-tetrahydrocannabinol) stock solution
- 9-Carboxy-Δ^8-THC-d6 (100 µg/mL) stock solution

4.4 Solutions - Different volumes may be prepared in the same proportions as specified.

- Potassium Hydroxide (11.8 *N*):

  Dissolve 6.62 g potassium hydroxide per 10 mL H_2O. (Prepare fresh.)
- Maleic Acid Solution (2.5 *M*)
- Ethyl Acetate/Hexane (1:9):

  Mix 10 mL ethyl acetate per 90 mL Hexane. (Prepare appropriate amount of fresh solution for size of batch.)
- TMS reagent (1:2, BSA:ethyl acetate):

  Mix 800 µL N,O-bis(trimethylsilyl)acetamide (BSA) per 1.6 mL ethyl acetate for thirty samples. (Prepare fresh.)
- 20 mg/L 9-carboxy-THC (9-carboxy-11-nor-Δ^9-tetrahydrocannabinol) working solution

  Dilute 2.0 mL 9-carboxy-THC (9-carboxy-11-nor-Δ^9-tetrahydrocannabinol) stock solution to 10 mL with methanol in a volumetric flask.
- 1 mg/L 9-carboxy-THC (9-carboxy-11-nor-Δ^9-tetrahydrocannabinol) working solution

  Dilute 500 µL of 20 mg/L 9-carboxy-THC (9-carboxy-11-nor-Δ^9-tetrahydrocannabinol) working solution to 10 mL with methanol in a volumetric flask.
- 9-Carboxy-Δ^8-THC-d6 ISTD (8 mg/L)

  Add 250 µL of 100 µg/mL solution to 2.875 mL of methanol for total volume of 3125 µL.
- THC working control solution (0.6 mg/L)

  See TOX-05-25 for preparation.

5 Standards, Controls, and Calibration

A. Calibrators:

1. Drug standard: 9-carboxy-THC (9-carboxy-11-nor-Δ^9-tetrahydrocannabinol)
2. Calibrator series: 10, 20, 100, 150, and 300 ng/mL
3. Working standard solutions: 20 mg/L and 1 mg/L

B. Internal Standard:

1. Drug Standard: 9-carboxy-Δ8-THC-d6 (9-carboxy- d6-11-nor-Δ8-tetrahydrocannabinol)
2. Working solution: 8 mg/L 9-carboxy-Δ8-THC-d6 (9-carboxy- d6-11-nor-Δ8-tetrahydrocannabinol)
3. 100 ng/mL final concentration in samples

C. Blanks

1. Calibrator matrix blank - urine
2. H_2O blank (optional)

D. Control

1. Analyte: 9-carboxy-THC
2. Prepared in-house coinciding with Blood THC analysis (TOX-05-25).
3. Target concentration: 0.075 mg/L

6 Procedure

1. Allow specimens, matrix blank, and working solutions to equilibrate to room temperature.
2. Aliquot 2 mL of matrix blank and case samples to clean, labeled 16x100 mm tubes.
3. Prepare calibrator samples by adding the specified amounts of working standard solution to 2 mL blank urine in clean, labeled 16X100 mm tubes.

   10 ng/mL: 20 µL of 1 mg/L working standard solution and 80 µL MeOH
   20 ng/mL: 40 µL of 1 mg/L working standard solution and 60 µL MeOH

   100 ng/mL: 10 µL of 20 mg/L working standard solution and 90 µL MeOH
   150 ng/mL: 15 µL of 20 mg/L working standard solution and 85 µL MeOH
   300 ng/mL: 30 µL of 20 mg/L working standard solution and 70 µL MeOH
4. Prepare control sample by adding 100 µL of THC control working solution to 2.0 mL blank urine in clean, labeled 16X100 mm tubes.
5. Add 100 µL MeOH to each sample and matrix blank.
6. Add 25 µL 8 mg/L 9-carboxy-Δ8-THC-d6 ISTD and 0.2 mL 11.8 N potassium hydroxide to each tube.
7. Vortex briefly to mix and incubate in water bath for 5 minutes at 50ºC.
8. Add 1 mL 2.5 *M* Maleic acid solution and allow to cool approximately 5 minutes.
9. Add 5 mL Ethyl acetate/hexane (1:9) and mix on rotator for at least 15 minutes.

10. Centrifuge to separate layers (e.g. 2629 x *g* for twelve minutes).
11. Transfer the organic layer to a clean, labeled 16X100 mm tube.
12. Evaporate to dryness in an evaporator (e.g. 45ºC at 5-20 psi for 20 minutes).
13. Add 50 µL TMS reagent (1:2, BSA:ethyl acetate) and vortex briefly to dissolve the analytes.
14. Cap and centrifuge (e.g. 1479 x *g* for two minutes) to drain the walls (optional).
15. Transfer solution to a microvolume insert in a labeled autosampler vial.

7 Interpretation

Proceed to Target Compound Analysis (TOX-06-02).

8 Limitations

Sensitivity (administrative cut-off): 5 ng/mL qualitative

All glassware used during extraction should be silanized to prevent loss of analytes.

9 Literature and Supporting Documentation

The DPS Crime Lab uses the procedure from the Mesa, Arizona, Laboratory Adapted from the Toxi-Lab procedure. Implemented at the DPS Austin Laboratory (10/19/90).

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue;
01	07/01/2003	Major Revision and renumbering
02	10/20/2006	Major Revision – restructuring of documents and substantive changes to content
03	06/03/2009	Major Revision – Section 6 Minor Revision – Sections 3, 4, 5, and 9 Implementation of LCMS, additional practices and clarification of text
04	02/03/2014	Major Revision – Section 4 Minor Revision – Sections 3, 5, 6, 9
05	09/19/2014	Minor Revision – Sections 4, 5, 8
06	04/02/2018	Major Revision – Sections 1, 2, 4, 5, 6, and 8

SPE FOR BENZODIAZEPINE MIX WITH LCMS

1 Scope

Benzodiazepines and their metabolites, hydrocodone, carisoprodol, and meprobamate content can be determined by analyzing blood and urine specimens. Quantitative analytes in blood include diazepam, nordiazepam, alprazolam, clonazepam, lorazepam, hydrocodone, carisoprodol, and meprobamate. Qualitative analytes in blood include chlordiazepoxide, flunitrazepam, 7-aminoflunitrazepam, desalkylflurazepam, temazepam, and midazolam. Additional qualitative analytes in urine include estazolam, triazolam, α-hydroxytriazolam, α-hydroxymidazolam, α-hydroxyalprazolam, flurazepam, oxazepam, and 7-aminoclonazepam.

Extraction and purification of analytes is accomplished with solid phase extraction. Specimens are analyzed using a liquid chromatograph mass spectrometer (LCMS).

Quantitative calculation is with an internal standard method based on a multipoint calibration.

Qualitative identification is based on multiple reaction monitoring (MRM) data that is supported by chromatographic retention time.

This procedure includes a process to hydrolyze urine specimens when the standard method fails to confirm benzodiazepine positive EMIT results.

2 Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Controls (TOX-01-04)

Target Compound Analysis (TOX-06-02)

Preparation of Sodium Acetate Buffer (TOX-07-07)

Preparation of Ammonium Formate (TOX-07-24)

Preparation of Potassium Carbonate Buffer (TOX-07-26)

Preparation of Mobile Phase for LCMS (TOX-07-27)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Pipette (50-250 µL) with disposable tips
- Repeater pipette with appropriate size tip
- Bottle top dispenser
- Vortexer
- Sonicator
- Centrifuge
- Sample evaporator
- Solid phase extraction manifold

4.2 Materials

- 16X100 mm tubes
- Polyethylene plug caps
- SPE columns: Trace-B
- Autosampler vials with microvolume inserts

4.3 Chemicals

- H_2O (HPLC grade)
- Acetonitrile (HPLC grade)
- Ethyl acetate (high purity solvent suitable for trace analysis)
- Methanol (high purity solvent suitable for trace analysis)
- β-glucuronidase: Type HP-2; H. Pomatia (≥100,000 units/mL)
- Ammonium formate, for mass spectrometry
- Formic acid, ACS grade
- Ammonium hydroxide (NH_4OH), ACS grade
- Sodium acetate trihydrate, ACS grade
- 1.0 mg/mL alprazolam stock solution
- 1.0 mg/mL flunitrazepam stock solution
- 1.0 mg/mL 7-aminoflunitrazepam stock solution
- 1.0 mg/mL clonazepam stock solution
- 1.0 mg/mL chlordiazepoxide stock solution
- 1.0 mg/mL diazepam stock solution
- 1.0 mg/mL desalkylflurazepam stock solution
- 1.0 mg/mL lorazepam stock solution
- 1.0 mg/mL midazolam stock solution
- 1.0 mg/mL nordiazepam stock solution
- 1.0 mg/mL temazepam stock solution
- 1.0 mg/mL hydrocodone stock solution
- 1.0 mg/mL carisoprodol stock solution

- 1.0 mg/mL meprobamate stock solution
- 1.0 mg/mL estazolam stock solution
- 1.0 mg/mL triazolam stock solution
- 1.0 mg/mL flurazepam stock solution
- 1.0 mg/mL oxazepam stock solution
- 1.0 mg/mL α-hydroxyalprazolam stock solution
- 100 µg/mL α-hydroxymidazolam stock solution
- 100 µg/mL α-hydroxytriazolam stock solution
- 100 µg/mL 7-aminoclonazepam stock solution
- 100 µg/mL hydrocodone-D_6 ISTD stock solution
- 100 µg/mL diazepam-D_5 ISTD stock solution
- 100 µg/mL alprazolam-D_5 ISTD stock solution
- 1 mg/mL nordiazepam-D_5 ISTD stock solution
- 100 µg/mL clonazepam-D_4 ISTD stock solution
- 100 µg/mL lorazepam-D_4 ISTD stock solution
- 1 mg/mL meprobamate-D_7 ISTD stock solution
- 1 mg/mL carisoprodol-D_3 ISTD stock solution

4.4 Solutions – Different volumes may be prepared in the same proportions as specified.

1. 0.1 M sodium actetate buffer (pH 4.5)
2. Potassium carbonate buffer (pH 9.0)
3. 1 M ammonium formate solution
4. Mobile Phase Eluent A
5. Mobile Phase Eluent B
6. Reconstitution Solution: 90% eluent A, 10% eluent B
7. Ethyl acetate: NH_4OH (98:2) prepare fresh
8. Carisoprodol-D_3 1.0 mg/mL solution from powder (May be used instead of stock solution)

   Weigh 5.0 mg of standard per 5 mL volumetric flask and dissolve in methanol.
9. Benzodiazepine mix ISTD (0.2 mg/L alprazolam-D_5, 0.4 mg/L hydrocodone-D_6, 0.4 mg/L clonazepam-D_4, 0.4 mg/L lorazepam-D_4, 2 mg/L diazepam-D_5, 2 mg/L nordiazepam-D_5, 8 mg/L meprobamate-D_7, and 8 mg/L carisoprodol-D_3)

   Add 50 µL 100 µg/mL alprazolam-D_5 stock solution, 500 µL 100 µg/mL diazepam-D_5 stock solution, 50 µL 1.0 mg/mL nordiazepam-D_5 stock solution, 100 µL 100 µg/mL hydrocodone-D_6 stock solution, 100 µL 100 µg/mL clonazepam-D_4 stock solution, 100 µL 100 µg/mL lorazepam-D_4 stock solution, 200 µL 1.0 mg/mL meprobamate-D_7 stock solution, and 200 µL 1.0 mg/mL carisoprodol-D_3 stock solution (1.0 mg/mL solution from powder can

be used instead) per 25 mL in a volumetric flask and dilute to volume with methanol.

10. Diazepam, nordiazepam, temazepam, chlordiazepoxide working solution (100 mg/L)

    a) *Dilute 500 µL diazepam stock solution, 500 µL nordiazepam stock solution, 500 µL temazepam stock solution, and 500 µL chlordiazepoxide stock solution to 5 mL with methanol in a volumetric flask.*

11. Low Soma Mix

    a) *Carisoprodol (10 mg/L)*

    b) *Meprobamate (20 mg/L)*

    c) *Diazepam, nordiazepam, temazepam, and chlordiazepoxide (1 mg/L)*

    d) *Dilute 50 µL carisoprodol stock solution, 100 µL meprobamate stock solution, and 50 µL of 100 mg/L diazepam, nordiazepam, temazepam, chlordiazepoxide working solution to 5 mL with methanol in a volumetric flask.*

12. Mid Soma Mix

    a) *Carisoprodol (40 mg/L)*

    b) *Meprobamate (80 mg/L)*

    c) *Diazepam, nordiazepam, temazepam, and chlordiazepoxide (4 mg/L)*

    d) *Dilute 200 µL carisoprodol stock solution, 400 µL meprobamate stock solution, and 200 µL of 100 mg/L diazepam, nordiazepam, temazepam, chlordiazepoxide working solution to 5 mL with methanol in a volumetric flask.*

13. High Soma Mix

    a) *Carisoprodol (100 mg/L)*

    b) *Meprobamate (200 mg/L)*

    c) *Diazepam, nordiazepam, temazepam, and chlordiazepoxide (10 mg/L)*

    d) *Dilute 500 µL carisoprodol stock solution, 1000 µL meprobamate stock solution, and 50 µL of diazepam **stock** solution, 50 µL of nordiazepam **stock** solution, 50 µL of temazepam **stock** solution, 50 µL of chlordiazepoxide **stock** solution to 5 mL with methanol in a volumetric flask.*

14. Hydrocodone, clonazepam, lorazepam, desalkylflurazepam working solution (50 mg/L)

    a) *Dilute 250 µL hydrocodone stock solution, 250 µL clonazepam stock solution, 250 µL lorazepam stock solution, and 250 µL desalkylflurazepam stock solution to 5 mL with methanol in a volumetric flask.*

15. Alprazolam working solution (25 mg/L)
    a) *Dilute 125 µL alprazolam stock solution to 5 mL with methanol in a volumetric flask.*
16. Flunitrazepam working solution (25 mg/L)
    a) *Dilute 125 µL flunitrazepam stock solution to 5 mL with methanol in a volumetric flask.*
17. 7-Aminoflunitrazepam working solution (10 mg/L)
    a) *Dilute 50 µL 7-aminoflunitrazepam stock solution to 5 mL with methanol in a volumetric flask.*
18. Midazolam working solution (50 mg/L)
    a) *Dilute 250 µL midazolam stock solution to 5 mL with methanol in a volumetric flask.*
19. Low Hydrocodone Mix
    a) *Hydrocodone, clonazepam, lorazepam, and desalkylflurazepam (0.2 mg/L)*
    b) *Alprazolam (0.1 mg/L)*
    c) *Flunitrazepam (0.1 mg/L)*
    d) *7-Aminoflunitrazepam (0.04 mg/L)*
    e) *Midazolam (0.2 mg/L)*
    f) *Dilute 20 µL of 50 mg/L hydrocodone, clonazepam, lorazepam, desalkylflurazepam working solution, 20 µL of 25 mg/L alprazolam working solution, 20 µL of 25 mg/L flunitrazepam working solution, 20 µL of 10 mg/L 7-aminoflunitrazepam working solution, and 20 µL of 50 mg/L midazolam working solution to 5 mL with methanol in a volumetric flask.*
20. Mid Hydrocodone Mix
    a) *Hydrocodone, clonazepam, lorazepam, and desalkylflurazepam (1.2 mg/L)*
    b) *Alprazolam (0.6 mg/L)*
    c) *Flunitrazepam (0.4 mg/L)*
    d) *7-Aminoflunitrazepam (0.2 mg/L)*
    e) *Midazolam (0.6 mg/L)*
    f) *Dilute 120 µL of 50 mg/L hydrocodone, clonazepam, lorazepam, desalkylflurazepam working solution, 120 µL of 25 mg/L alprazolam working solution, 80 µL of 25 mg/L flunitrazepam working solution, 100 µL of 10 mg/L 7-aminoflunitrazepam working solution, and 60 µL of 50 mg/L midazolam working solution to 5 mL with methanol in a volumetric flask.*

21. High Hydrocodone Mix
    a) *Hydrocodone, clonazepam, lorazepam, and desalkylflurazepam (6.4 mg/L)*
    b) *Alprazolam (3.2 mg/L)*
    c) *Flunitrazepam (1.6 mg/L)*
    d) *7-Aminoflunitrazepam (1 mg/L)*
    e) *Midazolam (3 mg/L)*
    f) *Dilute 640 µL of 50 mg/L hydrocodone, clonazepam, lorazepam, desalkylflurazepam working solution, 640 µL of 25 mg/L alprazolam working solution, 320 µL of 25 mg/L flunitrazepam working solution, 500 µL of 10 mg/L 7-aminoflunitrazepam working solution, and 300 µL of 50 mg/L midazolam working solution to 5 mL with methanol in a volumetric flask.*
22. Diazepam and nordiazepam working control (100 mg/L)
    a) *Dilute 500 µL diazepam stock solution and 500 µL nordiazepam stock solution to 5 mL with methanol in a volumetric flask.*
23. Low Soma Control Mix
    a) *Carisoprodol (25 mg/L)*
    b) *Meprobamate (50 mg/L)*
    c) *Diazepam and nordiazepam (3 mg/L)*
    d) *Dilute 125 µL carisoprodol stock solution, 250 µL meprobamate stock solution, and 150 µL of 100 mg/L diazepam and nordiazepam working control to 5 mL with methanol in a volumetric flask.*
24. High Soma Control Mix
    a) *Carisoprodol (50 mg/L)*
    b) *Meprobamate (100 mg/L)*
    c) *Diazepam and nordiazepam (5 mg/L)*
    d) *Dilute 250 µL carisoprodol stock solution, 500 µL meprobamate stock solution, and 250 µL of 100 mg/L diazepam and nordiazepam working control to 5 mL with methanol in a volumetric flask.*
25. Clonazepam, lorazepam, and hydrocodone working control (50 mg/L)
    a) *Dilute 250 µL clonazepam stock solution, 250 µL lorazepam stock solution, and 250 µL hydrocodone stock solution to 5 mL with methanol in a volumetric flask.*
26. Alprazolam working control (50 mg/L)
    a) *Dilute 250 µL alprazolam stock solution to 5 mL with methanol in a volumetric flask.*
27. Low Hydrocodone Control Mix
    a) *Clonazepam, lorazepam, and hydrocodone (1 mg/L)*

b) *Alprazolam (0.3 mg/L)*

c) *Dilute 100 µL clonazepam, lorazepam, and hydrocodone working control and 30 µL alprazolam working control to 5 mL with methanol in a volumetric flask.*

28. High Hydrocodone Control Mix

a) *Clonazepam, lorazepam, and hydrocodone (1.6 mg/L)*

b) *Alprazolam (0.6 mg/L)*

c) *Dilute 160 µL clonazepam, lorazepam, and hydrocodone working control and 60 µL alprazolam working control to 5 mL with methanol in a volumetric flask.*

29. Urine benzodiazepine working solution (1.0/10 mg/L)

a) *Dilute 10 µL 7-aminoflunitrazepam, alprazolam, chlordiazepoxide, clonazepam, desalkylflurazepam, diazepam, flunitrazepam, lorazepam, midazolam, nordiazepam, temazepam, hydrocodone, estazolam, triazolam, oxazepam, flurazepam, α-hydroxyalprazolam stock solution, and 100 µL stock solution carisoprodol, meprobamate, α-hydroxymidazolam, α-hydroxytriazolam, 7-aminoclonazepam stock solution to 10 mL with methanol in a volumetric flask.*

30. Urine benzodiazepine working solution (0.025/0.25 mg/L)

a) *Dilute 250 µL of 1.0/10 mg/L urine benzodiazepine working solution to 10 mL with methanol in a volumetric flask.*

31. The stock solutions in the working solution must be from a different source than those used in the working control. The working solutions are for blood unless otherwise specified.

5 Standards, Controls, and Calibration

5.1 Calibrators

A. Drug standards: diazepam, nordiazepam, alprazolam, chlordiazepoxide, temazepam, clonazepam, desalkylflurazepam, lorazepam, midazolam, flunitrazepam, 7-aminoflunitrazepam, hydrocodone, carisoprodol, and meprobamate. Additionally for urine specimens, estazolam, triazolam, α-hydroxytriazolam, α-hydroxymidazolam, α-hydroxyalprazolam, flurazepam, oxazepam, and 7-aminoclonazepam

B. Blood calibrator series: 0.050, 0.10, 0.30, 0.50, 1.0, and 2.0 mg/L for diazepam, nordiazepam, chlordiazepoxide, and temazepam

C. Blood calibrator series: 0.010, 0.020, 0.060, 0.18, 0.32, and 0.64 mg/L for clonazepam, lorazepam, hydrocodone, and desalkylflurazepam

D. Blood calibrator series: 0.005, 0.010, 0.030, 0.090, 0.16, and 0.32 mg/L for alprazolam

E. Blood calibrator series: 0.005, 0.010, 0.020, 0.060, 0.080, and 0.16 mg/L for flunitrazepam

F. Blood calibrator series: 0.002, 0.004, 0.010, 0.030, 0.050, 0.10 mg/L for 7-aminoflunitrazepam

G. Blood calibrator series: 0.010, 0.020, 0.030, 0.090, 0.15, and 0.30 mg/L for midazolam

H. Blood calibrator series: 0.50, 1.0, 3.0, 5.0, 10, and 20 mg/L for carisoprodol

I. Blood calibrator series: 1.0, 2.0, 6.0, 10, 20, and 40 mg/L for meprobamate

J. Urine calibrator series: 0.005, 0.05, 0.20 mg/L for diazepam, nordiazepam, alprazolam, chlordiazepoxide, temazepam, clonazepam, desalkylflurazepam, lorazepam, midazolam, flunitrazepam, 7-aminoflunitrazepam, hydrocodone, estazolam, triazolam, α-hydroxytriazolam, α-hydroxymidazolam, α-hydroxyalprazolam, flurazepam, oxazepam, and 7-aminoclonazepam

K. Urine calibrator series: 0.05, 0.50, 2.0 mg/L for carisoprodol and meprobamate

L. Sample volume: 0.2 mL

M. Blood calibration plots for analytes are as follows:

1. Linear function with inverse weighting – alprazolam, temazepam, and hydrocodone
2. Quadratic function with inverse weighting – chlordiazepoxide, clonazepam, desalkylflurazepam, diazepam, flunitrazepam, lorazepam, and nordiazepam
3. Quadratic function with inverse squared weighting – 7-aminoflunitrazepam, midazolam, carisoprodol, and meprobamate

N. Urine calibration plots for all analytes are quadratic function with no weighting

5.2 Internal Standard

A. Benzodiazepine mix ISTD working solution containing: diazepam-D_5, nordiazepam-D_5, alprazolam-D_5, hydrocodone-D_6, clonazepam-D_4, lorazepam-D_4, carisoprodol-D_3, and meprobamate-D_7

B. Diazepam-D_5 ISTD

1. For the quantitation of diazepam in blood and qualitative ISTD for urine samples
2. Final concentration of the diazepam-D_5 ISTD in the sample is 0.25 mg/L

C. Nordiazepam-D_5 ISTD

1. For the quantitation of nordiazepam
2. Final concentration of the nordiazepam-D_5 ISTD in the sample is 0.25 mg/L

D. Alprazolam-D5 ISTD

1. For the quantitation of alprazolam and qualitative assessment of flunitrazepam, 7-aminoflunitrazepam, and desalkylflurazepam
2. Final concentration of the alprazolam-D_5 ISTD in the sample is 0.025 mg/L

E. Hydrocodone-D_6 ISTD

1. For the quantitation of hydrocodone
2. Final concentration of the hydrocodone-D_5 ISTD in the sample is 0.05 mg/L

F. Clonazepam–D_4 ISTD

1. For the quantitation of clonazepam and qualitative assessment of chlordiazepoxide, temazepam, and midazolam
2. Final concentration of the clonazepam-D_4 ISTD in the sample is 0.05 mg/L

G. Lorazepam-D_4 ISTD

1. For the quantitation of lorazepam
2. Final concentration of the lorazepam- D_4 ISTD in the sample is 0.05 mg/L

H. Carisoprodol-D_3 ISTD

1. For the quantitation of carisoprodol
2. Final concentration of the carisoprodol-D_3 ISTD in the sample is 1.0 mg/L

I. Meprobamate-D_7 ISTD

1. For the quantitation of meprobamate
2. Final concentration of the meprobamate-D_7 ISTD in the sample is 1.0 mg/L

J. Negative Control

1. Calibrator matrix blank – blood and/or urine

K. Standard spiked positive blood control

1. Standards: diazepam, nordiazepam, alprazolam, clonazepam, lorazepam, hydrocodone, carisoprodol, and meprobamate.
2. Control may be purchased (e.g. UTAK Alprazolam Plus Mid Whole Blood Control or UTAK Alprazolam Plus High Whole Blood Control) or prepared in-house.
3. Target concentration for benzodiazepine mix control #1:
   a) *0.30 mg/L for diazepam and nordiazepam*
   b) *0.10 mg/L for clonazepam, lorazepam, and hydrocodone*
   c) *0.030 mg/L for alprazolam*
   d) *2.5 mg/L for carisoprodol*
   e) *5.0 mg/L for meprobamate*
4. Target concentration for benzodiazepine mix control #2:
   a) *0.50 mg/L for diazepam and nordiazepam*
   b) *0.16 mg/L for clonazepam, lorazepam, and hydrocodone*
   c) *0.060 mg/L for alprazolam*
   d) *5.0 mg/L for carisoprodol*
   e) *10 mg/L for meprobamate*

6 Procedure

1. Allow specimens, matrix blank, and working solutions to equilibrate to room temperature.

2. Aliquot 0.2 mL of case samples and matrix blanks to clean, labeled 16X100 mm tubes.
3. If blood specimens are being analyzed, prepare calibrator samples by adding the specified amounts of working solution to 0.2 mL blank blood in clean, labeled 16x100 mm tubes.
   a) *Level 1: 10 µL of Low Hydrocodone Mix, 10 µL of Low Soma Mix, and 40 µL of methanol*
   b) *Level 2: 20 µL of Low Hydrocodone Mix, 20 µL of Low Soma Mix, and 20 µL of methanol*
   c) *Level 3: 10 µL of Mid Hydrocodone Mix, 15 µL of Mid Soma Mix, and 35 µL of methanol*
   d) *Level 4: 30 µL of Mid Hydrocodone Mix, 25 µL of Mid Soma Mix, and 5 µL of methanol*
   e) *Level 5: 10 µL of High Hydrocodone Mix, 20 µL of High Soma Mix, and 30 µL of methanol*
   f) *Level 6: 20 µL of High Hydrocodone Mix, 40 µL of High Soma Mix, and no methanol*
4. If blood specimens are being analyzed, prepare control samples by adding the specified amounts of working solution to 0.2 mL blank blood in clean, labeled 16x100 mm tubes.
   a) *Control 1: 20 µL of Low Hydrocodone Control Mix, 20 µL of Low Soma Control Mix, and 20 µL of methanol*
   b) *Control 2: 20 µL of High Hydrocodone Control Mix, 20 µL of High Soma Control Mix, and 20 µL of methanol*
5. Add 60 µL methanol to each blood case sample and blank blood matrix.
6. If urine specimens are being analyzed, prepare calibrator samples by adding the specified amounts of working solution to 0.2 mL blank urine in clean, labeled 16X100 mm tubes.
   a) *0.005/0.05 mg/L: 40 µL of urine benzodiazepine working solution (0.025/0.25 mg/L)*
   b) *0.05/0.50 mg/L: 10 µL of urine benzodiazepine working solution (1.0/10 mg/L) and 30 µL MeOH*
   c) *0.20/2.0 mg/L: 40 µL of urine benzodiazepine working solution (1.0/10 mg/L)*
7. Add 40 µL methanol to each urine case sample and blank urine matrix.
8. Add to each tube 25 µL of the benzodiazepine mix ISTD, vortex briefly to mix, and allow mixture to equilibrate for approximately 10 minutes.
9. For urine specimens:
   a) *Add 1 mL of 0.1 M sodium acetate buffer and 10 µL β-glucuronidase (H. Pomatia, Type HP-2) to each sample and calibrator tube and vortex to mix*

b) *Heat for 2 hours at 56 ± 1°C*

c) *Cool to room temperature*

10. For blood specimens:

a) *Add 1 mL of 0.1 M sodium acetate buffer*

b) *Sonicate for 15 minutes*

11. Centrifuge to separate layers (e.g. 2629 x *g* for twelve minutes). Prepare SPE column:

a) *1 mL ethyl acetate pushed through under pressure*

b) *1 mL methanol pushed through under pressure*

c) *15 seconds of nitrogen flow at approximately 25 psi*

12. Load samples onto the columns and push through under pressure.

13. Rinse SPE column:

a) *1 mL of potassium carbonate buffer pushed through under pressure*

b) *1 mL H_2O pushed through under pressure*

c) *Dry column for 20 minutes at approximately 25 psi*

14. Elute analytes:

a) *2 mL ethyl acetate: NH_4OH (98:2) pushed through under pressure*

b) *Collect eluate in clean, labeled 16X100 mm tubes*

15. Evaporate eluate to dryness in an evaporator (e.g. 45°C at 5-20 psi for 15 minutes).

16. Add 100 µL of Reconstitution Solution and vortex briefly to dissolve the analytes.

17. Cap and centrifuge (e.g. 2629 x g for six minutes) to drain the walls and pack down any residue.

18. Transfer solution to a microvolume insert in a labeled autosampler vial.

19. Data is acquired for analysis of all vials using a liquid chromatograph mass spectrometer (LCMS).

7 Interpretation

A. Proceed to Target Compound Analysis (TOX-06-02).

B. Sensitivity

1. Blood administrative cut-offs:

a) *0.05 mg/L quantitative for diazepam and nordiazepam*

b) *0.005 mg/L quantitative for alprazolam*

c) *0.01 mg/L quantitative for clonazepam, hydrocodone, and lorazepam*

d) *0.50 mg/L quantitative for carisoprodol*

e) *1.0 mg/L quantitative for meprobamate*

f) *0.025 mg/L qualitative for diazepam, nordiazepam, temazepam, and chlordiazepoxide*

g) *0.0025 mg/L qualitative for alprazolam and flunitrazepam*

h) *0.005 mg/L qualitative for clonazepam, hydrocodone, lorazepam, desalkylflurazepam, and midazolam*

i) *0.25 mg/L qualitative for carisoprodol*

j) *0.50 mg/L qualitative for meprobamate*

k) *0.001 mg/L qualitative for 7-aminoflunitrazepam*

2. Urine administrative cut-offs:

a) *Sexual assault cases: 0.0025 mg/L qualitative for all analytes except for carisoprodol and meprobamate*

b) *Other cases: 0.025 mg/L qualitative for all analytes except for carisoprodol and meprobamate*

c) *All cases: 0.25 mg/L qualitative for carisoprodol and meprobamate*

8 Literature and Supporting Documentation

Baselt RC. Disposition of Toxic Drugs and Chemicals in Man, 7th edition. 2004.

SPEware Corporation. Trace Applications Manual: Benzodiazepines from Whole Blood for LC/MS Confirmations. November 2006.

Applied Biosystems. Experimental Conditions to Analyze Benzodiazepines.

Meatherall, Robert. Optimal Enzymatic Hydrolysis of Urinary Benzodiazepine Conjugates. J Anal Tox. 1994. 18, pp. 382-384.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue
01	01/20/2010	Major Revision – Sections 1 and 3 - 7 Advisory Board 01/11/2010
02	03/22/2010	Major Revision – Sections 1, 6, and 8
03	02/15/2013	Major Revision – Sections 4, 5, 6, and 9
04	02/03/2014	Major Revision – Sections 3-6
05	11/12/2014	Major Revision – Sections 4.3, 5, and 6
06	10/26/2015	Minor Revision – Section 4.3
07	04/02/2018	Major Revision - Sections 4, 6, and 8 Minor Revision – Sections 1 and 2

EXTRACTION FOR LCMS SCREEN

1 Scope

Extraction and purification of analytes in blood specimens is accomplished with a protein precipitate extraction. Specimens are analyzed using a liquid chromatograph mass spectrometer (LCMS).

Qualitative identification is based on multiple reaction monitoring (MRM) data that is supported by an Enhanced Product Ion (EPI) scan and chromatographic retention time.

If the LCMS screening method will be combined with EMIT analysis, a portion of the resulting supernatant will be used for that purpose. Include calibrators specifically for EMIT in this extraction and follow TOX-03-01, EMIT in blood, for equipment and set-up. Then continue with the Procedure for TOX-05-20.

2 Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Controls (TOX-01-04)

Screening By LCMS (TOX-06-03)

Preparation of Acidified Ethanol (TOX-07-02)

Preparation of Tris Buffer (TOX-07-36)

Preparation of Tris Buffer in Methanol (TOX-07-37)

Preparation of Ammonium Formate (TOX-07-24)

Preparation of Mobile Phase for LCMS (TOX-07-27)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Pipette (0.1 – 1.0 mL) with disposable tips
- Repeater pipette with appropriate size tip
- Micropipette with disposable tips
- Vortexer
- Centrifuge
- Sample Evaporator

4.2 Materials

- 16X100 mm tubes
- Polyethylene plug caps
- Autosampler vials with microvolume inserts

4.3 Chemicals

- Acetonitrile (HPLC grade)
- Methanol (high purity solvent suitable for trace analysis)
- Ammonium formate, for mass spectrometry
- Formic acid, ACS grade
- Hydrochloric acid (HCl): ACS grade
- Ethanol (190-200 proof)
- Acetone (high purity solvent suitable for trace analysis)
- 1.0 mg/mL Carisoprodol stock solution
- 1.0 mg/mL Phencyclidine stock solution
- 1.0 mg/mL Methadone stock solution
- 1.0 mg/mL Nortriptyline stock solution
- 1.0 mg/mL Codeine stock solution
- 1.0 mg/mL Zolpidem stock solution
- 1.0 mg/mL Mepivacaine stock solution

4.4 Solutions – Different volumes may be prepared in the same proportions as specified

1. 1 M ammonium formate solution
2. Mobile Phase Eluent A
3. Mobile Phase Eluent B
4. Tris buffer
5. Tris buffer:MeOH
6. Acidified Ethanol
7. 100 mg/L phencyclidine solution
   a) *Dilute 500 µL phencyclidine stock solution to 5 mL with methanol in volumetric flask.*
8. Screens control mix (10 mg/L carisoprodol, 0.8 mg/L phencyclidine, 3 mg/L methadone, 8 mg/L nortriptyline, 2 mg/L codeine, and 2 mg/L zolpidem)
   a) *Dilute 100 µL carisoprodol stock solution, 80 µL phencyclidine 100 mg/L solution, 30 µL methadone stock solution, 80 µL nortriptyline stock solution, 20 µL codeine stock solution, and 20 µL zolpidem stock solution to 10 mL with methanol in volumetric flask.*
9. Mepivacaine ISTD (3 mg/L)
   a) *Add 0.15 mL of 1 mg/mL stock solution per 50 mL in a volumetric flask*

and dilute to volume with methanol.

5 Standards, Controls, and Calibration

A. Internal Standard:

1. Internal standard: mepivacaine
2. Working solution: 3.0 mg/L mepivacaine
3. Final concentration in samples: 0.15 mg/L

B. Blank

1. Matrix blank - blood

C. Standard spiked positive blood control:

1. Analytes: codeine, carisoprodol, phencyclidine, nortriptyline, methadone, and zolpidem.
2. Screens control mix: 10 mg/L carisoprodol, 0.8 mg/L phencyclidine, 3 mg/L methadone, 8 mg/L nortriptyline, 2 mg/L codeine, and 2 mg/L zolpidem
3. Target Concentration: 0.5 mg/L carisoprodol, 0.04 mg/L phencyclidine, 0.15 mg/L methadone, 0.4 mg/L nortriptyline, 0.1 mg/L codeine, and 0.1 mg/L zolpidem

6 Procedure

1. Allow specimens, matrix blank, and working solutions to equilibrate to room temperature.
2. If EMIT analysis will be performed, include prepared EMIT calibrators and prepared hydrocodone cut-off in the batch. The prepared calibrators include a matrix blank for the batch.
3. Add a 1 mL aliquot of the respective samples and blood blank, if EMIT analysis will not be performed, to clean, labeled 16 x 100 mm tubes.
4. Prepare a standard spiked positive control by adding 50 µL screens control working solution to 1 mL blank blood in a clean, labeled 16 x 100 mm tube.
5. Add 50 µL methanol to each sample and blank. Add 50 µL of 3.0 mg/L mepivacaine ISTD to each tube. Vortex briefly, and allow mixture to equilibrate for approximately 15 minutes.
6. Add 3 mL acetone, cap, shake or vortex.
7. Vortex all samples for approximately 30 seconds.
8. Centrifuge to pack solids (e.g. 2629 x g for twelve minutes).
9. Place a 4 mL reservoir containing a frit into a clean, labeled tube.
10. Decant the supernatant into the reservoir and allow it to completely drain into a clean, labeled tube.
11. Add 0.5 mL acetone to each reservoir and allow it to drain into the tube.
12. Discard the reservoirs.
13. Add 50 µL of acidified Ethanol.

14. Evaporate to dryness in an evaporator (e.g. 55°C for 30 minutes at 5-20 psi).
15. Add 0.5 mL Tris buffer:MeOH to each sample and cap.
16. Vortex for approximately 30 seconds to dissolve residue, centrifuge to pack solids (e.g. 2629 x g for twelve minutes), and transfer at least 60 µL of the supernatant to a microvolume insert in a labeled autosampler vial.
17. If the LCMS screening method will be combined with EMIT analysis, the remaining supernatant for the EMIT calibrators, hydrocodone cut-off, and specimens will be used for blood EMIT analysis (TOX-03-01). The LCMS positive control will be excluded from EMIT analysis, and the EMIT calibrators and hydrocodone cut-off will be excluded from LCMS analysis.

8 Interpretation

Proceed to Screening by LCMS (TOX-06-03).

9 Literature and Supporting Documentation

Baselt RC. Disposition of Toxic Drugs and Chemicals in Man, 7th edition. 2004.

Toxicology Analysis for Drugs in Blood Using Liquid Chromatography Tandem Mass Spectrometry presented by Georgia Bureau of Investigation Division of Forensic Sciences.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	01/20/2010	Original Issue
01	02/15/2013	Major Revision – Sections 1 and 6
02	02/03/2014	Minor Revision – Sections 3-6 and Title
03	09/19/2014	Minor Revision – Section 4.4
04	04/02/2018	Major Revision – Sections 4 and 6 Minor Revision – Sections 2 and 3

SPE FOR OPIATES, COCAINE, AND COCAINE METABOLITES

1 Scope

The content of opiates, cocaine, and cocaine metabolites can be determined by analyzing blood and urine specimens. Quantitative analytes include cocaine, benzoylecgonine, cocaethylene, codeine, morphine, hydrocodone, and oxycodone. Qualitative analytes include hydromorphone, oxymorphone, and 6-acetylmorphine.

Extraction and purification of analytes is accomplished with solid phase extraction (SPE). Specimens are analyzed using a liquid chromatograph mass spectrometer (LCMS).

Quantitative calculation is with an internal standard method based on a multipoint calibration. Qualitative identification is based on multiple reaction monitoring (MRM) data that is supported by chromatographic retention time.

2 Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Controls (TOX-01-04)

Target Compound Analysis (TOX-06-02)

Preparation of 0.1 M Potassium Phosphate Buffer (TOX-07-06)

Preparation of 1M Ammonium Formate (TOX-07-24)

Preparation of Mobile Phase for LCMS (TOX-07-27)

Preparation of 0.1 M Hydrochloric Acid (TOX-07-29)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Pipette (50 - 250 µL) with disposable tips
- Micropipette with disposable tips
- Repeater pipette with appropriate size tip
- Bottle top dispenser
- Vortexer
- Centrifuge
- Sample evaporator
- Solid phase extraction manifold

4.2 Materials

- 16X100 mm tubes
- Polyethylene plug caps
- SPE Columns: World Wide Monitoring Clean Screen UCT columns (CSDAU203 200 mg/3mL)
- Autosampler vials with microvolume inserts

4.3 Chemicals – Different concentrations of stock solutions may be purchased if stated concentration is unavailable or pricing is different. Working solutions must be prepared to the stated concentration.

- H_2O (purified water)
- Acetonitrile (HPLC grade)
- Hydrochloric acid (HCl): ACS grade
- Methanol (high purity solvent suitable for trace analysis)
- Methylene chloride (high purity solvent suitable for trace analysis)
- Isopropanol (ACS grade)
- Ammonium formate, for mass spectrometry
- Formic acid, ACS grade
- Ammonium hydroxide (NH_4OH): ACS grade
- 1.0 mg/mL benzoylecgonine-D_8 stock solution
- 100 µg/mL cocaethylene-D_8 stock solution
- 100 µg/mL cocaine-D_3 stock solution
- 100 µg/mL codeine-D_6 stock solution
- 100 µg/mL morphine-D_6 stock solution
- 1.0 mg/mL hydrocodone-D_6 stock solution
- 1.0 mg/mL oxycodone-D_6 stock solution
- 1.0 mg/mL benzoylecgonine stock solution
- 1.0 mg/mL cocaine stock solution
- 1.0 mg/mL cocaethylene stock solution
- 1.0 mg/mL hydromorphone stock solution
- 1.0 mg/mL morphine stock solution
- 1.0 mg/mL codeine stock solution
- 1.0 mg/mL hydrocodone stock solution
- 1.0 mg/mL oxymorphone stock solution
- 1.0 mg/mL oxycodone stock solution
- 1.0 mg/mL 6-acetylmorphine stock solution

4.4 Solutions – Different volumes may be prepared in the same proportions as specified

1. 0.1 M Potassium phosphate buffer (pH 6.0)
2. 0.1 M Hydrochloric acid

3. 1 M Ammonium formate solution
4. Mobile Phase Eluent A
5. Mobile Phase Eluent B
6. Reconstitution Solution: 90% eluent A, 10% eluent B
7. Methylene chloride/isopropanol/ammonium hydroxide (78:20:2) solution: (Prepare fresh.)
8. Opiate and cocaine ISTD (0.4 mg/L morphine-D_6, 0.4 mg/L hydrocodone-D_6, 0.4 mg/L codeine-D_6, 0.4 mg/L oxycodone-D_6, 2 mg/L cocaine-D_3, 2 mg/L benzoylecgonine-D_8, 2 mg/L cocaethylene-D_8)
   a) *Add 100 µL 100 µg/mL codeine-D_6 stock solution, 100 µL 100 µg/mL morphine-D_6 stock solution, 10 µL 1.0 mg/mL oxycodone-D_6 stock solution, 10 µL 1.0 mg/mL hydrocodone-D_6 stock solution, 500 µL 100 µg/mL cocaine-D_3 stock solution, 500 µL 100 µg/mL cocaethylene-D_8 stock solution, 50 µL 1.0 mg/mL benzoylecgonine-D_8 stock solution per 25 mL in a volumetric flask and dilute to volume with methanol.*
9. Opiates (2.0 mg/L) and cocaine (10 mg/L) working solution
   a) *Dilute 100 µL cocaine stock solution, 100 µL cocaethylene stock solution, 100 µL benzoylecgonine stock solution, 20 µL hydromorphone stock solution, 20 µL morphine stock solution, 20 µL codeine stock solution, 20 µL hydrocodone stock solution, 20 µL oxymorphone stock solution, 20 µL oxycodone stock solution, 20 µL 6-acetylmorphine stock solution to 10 mL with methanol in a volumetric flask.*
10. Opiates (0.2 mg/L) and cocaine (1.0 mg/L) working solution
    a) *Dilute 1.0 mL of opiates (2.0 mg/L) and cocaine (10 mg/L) working solution to 10 mL with methanol in a volumetric flask.*
11. UTAK Drugs of Abuse Low Whole Blood Toxicology Control
12. UTAK Drugs of Abuse High Whole Blood Toxicology Control

5 Standards, Controls, and Calibration

A. Calibrators:

1. Drug standards: cocaine, cocaethylene, benzoylecgonine, hydromorphone, morphine, codeine, hydrocodone, oxymorphone, oxycodone, and 6-acetylmorphine
2. Calibrator series: 0.05, 0.10, 0.30, 0.50, 1.25, and 2.50 mg/L for cocaine, cocaethylene, and benzoylecgonine
3. Calibrator series: 0.01, 0.02, 0.06, 0.10, 0.25, and 0.50 mg/L for hydromorphone, morphine, codeine, hydrocodone, oxymorphone, oxycodone, and 6-acetylmorphine
4. Sample volume: 0.2 mL
5. Calibration plots for analytes are as follows:
   a) *Linear function with inverse weighting – morphine, oxymorphone, oxycodone, codeine, cocaine, and benzoylecgonine*

b) *Quadratic function with inverse weighting – hydromorphone, hydrocodone, and cocaethylene*

c) *Quadratic function with inverse squared weighting – 6-acetylmorphine*

B. Internal standard:

1. Opiate and cocaine ISTD working solution containing: morphine-D_6, hydrocodone-D_6, codeine-D_6, oxycodone-D_6, cocaine-D_3, benzoylecgonine-D_8, and cocaethylene-D_8

2. Benzoylecgonine-D_8 ISTD

   a) *For the quantitation of benzoylecgonine*

   b) *Final sample concentration of the benzoylecgonine-D_8 ISTD is 0.25 mg/L.*

3. Cocaine-D_3 ISTD

   a) *For the quantitation of cocaine*

   b) *Final sample concentration of the cocaine-D_3 ISTD is 0.25 mg/L.*

4. Cocaethylene-D_8 ISTD

   a) *For the quantitation of cocaethylene*

   b) *Final sample concentration of the cocaethylene-D_8 ISTD is 0.25 mg/L.*

5. Hydrocodone-D_6 ISTD

   a) *For the quantitation of hydrocodone and qualitative assessment of hydromorphone*

   b) *Final sample concentration of the hydrocodone-D_6 ISTD is 0.05 mg/L.*

6. Codeine-D_6 ISTD

   a) *For the quantitation of codeine*

   b) *Final sample concentration of the codeine-D_6 ISTD is 0.05 mg/L.*

7. Morphine-D_6 ISTD

   a) *For the quantitation of morphine and qualitative assessment of 6-acetylmorphine*

   b) *Final sample concentration of the morphine-D_6 ISTD is 0.05 mg/L.*

8. Oxycodone-D_6 ISTD

   a) *For the quantitation of oxycodone and qualitative assessment of oxymorphone*

   b) *Final sample concentration of the oxycodone-D_6 ISTD is 0.05 mg/L.*

C. Negative control

1. Calibrator matrix blank – blood

D. Positive Blood Controls

1. Standards: cocaine, cocaethylene, benzoylecgonine, morphine, codeine, hydrocodone, and oxycodone

2. Target concentration for UTAK Drugs of Abuse Low Whole Blood Toxicology Control
   a) *0.05 mg/L morphine, codeine, hydrocodone, and oxycodone*
   b) *0.10 mg/L cocaine, cocaethylene, and benzoylecgonine*
3. Target concentration for UTAK Drugs of Abuse High Whole Blood Toxicology Control
   a) *0.15 mg/L morphine, codeine, hydrocodone, and oxycodone*
   b) *0. 40 mg/L cocaine, cocaethylene, and benzoylecgonine*

6 Procedure

1. Allow specimens, matrix blank, controls, and working solutions to equilibrate to room temperature.
2. Aliquot 0.2 mL of case samples and matrix blanks to clean, labeled 16x100 mm tubes.
3. Prepare control samples by adding 0.2 mL of each control to a clean, labeled 16X100 mm tubes.
   a) *UTAK Drugs of Abuse Low Whole Blood Toxicology Control*
   b) *UTAK Drugs of Abuse High Whole Blood Toxicology Control*
4. Prepare calibrator samples by adding the specified amounts of working standard solution to 0.2 mL blank blood in clean, labeled 16X100 mm tubes.
   a) *Level 1: 10 µL of opiates (0.2 mg/L) and cocaine (1.0 mg/L) WS and 50 µL methanol*
   b) *Level 2: 20 µL of opiates (0.2 mg/L) and cocaine (1.0 mg/L) WS and 40 µL methanol*
   c) *Level 3: 60 µL of opiates (0.2 mg/L) and cocaine (1.0 mg/L) WS and no methanol*
   d) *Level 4: 10 µL of opiates (2.0 mg/L) and cocaine (10 mg/L) WS and 50 µL methanol*
   e) *Level 5: 25 µL of opiates (2.0 mg/L) and cocaine (10 mg/L) WS and 35 µL methanol*
   f) *Level 6: 50 µL of opiates (2.0 mg/L) and cocaine (10 mg/L) WS and 10 µL methanol*
5. Add 60 µL methanol to each case sample, control, and matrix blank.
6. Add to each tube 25 µL opiate and cocaine ISTD.
7. Add to each tube 1.0 mL 0.1M potassium phosphate buffer, vortex briefly to mix, and allow mixture to equilibrate approximately 10 minutes.
8. If any urine samples are included in the batch, check their pH and adjust to pH 6 ±0.5 with dilute phosphoric acid to lower pH or 1.0 M KOH to raise pH.
9. Centrifuge to pack solids (e.g. 2629 x *g* for twelve minutes)
10. Prepare SPE Column: [**Caution**: Do not let the columns dry out before

sample loading. Sample capacity is severely reduced on a dry column.]

a) *1 mL methanol pushed through under pressure*

b) *1 mL 0.1M potassium phosphate buffer (pH 6.0) pushed through under pressure*

11. Load samples onto the columns and push through under pressure.

12. Rinse SPE Column:

    a) *1 mL H_2O pushed through under pressure*

    b) *1 mL 0.1 M HCl pushed through under pressure*

    c) *Dry column for 10 minutes at approximately 25 psi*

    d) *0.5 mL methanol pushed through under pressure*

13. Elute analytes:

    a) *Shake elution solvent vigorously to assure mixing shortly before usage.*

    b) *2 mL methylene chloride/isopropanol/ammonium hydroxide (78/20/2) pushed through under pressure*

    c) *Collect eluate in clean, labeled 16X100 mm tubes*

14. Evaporate eluate to dryness in an evaporator (e.g. 40°C at 5-20 psi for 15 minutes).

15. Add 100 µL of Reconstitution Solution and vortex briefly to dissolve the analytes.

16. Cap and centrifuge (e.g. 2629 x g for six minutes) to drain the walls and pack down any residue.

17. Transfer solution to a microvolume insert in a labeled autosampler vial.

18. Data is acquired for analysis of all vials using a liquid chromatograph mass spectrometer (LCMS).

7 Interpretation

A. Proceed to Target Compound Analysis (TOX-06-02).

B. Sensitivity (Administrative cut-offs):

    a) *0.05 mg/L for quantitative analysis of cocaine, cocaethylene, and benzoylecgonine*

    b) *0.025 mg/L for qualitative analysis of cocaine, cocaethylene, and benzoylecgonine*

    c) *0.01 mg/L for quantitative analysis of morphine, oxycodone, codeine, and hydrocodone*

    d) *0.005 mg/L for qualitative analysis of morphine, oxycodone, codeine, hydrocodone, hydromorphone, oxymorphone, and 6-acetylmorphine*

8 Limitations

Cocaine degrades to benzoylecgonine in stored blood specimens. Cocaine and benzoylecgonine concentrations determined are the concentration at the time of analysis

and may not be the concentration at the time the sample was drawn.

Standard solutions for cocaine and its metabolites are prepared in methanol to minimize degradation.

9 Literature and Supporting Documentation

Varian Bond Elut Certify Methods Manual.

UCT Clean Screen Extraction Columns Applications Manual.

Telepchak M, August T, and Chaney G. Forensic and Clinical Applications of Solid Phase Extraction. 2004.

Solid-Phase Extraction and GC/MS Quantitation of Cocaine, Ecgonine Methyl Ester, Benzoylecgonine, and Cocaethylene from Meconium, Whole Blood, and Plasma. J Anal Tox. 1993. 17, pp.353-358.

Skopp G, Pötsch L, Klingmann A, and Mattern R. Stability of Morphine, Morphine-3-Glucuronide, and Morphine-6-Glucuronide in Fresh Blood and Plasma and Postmortem Blood Samples. J Anal Tox. 2001. 25, pp. 2-7.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	03/22/2010	Original Issue;
01	02/15/2013	Major Revision – Section 4.3
02	02/03/2014	Major Revision – Sections 3-6
03	09/19/2014	Minor Revision – Sections 4.3, 4.4, 6
04	04/02/2018	Major Revision – Sections 4, 6, and 8

EXTRACTION FOR BARBITURATES

1 Scope

The content of barbiturates and certain anticonvulsants can be determined by analyzing blood and urine specimens for phenobarbital, butalbital, secobarbital, and phenytoin. Quantitative analytes include phenobarbital, butalbital, and secobarbital. Phenytoin is a qualitative analyte.

Extraction and purification of analytes is accomplished with liquid-liquid extraction. Chromatographic performance is improved with methylation (TMAH derivatization). Specimens are analyzed using a gas chromatograph mass spectrometer (GCMS) in selected ion monitoring (SIM) mode.

Quantitative calculation is performed with an internal standard method based on a multipoint calibration. Qualitative identification is based on comparison of case sample and standard mass spectra supported by chromatographic retention time.

2 Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Sodium Acetate Buffer (TOX-07-07)

Target Compound Analysis (TOX-06-02)

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Controls (TOX-01-04)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Pipettes with disposable tips
- Vortexer
- Sample rotator, platform type
- Centrifuge
- Sample evaporator

4.2 Materials

- 16X100 mm tubes
- Polyethylene plug caps
- Autosampler vials with microvolume inserts
- Pasteur pipettes and bulbs

4.3 Chemicals

- Dichloromethane (high purity solvent suitable for trace analysis)
- Methanol (high purity solvent suitable for trace analysis)
- MethElute™ Reagent
- Cerilliant Barbiturate Mix-5 stock solution (250 µg/mL)
- 1.0 mg/mL butalbital-D_5 in methanol stock solution
- 1.0 mg/mL secobarbital-D_5 in methanol stock solution
- 1.0 mg/mL phenobarbital-D_5 in methanol stock solution
- 1.0 mg/mL butalbital in methanol stock solution
- 1.0 mg/mL secobarbital in methanol stock solution
- 1.0 mg/mL phenobarbital in methanol stock solution
- 1.0 mg/mL phenytoin in methanol stock solution

4.4 Solutions – Different volumes may be prepared in the same proportions as specified.

1. 0.1 M Sodium acetate buffer (pH 4.5)
2. Butalbital-D_5, secobarbital-D_5, and phenobarbital-D_5 ISTD working solution (100 mg/L)
   a) *Add 0.5 mL of 1 mg/mL stock solution for each standard per 5 mL in one volumetric flask and dilute to volume with methanol.*
3. High barbiturate working solution (100 mg/L)
   a) *Add 1 mL phenobarbital stock solution, 1 mL butalbital stock solution, 1 mL secobarbital stock solution, and 1 mL phenytoin stock solution per 10 mL in a volumetric flask and dilute to volume with methanol.*

NOTE: The stock solutions must be from a different source than those used in the control mix.

4. Low barbiturate working solution (10 mg/L)
   a) *Add 1 mL of high barbiturate working solution per 10 mL in a volumetric flask and dilute to volume with methanol.*
5. Mixed barbiturates control solution (50 mg/L)
   a) *Add 1 mL of 250 µg/mL Barbiturate Mix-5 stock solution per 5 mL in a volumetric flask and dilute to volume with methanol.*

5 Standards, Controls, and Calibration

A. Calibrators:

1. Drug standards: phenobarbital, butalbital, secobarbital, and phenytoin
2. Calibrator series: 1.0, 3.0, 5.0, 10.0, 20.0, and 30.0 mg/L for butalbital, secobarbital, phenobarbital, and phenytoin
3. Working solutions: 100 mg/L and 10 mg/L for butalbital, secobarbital, phenobarbital, and phenytoin
4. Sample volume: 0.5 mL

5. Calibration plots for analytes are as follows:
6. Quadratic function with inverse weighting – butalbital, secobarbital, phenytoin, and phenobarbital

B. Internal Standard:

1. Butalbital-D_5, secobarbital-D_5, and phenobarbital-D_5 ISTD working solution
   a) *Phenobarbital-D_5 for the quantitation of phenobarbital, butalbital-D_5 for butalbital, and secobarbital-D_5 for secobarbital*
   b) *Final concentration of the ISTD in the sample is 2.0 mg/L.*

C. Negative Control

1. Calibrator matrix blank - blood
2. Solvent blank

D. Positive Controls:

1. Standard spiked mixed barbiturate control
   a) *Mixed barbiturate control solution (50 mg/L)*
   b) *Analytes: butalbital, secobarbital, phenobarbital*
   c) *Target concentration: 2.0 mg/L of each analyte for the low concentration control, 15 mg/L of each analyte for the mid-range concentration control*

6 Procedure

1. Allow specimens, matrix blank, and working solutions to equilibrate to room temperature.
2. Aliquot 0.5 mL of the respective case samples to clean, labeled 16x100 mm tubes.
3. Prepare a calibrator matrix blank by adding 0.5 mL blank blood to a clean, labeled 16X100 mm tube.
4. Prepare a low standard spiked mixed barbiturate control by adding 20 µL 50 mg/L mixed barbiturate control solution and 130 µL methanol to 0.5 mL blank blood in a clean, labeled 16X100 mm tube.
5. Prepare a mid-range standard spiked mixed barbiturate control by adding 150 µL 50 mg/L mixed barbiturate control solution to 0.5 mL blank blood in a clean, labeled 16X100 mm tube.
6. Prepare calibrator samples by adding the specified amounts of working solution to 0.5 mL blank blood in clean, labeled 16X100 mm tubes.
   a) *1.0mg/L: 50 µL of low barbiturate working solution and 100 µL MeOH*
   b) *3.0 mg/L:150 µL of low barbiturate working solution and no MeOH*
   c) *5.0mg/L: 25 µL of high barbiturate working solution and 125 µL MeOH*
   d) *10.0mg/L: 50 µL of high barbiturate working solution and 100 µL MeOH*
   e) *20.0 mg/L: 100 µL of high barbiturate working solution and 50 µL MeOH*

f) 30.0 mg/L: 150 µL of high barbiturate working solution and no MeOH

7. Add 150 µL MeOH to each case sample and calibrator matrix blank
8. Add to each tube 10 µL 100 mg/L barbiturate ISTD, vortex briefly to mix, and allow mixture to equilibrate for approximately 10 minutes.
9. Add 1 mL sodium acetate buffer (pH 4.5) to each tube.
10. Add 5 mL dichloromethane, cap tubes, and rotate for approximately 10 minutes.
11. Centrifuge to separate layers (e.g. 2629 x *g* for twelve minutes).
12. Remove the aqueous layer and discard. Transfer organic layer to a clean, labeled 16X100 mm tube.
13. Evaporate organic layer to dryness in an evaporator(e.g. 36ºC at 5-20 psi for 15 minutes).
14. Dissolve residue in 100 µL MethElute derivatizing reagent and 50 µL additional methanol, vortex briefly.
15. Transfer solution to a microvolume insert in a labeled autosampler vial.

7 Interpretation

A. Proceed to Target Compound Analysis (TOX-06-02)

B. Sensitivity (Administrative cut-offs):

1.0 mg/L for quantitative analysis of butalbital, secobarbital, and phenobarbital

0.50 mg/L for qualitative analysis of butalbital, secobarbital, phenobarbital, and phenytoin

8 Literature and Supporting Documentation

Foerster EH, Dempsey J, and Garriott JC. A Gas Chromatographic Screening Procedure for Acid and Neutral Drugs in Blood. J Anal Tox. 1979. 3, pp. 87-91.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	03/22/2010	Original Issue
01	02/15/2013	Major Revision – Sections 1, 4.3, 4.4, 5, 6, and 7
02	02/03/2014	Major Revision – Sections 1,3,4,5,6,7
03	09/19/2014	Minor Revision – Section 4.4
04	04/02/2018	Major Revision - Sections 4 and 6 Minor Revision – Section 2

LCMS TARGET QUALITATIVE ANALYSIS (TQUAL)

1 Scope

Many analytes can be identified in the blood by the LCMS screening procedure (TOX-05-20). The analytes with standards included below can be confirmed above an established cut-off level using this method.

Extraction and purification of analytes is accomplished with solid phase extraction. Specimens are analyzed using a liquid chromatograph mass spectrometer (LCMS).

The cut-off value is established with an internal standard method based on a two-point calibration.

Qualitative identification is based on multiple reaction monitoring (MRM) data that is supported by chromatographic retention time.

2 Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Target Compound Analysis (TOX-06-02)

Preparation of Sodium Acetate Buffer (TOX-07-07)

Preparation of Ammonium Formate (TOX-07-24)

Preparation of Potassium Carbonate Buffer (TOX-07-26)

Preparation of Mobile Phase for LCMS (TOX-07-27)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Pipette (50-250 µL) with disposable tips
- Repeater pipette with appropriate size tip
- Bottle top dispenser
- Vortexer
- Sonicator
- Centrifuge
- Sample evaporator
- Solid phase extraction manifold

4.2 Materials

- 16X100 mm tubes
- Polyethylene plug caps
- SPE columns: Trace-B
- Autosampler vials with microvolume inserts

4.3 Chemicals

- H_2O (HPLC grade)
- Acetonitrile (HPLC grade)
- Ethyl acetate (high purity solvent suitable for trace analysis)
- Methanol (high purity solvent suitable for trace analysis)
- Ammonium formate, for mass spectrometry
- Formic acid, ACS grade
- Ammonium hydroxide (NH_4OH), ACS grade
- Sodium acetate trihydrate, ACS grade
- 1.0 mg/mL amitriptyline stock solution
- 1.0 mg/mL citalopram stock solution
- 1.0 mg/mL cyclobenzaprine stock solution
- 1.0 mg/mL dextromethorphan stock solution
- 1.0 mg/mL dihydrocodeine stock solution
- 1.0 mg/mL diphenhydramine stock solution
- 1.0 mg/mL fentanyl stock solution
- 1.0 mg/mL methadone stock solution
- 1.0 mg/mL nortriptyline stock solution
- 1.0 mg/mL promethazine stock solution
- 1.0 mg/mL tramadol stock solution
- 1.0 mg/mL trazodone stock solution
- 1.0 mg/mL zolpidem stock solution
- 1.0 mg/mL lamotrigine stock solution
- 1.0 mg/mL quetiapine stock solution
- 100 µg/mL o-desmethylvenlafaxine stock solution
- 1.0 mg/mL nordoxepin stock solution
- 1.0 mg/mL venlafaxine stock solution
- 1.0 mg/mL chlorpheniramine stock solution
- 1.0 mg/mL doxylamine stock solution
- 1.0 mg/mL mirtazapine stock solution
- 1.0 mg/mL doxepin stock solution
- 1.0 mg/mL hydroxyzine stock solution
- 1.0 mg/mL phencyclidine stock solution
- 1.0 mg/mL sertraline stock solution

- 1.0 mg/mL mepivacaine stock solution

4.4 Solutions – Different volumes may be prepared in the same proportions as specified

1. 0.1 M sodium acetate buffer (pH 4.5)
2. Potassium carbonate buffer (pH 9.0)
3. 1 M ammonium formate solution
4. Mobile Phase Eluent A
5. Mobile Phase Eluent B
6. Reconstitution Solution: 90% eluent A, 10% eluent B
7. Ethyl acetate: NH_4OH (98:2) prepare fresh
8. TQual Mix #1 Medium working solution (0.40/4.0/20 mg/L)
   a) *Dilute 10 µL fentanyl stock solution, 100 µL citalopram stock solution, 100 µL cyclobenzaprine stock solution, 100 µL dextromethorphan stock solution, 100 µL dihydrocodeine stock solution, 100 µL diphenhydramine stock solution, 100 µL methadone stock solution, 100 µL promethazine stock solution, 100 µL trazodone stock solution, 100 µL zolpidem stock solution, 500 µL amitriptyline stock solution, 500 µL nortriptyline stock solution, and 500 µL tramadol stock solution to 25 mL with methanol in a volumetric flask.*
9. TQual Mix #1 Low working solution (0.04/0.40/2.0 mg/L)
   a) *Dilute 1.0 mL of TQual Mix #1 Medium working solution (0.40/4.0/20 mg/L) to 10 mL with methanol in a volumetric flask.*
10. TQual Mix #2 Medium working solution (4.0/20 mg/L)
    a) *Dilute 100 µL lamotrigine stock solution, 100 µL quetiapine stock solution, 200 µL o-desmethylvenlafaxine stock solution, 20 µL nordoxepin stock solution, 20 µL venlafaxine stock solution, 20 µL chlorpheniramine stock solution, 20 µL doxylamine stock solution, 20 µL mirtazapine stock solution, 20 µL doxepin stock solution, 20 µL hydroxyzine stock solution, 20 µL phencyclidine stock solution, and 20 µL sertraline stock solution to 5 mL with methanol in a volumetric flask.*
11. TQual Mix #2 Low working solution (0.40/2.0 mg/L)
    a) *Dilute 500 µL of TQual Mix #2 Medium working solution (4.0/20 mg/L) to 5 mL with methanol in a volumetric flask.*
12. Mepivacaine ISTD (3 mg/L)
    a) *Add 0.15 mL of 1 mg/mL stock solution per 50 mL in a volumetric flask and dilute to volume with methanol.*

5 Standards, Controls, and Calibration

A. Calibrators

1. Analytes for Mix #1: amitriptyline, citalopram, cyclobenzaprine, dextromethorphan, dihydrocodeine, diphenhydramine, fentanyl, methadone, nortriptyline, promethazine, tramadol, trazodone, and zolpidem

   a) *Blood calibrator series: 0.020 and 0.20 mg/L for citalopram, cyclobenzaprine, dextromethorphan, dihydrocodeine, diphenhydramine, methadone, promethazine, trazodone, and zolpidem*

   b) *Blood calibrator series: 0.10 and 1.0 mg/L for amitriptyline, nortriptyline, and tramadol*

   c) *Blood calibrator series: 0.002 and 0.020 mg/L for fentanyl*

2. Analytes for Mix #2: lamotrigine, quetiapine, o-desmethylvenlafaxine, nordoxepin, venlafaxine, chlorpheniramine, doxylamine, mirtazapine, doxepin, hydroxyzine, phencyclidine, and sertraline

   a) *Blood calibrator series: 0.020 and 0.20 mg/L for o-desmethylvenlafaxine, nordoxepin, venlafaxine, chlorpheniramine, doxylamine, mirtazapine, doxepin, hydroxyzine, phencyclidine, and sertraline*

   b) *Blood calibrator series: 0.10 and 1.0 mg/L for lamotrigine and quetiapine*

3. Sample volume: 0.2 mL

4. Blood calibration plots for analytes are Linear function

B. Internal Standard

1. Internal Standard: mepivacaine

2. Working Solution: 3.0 mg/L mepivacaine

3. Final concentration in samples: 0.15 mg/L

C. Negative Control

1. Calibrator matrix blank – blood

6 Procedure

1. Allow specimens, matrix blank, and working solutions to equilibrate to room temperature.

2. Aliquot 0.2 mL of case samples and matrix blank to clean, labeled 16X100 mm tubes.

3. Prepare calibrator samples by adding the specified amounts of working solution to 0.2 mL blank blood in clean, labeled 16X100 mm tubes.

   a) *TQual Low calibrator: 10 µL TQual Mix #1 Low working solution (0.04/0.40/2.0 mg/L); 10 µL TQual Mix #2 Low working solution (0.40/2.0 mg/L)*

   b) *TQual Medium calibrator: 10 µL TQual Mix #1 Medium working solution (0.40/4.0/20 mg/L); 10 µL TQual Mix #2 Medium working solution (4.0/20 mg/L)*

4. Add 20 µL methanol to each case sample and matrix blank.
5. Add to each tube 10 µL of the mepivacaine ISTD solution, vortex briefly to mix, and allow mixture to equilibrate for approximately 10 minutes.
6. Add 1 mL of 0.1 M sodium acetate buffer and sonicate for 15 minutes
7. Centrifuge at 2629 x *g* for twelve minutes.
8. Prepare SPE column:
   a) *1 mL ethyl acetate pushed through under pressure*
   b) *1 mL methanol pushed through under pressure*
   c) *15 seconds of nitrogen flow at approximately 25 psi*
9. Load samples onto the columns and push through under pressure.
10. Rinse SPE column:
    a) *1 mL of potassium carbonate buffer pushed through under pressure*
    b) *1 mL H_2O pushed through under pressure*
    c) *Dry column for 20 minutes at approximately 25 psi*
11. Elute analytes:
    a) *2 mL ethyl acetate: NH_4OH (98:2) pushed through under pressure*
    b) *Collect eluate in clean, labeled 16X100 mm tubes*
12. Evaporate eluate to dryness in an evaporator (e.g. 45ºC at 5-20 psi for 15 minutes).
13. Add 100 µL of Reconstitution Solution and vortex briefly to dissolve the analytes.
14. Cap and centrifuge (e.g. 2629 x g for six minutes) to drain the walls and pack down any residue.
15. Transfer solution to a microvolume insert in a labeled autosampler vial.
16. Data is acquired for analysis of all vials using a liquid chromatograph mass spectrometer (LCMS).

7 Interpretation

A. Proceed to Target Compound Analysis (TOX-06-02).

B. Sensitivity

1. Blood administrative cut-offs:
   a) *0.010 mg/L qualitative for citalopram, cyclobenzaprine, dextromethorphan, dihydrocodeine, diphenhydramine, methadone, promethazine, trazodone, zolpidem, o-desmethylvenlafaxine, nordoxepin, venlafaxine, chlorpheniramine, doxylamine, mirtazapine, doxepin, hydroxyzine, phencyclidine, and sertraline*
   b) *0.050 mg/L qualitative for amitriptyline, nortriptyline, tramadol, lamotrigine, and quetiapine*

c) 0.001 mg/L qualitative for fentanyl

8 Limitations

Ion suppression may occur with drugs that elute at similar retention times. A high concentration of a drug may suppress other drugs that elute at similar times. If a drug is suppressed due to this reason and is unable to be confirmed, then another method should be used for confirmation.

9 Literature and Supporting Documentation

Baselt RC. Disposition of Toxic Drugs and Chemicals in Man, 7th edition. 2004 (or later edition).

SPEware Corporation. Trace Applications Manual: Benzodiazepines from Whole Blood for LC/MS Confirmations. November 2006.

Toxicology Analysis for Drugs in Blood Using Liquid Chromatography Tandem Mass Spectrometry presented by Georgia Bureau of Investigation Division of Forensic Sciences.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	02/15/2013	Original Issue
01	02/03/2014	Major Revision – Sections 3, 4 Minor Revision – Sections 5, 6
02	09/19/2014	Minor Revision – Sections 4.4, 6
03	04/01/2016	Minor Revision – Sections 4.3, 4.4, 5.6, 7
04	04/02/2018	Major Revision – Sections 4, 6, and 8

EXTRACTION FOR Δ^9-THC AND Δ^9-CARBOXY-THC IN BLOOD

1 Scope

The presence of Δ^9-tetrahydrocannabinol (THC) and Δ^9-carboxy-tetrahydrocannabinol (carboxy-THC), a THC metabolite, can be determined by analyzing blood specimens.

Extraction and purification of analytes is accomplished with liquid-liquid extraction (LLE). Specimens are analyzed using a liquid chromatograph mass spectrometer (LCMS).

Quantitative calculation is with an internal standard method based on a multipoint calibration. Qualitative identification is based on multiple reaction monitoring (MRM) data that is supported by chromatographic retention time.

2 Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

When dry ice is used, it should be handled with protective gloves and never be in contact with skin.

A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)
Controls (TOX-01-04)
Target Compound Analysis (TOX-06-02)
Preparation of Mobile Phase for LCMS (TOX-07-27)
Preparation Hexane:Ethyl Acetate Extraction Solvent (TOX-07-31)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Appropriate sized pipette with disposable tips
- Repeater pipette with appropriate size tip
- Bottle top dispenser
- Vortexer
- Sample Rotator, platform type
- Heating block
- Centrifuge
- Sample evaporator
- CO_2 tank (any grade)

4.2 Materials

- 16X100 mm tubes (silanized)
- 16X100 mm screw cap tubes (silanized)
- Screw caps
- Polyethylene plug caps
- Autosampler vials with micro-volume inserts (silanized)
- Pasteur pipettes and bulbs

4.3 Chemicals

- H_2O (purified water)
- Hexane (high purity solvent suitable for trace analysis)
- Ethyl acetate (high purity solvent suitable for trace analysis)
- Methanol (HPLC grade or greater)
- Acetone (any grade)
- Dry ice
- Formic acid, ACS grade
- 1.0 mg/mL Δ^9-tetrahydrocannabinol (THC) stock solution
- 100 µg/mL Δ^9-tetrahydrocannabinol (THC) stock solution
- 100 µg/mL Δ^9-carboxy-tetrahydrocannabinol (carboxy-THC) stock solution
- 100 µg/mL THC-D_3 stock solution
- 100 µg/mL carboxy-THC-D_3 stock solution

4.4 Solutions - Different volumes may be prepared in the same proportions as specified

1. Hexane: Ethyl Acetate (80:20) extraction solvent
2. Mobile Phase Eluent A
3. Reconstitution Solution/Mobile Phase THC Eluent B: 99.9% methanol, 0.1% formic acid
4. THC ISTD (0.8 mg/L THC-D_3 and 1.6 mg/L carboxy-THC-D_3)
   a) *Add 200 µL 100 µg/mL THC-D_3 stock solution and 400 µL 100 µg/mL carboxy-THC-D_3 stock solution per 25 mL in a volumetric flask and dilute to volume with methanol.*
5. THC High working solution (1.0/2.5 mg/L)
   a) *Dilute 10 µL 1.0 mg/mL THC stock solution and 250 µL carboxy-THC stock solution to 10 mL with methanol in a volumetric flask.*
6. THC Low working solution (0.1/0.25 mg/L)
   a) *Dilute 1.0 mL of THC High working solution (1.0/2.5 mg/L) to 10 mL with methanol in a volumetric flask.*
7. THC working control solution (0.6/1.5 mg/L)
   a) *Dilute 30 µL 100 µg/mL THC stock solution, and 75 µL carboxy-THC stock solution to 5 mL with methanol in a volumetric flask.*

8. The stock solutions in the working solution must be from a different source than those used in the working control solution.

5 Standards, Controls, and Calibration

A. Calibrators

1. Drug Standards: THC and carboxy-THC
2. Blood calibrator series: 2, 5, 10, 25, 50, and 100 ng/mL for THC
3. Blood calibrator series: 5, 12.5, 25, 62.5, 125, and 250 ng/mL for carboxy-THC
4. Sample volume: 1.0 mL
5. Blood calibration plots for analytes are linear function with inverse weighting

B. Internal Standard:

1. THC ISTD working solution containing: THC-D_3 and carboxy-THC-D_3
2. THC-D_3 ISTD
   a) *For the quantitation of THC*
   b) *Final concentration of the THC-D_3 ISTD in the sample is 20 ng/mL*
3. Carboxy-THC-D_3 ISTD
   a) *For the quantitation of carboxy-THC*
   b) *Final concentration of the carboxy-THC-D_3 ISTD in the sample is 40 ng/mL*

C. Controls

1. Standards: THC and carboxy-THC
2. Control may be purchased or prepared in-house.
3. Target concentration for THC control #1
   a) *15 ng/mL for THC*
   b) *37.5 ng/mL for carboxy-THC*
4. Target concentration for THC control #2
   a) *45 ng/mL for THC*
   b) *112.5 ng/mL for carboxy-THC*

D. Negative Control

1. Calibrator matrix blank – blood

6 Procedure

1. Allow specimens matrix blank and working solutions to equilibrate to room temperature.
2. Aliquot 1.0 mL of case samples and matrix blanks to clean, labeled 16X100 mm tubes.

3. Prepare calibrator samples by adding the specified amounts of working solution to 1.0 mL blank blood in clean, labeled 16X100 mm tubes.

   Level 1: 20 µL of THC (0.1/0.25 mg/L) WS and 80 µL methanol
   Level 2: 50 µL of THC (0.1/0.25 mg/L) WS and 50 µL methanol
   Level 3: 100 µL of THC (0.1/0.25 mg/L) WS and no methanol
   Level 4: 25 µL of THC (1.0/2.5 mg/L) WS and 75 µL methanol
   Level 5: 50 µL of THC (1.0/2.5 mg/L) WS and 50 µL methanol
   Level 6: 100 µL of THC (1.0/2.5 mg/L) WS and no methanol

4. If using purchased controls, aliquot 1.0 mL to clean labeled 16x100 mm tubes. If preparing controls in-house, prepare control samples by adding the specified amounts of working control solution to 1.0 mL blank blood in clean, labeled 16X100 mm tubes.

   Control #1: 25 µL of THC (0.6/1.5 mg/L) WC and 75 µL methanol
   Control #2: 75 µL of THC (0.6/1.5 mg/L) WC and 25 µL methanol

5. Add 100 µL methanol to each case sample and matrix blanks.
6. Add to each tube 25 µL of the THC ISTD working solution and 1 mL H_2O, vortex briefly to mix, and allow mixture to equilibrate for approximately 10 minutes.
7. Add 2.5 mL of hexane: ethyl acetate (80:20) and cap.
8. Mix on the rotator for 15 minutes.
9. Centrifuge to separate layers (e.g. 2629 x g for six minutes).
10. Prepare a cooling bath with acetone and dry ice. Add tray of samples to cooling bath and after blood component has frozen, transfer the organic layer to clean, labeled 16X100 mm tubes.
11. Evaporate to dryness in an evaporator (e.g. 40ºC at 5-10 psi for 7-10 minutes).
12. Add 50 µL of Reconstitution Solution and vortex briefly to dissolve the analytes.
13. Cap and centrifuge (e.g. 1479 x g for two minutes) to drain the walls and pack down any residue.
14. Transfer solution to an insert in a labeled autosampler vial.
15. Data is acquired for analysis of all vials using a liquid chromatograph mass spectrometer (LCMS).

7 Interpretation

A. Proceed to Target Compound Analysis (TOX-06-02).

B. Sensitivity (administrative cut-offs):

   1. 2 ng/mL quantitative for THC and 5 ng/mL quantitative for carboxy-THC
   2. 1 ng/mL qualitative for THC and 2.5 ng/mL qualitative for carboxy-THC

8 Limitations

All glassware used during extraction should be silanized to prevent loss of analytes.

9 Literature and Supporting Documentation

T. Sasaki, A. Elian, *Analysis of THC, OH-THC, and COOH-THC in Blood Utilizing LC/MS/MS*. Presented at Society of Forensic Toxicologists annual meeting, Austin, TX, 2006.

Multi-Target Screening for 300 Drugs Using a Q Trap LC/MS/MS System and Library Searching. Applied Biosystems.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	04/14/2014	Original Issue
01	09/19/2014	Major Revision – Section 4.4
02	04/02/2018	Major Revision – Sections 4, 6, and 8

EXTRACTION FOR AMINES IN BLOOD BY SPE

1 Scope

The content of amine drugs can be determined by analyzing blood specimens. Quantitative analytes include amphetamine, methamphetamine, MDA (methylenedioxyamphetamine), and MDMA (methylenedioxymethamphetamine). Qualitative analytes include phentermine, phenylpropanolamine, ephedrine, and pseudoephedrine. Additional drugs may be qualitatively identified (e.g. phenethylamine).

Extraction and purification of analytes is accomplished with solid phase extraction (SPE). Chromatographic performance is improved with Trifluoroacetic anhydride (TFAA) derivatization. Specimens are analyzed using a gas chromatograph/mass spectrometer (GCMS) in full scan/selected ion monitoring (SIM) mode.

Quantitative calculation is with an internal standard method based on a multipoint calibration.

Qualitative identification is based on comparison of case sample and standard mass spectra supported by chromatographic retention time.

2 Safety

Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Controls (TOX-01-04)

Target Compound Analysis (TOX-06-02)

Preparation of 1 M Acetic Acid (TOX-07-33)

Preparation of Acidified Methanol (TOX-07-34)

Preparation of Sodium Phosphate Buffer (pH 6.0) (TOX-07-35)

4 Equipment, Material, and Reagents

4.1 Equipment

- Pipette (0.1 - 1.0 mL) with disposable tips
- Micropipette with disposable tips
- Repeater pipette with appropriate size tips
- Bottle top dispenser
- Vortexer

- Centrifuge
- Sample evaporator
- Solid phase extraction manifold

4.2 Materials

- 16X100 mm tubes
- Polyethylene plug caps
- SPE Columns: SPEware Trace-B columns (711-335M, 35 mg/ 3mL)
- Autosampler vials with microvolume inserts
- Pasteur pipettes and bulbs

4.3 Chemicals

- H_2O (purified water)Hydrochloric acid (HCl), concentrated (ACS reagent grade)
- Acetonitrile (HPLC grade)
- Methanol (high purity solvent suitable for trace analysis)
- Ethyl acetate (high purity solvent suitable for trace analysis)
- Trifluoroacetic anhydride (TFAA)
- UTAK Drugs of Abuse Low Whole Blood Toxicology Control
- UTAK Drugs of Abuse High Whole Blood Toxicology Control
- Amine ISTD stock solutions:
  - 1.0 mg/mL (±)-Amphetamine-D_{11} stock solution
  - 1.0 mg/mL (±)-Methamphetamine-D_{14} stock solution
  - 1.0 mg/mL (±)-MDA-D_5 stock solution
  - 1.0 mg/mL (±)-MDMA-D_5 stock solution
- Amine stock solutions:
  - 1.0 mg/mL S-(+)-Methamphetamine stock solution
  - 1.0 mg/mL (±)-Amphetamine stock solution
  - 1.0 mg/mL (±)-MDMA stock solution
  - 1.0 mg/mL Phentermine stock solution
  - 1.0 mg/mL (±)-MDA stock solution
  - 1.0 mg/mL (±)-Phenylpropanolamine HCl stock solution
  - 1.0 mg/mL (-)-Ephedrine HCl stock solution
  - 1.0 mg/mL S,S-(+)-Pseudoephedrine stock solution

4.4 Solutions – Different volumes may be prepared in the same proportions as specified.

- Sodium Phosphate buffer (pH 6.0)
- 1 M Acetic Acid

- Elution Solvent – Ethyl Acetate:Ammonium Hydroxide (49:1) then add 2 mL methanol per 50 mL elution solvent . (Prepare fresh)
- TFAA/ethyl acetate (1:1) (derivatizing agent):

  Add 1.5 mL ethyl acetate per 1.5 mL TFAA for 48 samples. (Prepare fresh)
- Amines ISTD working solution: 5 mg/L

  Add 500 µL of each of the Amine internal standard stock solutions (see list under Chemicals) to a 100 mL volumetric flask. Dilute to 100 mL with methanol.
- 20 mg/L amine working solution

  Add 200 µL of each Amine stock solution (see list under Chemicals) to a 10-mL volumetric flask and dilute to volume with methanol.

  NOTE: the stock solutions must be from a different source than those used in the control mix.
- 2 mg/L amine working solution

  Dilute 1.0 mL of 20 mg/L amine working solution to 10 mL with methanol in a volumetric flask.

5 Standards, Controls, and Calibration

A. Calibrators

1. Drug standards: Amphetamine, Phentermine, Phenylpropanolamine, Ephedrine, Methamphetamine, Pseudoephedrine, MDA, and MDMA
2. The stock solutions must be from a different source than those used in the control mix.
3. Calibrator series: 0.05, 0.10, 0.20, 0.40, 0.80, and 1.2 mg/L
4. Working solutions: 20 mg/L and 2 mg/L
5. Sample volume: 1 mL
6. Calibration plots for analytes are as follows:

   a) *Linear function with inverse weighting – Amphetamine, Ephedrine, Methamphetamine, Pseudoephedrine, and MDA*

   b) *Quadratic function with inverse weighting – Phenylpropanolamine*

   c) *Quadratic function with equal weighting – Phentermine and MDMA*

B. Internal Standard

1. Amines ISTD working solution containing: (±) Amphetamine-D11, (±) Methamphetamine-D14, (±) MDA-D5, and (±) MDMA-D5
2. (±) Amphetamine-D_{11} ISTD

   a) *For the quantitation of Amphetamine and qualitative assessment of Phentermine and Phenylpropanolamine*

   b) *Final sample concentration of the (±) Amphetamine-D_{11} in the sample is 0.25 mg/L.*

3. (±) Methamphetamine-D_{14} ISTD
   a) *For the quantitation of Methamphetamine and qualitative assessment of Ephedrine and Pseudoephedrine*
   b) *Final sample concentration of the (±) Methamphetamine-D_{14} in the sample is 0.25 mg/L.*
4. (±) MDA-D_5 ISTD
   a) *For the quantitation of MDA*
   b) *Final sample concentration of the (±) MDA-D_5 in the sample is 0.25 mg/L.*
5. (±) MDMA-D_5 ISTD
   a) *For the quantitation of MDMA*
   b) *Final sample concentration of the (±) MDMA-D_5 in the sample is 0.25 mg/L.*

C. Negative Control
1. Calibrator matrix blank - blood to match calibrator matrix
2. Solvent blank

D. Positive Blood Controls
1. Standards: Amphetamine, Methamphetamine, MDA, and MDMA
2. Target concentration for UTAK Drugs of Abuse Low Whole Blood Toxicology Control: 0.10 mg/L
3. Target concentration for UTAK Drugs of Abuse High Whole Blood Toxicology Control: 0.50 mg/L

6 Procedure

1. Allow specimens, matrix blank, controls and working solutions to equilibrate to room temperature.
2. Aliquot 1 mL of the respective case samples and matrix blank to clean, labeled 16x100 mm tubes.
3. Prepare calibrator samples by adding the specified amounts of calibrator working solution to 1 mL blank blood in clean, labeled 16x100 mm tubes.

   0.05 mg/L: 25 µL 2 mg/L working solution and 75 µL MeOH

   0.10 mg/L: 50 µL 2 mg/L working solution and 50 µL MeOH

   0.20 mg/L: 100 µL 2 mg/L working solution and no MeOH

   0.40 mg/L: 20 µL 20 mg/L working solution and 80 µL MeOH

   0.80 mg/L: 40 µL 20 mg/L working solution and 60 µL MeOH

   1.20 mg/L: 60 µL 20 mg/L working solution and 40 µL MeOH
4. Add a 1 mL aliquot of the UTAK Low Whole Blood Control to a clean, labeled 16X100 mm tube. To another clean, labeled 16X100 mm tube, add a 1 mL aliquot of the UTAK High Whole Blood Control.

5. Add 100 µL methanol to each case sample, matrix blank, and UTAK controls.
6. Add to each tube 50 µL ISTD, vortex briefly, and allow mixture to equilibrate for approximately 15 minutes.
7. To each blood sample, calibrator, and control, add 2 mL COLD acetonitrile, vortex for approximately 10 seconds, and let stand for a couple of minutes.
8. Centrifuge to pack precipitate (e.g. 12 minutes at 2629 x g). Pour supernatant into a fresh tube and discard solids.
9. Partially evaporate the supernatant to about 0.5 mL at 45°C in the SPEware concentrator (~20 minutes). **Do not evaporate to dryness.**
10. Add 2 mL phosphate buffer (pH 6.0) and vortex briefly.
11. Add samples to 3cc Trace B columns on SPEware manifold (or equivalent) with a waste reservoir below. Use sufficient vacuum/pressure to draw blood samples through the column.
12. Wash columns with 1 mL deionized H_2O.
13. Wash columns with 1 mL 1M acetic acid. Dry column on full vacuum/pressure for 5 minutes. Dispose of waste from reservoir.
14. Wash columns with 1 mL hexane.
15. Wash columns with 1 mL ethyl acetate.
16. Wash columns with 1 mL methanol. Dispose of waste from reservoir.
17. Place collection tubes below columns.
18. Elute drugs with 1 mL freshly prepared Elution Solvent (shake vigorously before using). No pressure is required to reach a steady flow of solvent through the column. Turn on pressure for a few seconds at the end to ensure all elution solvent has drained from the columns.
19. Add 100 µL 1% acidified methanol to each collection tube and vortex briefly.
20. Evaporate to dryness in SPEware concentrator at 40°C (~ 8-9 minutes).
21. Add 50 µL 1:1 TFAA:EtAc to each tube. Vortex briefly. Allow to derivatize at room temperature, uncapped, for 3 minutes.
22. Cap and centrifuge tubes (e.g. 1479 x g for 2 minutes) to drain the walls.
23. Evaporate to dryness in evaporator (e.g. 35°C at 4 psi for ~2-3 minutes).

Note: Maintain low temperature and pressure as TFAA excess is killed. Remove immediately as sample reaches dryness.

24. Reconstitute in 50 µL ethyl acetate and vortex briefly.
25. Transfer solution to a microvolume insert in a labeled autosampler vial.

7 Interpretation

A. Proceed to “Target Compound Analysis” (TOX-06-02)

B. Reporting: Administrative cut-offs:

1. 0.05 mg/L quantitative Amphetamine, Methamphetamine, MDA, and MDMA

2. 0.025 mg/L qualitative Amphetamine, Phentermine, Phenylpropanolamine, Ephedrine, Methamphetamine, Pseudoephedrine, MDA, and MDMA

8 Literature and Supporting Documentation

Baselt RC. Disposition of Toxic Drugs and Chemicals in Man, 7th edition. 2004, or later edition.

Method developed by Sam Houston State University Regional Crime Laboratory: DRN TX-04-04, Version 03. Confirmation of Stimulants by Gas Chromatography/Mass Spectrometry (GC/MS).

Method developed by Arizona Department of Public Safety Scientific Analysis Bureau: TBP 2013.1, Revision 12. Sympathomimetic Amine Confirmation in Blood by GC/MS.

S. Valtier and J. Cody, Evaluation of Internal Standards for the Analysis of Amphetamine and Methamphetamine, J. Anal. Toxicol. 19: 375-380 (1995)

A. Dobos, E. Hidvégi and G.P. Somogyi, Comparison of Five Derivatizing Agents for the Determination of Amphetamine-Type Stimulants in Human Urine by Extractive Acylation and Gas Chromatography-Mass Spectrometry, J. Anal. Toxicol. 36: 340-344 (2012)

M.J. Telepchak, T.F. August and G. Chaney, Eds. Forensic and Clinical Applications of Solid Phase Extraction. Humana Press Inc. 2004.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	05/19/2014	Original Issue
01	09/19/2014	Minor Revision – Sections 4.4 and 6
02	10/26/2015	Minor Revision – Section 4.4
03	04/02/2018	Major Revision – Sections 4, 6, and 7

EXTRACTION FOR AMINES IN URINE BY SPE

1 Scope

The presence of amine drugs can be determined by analyzing urine specimens. Qualitative analytes include amphetamine, phentermine, phenylpropanolamine, ephedrine, methamphetamine, pseudoephedrine, 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA).

Extraction and purification of analytes is accomplished with solid phase extraction (SPE). Chromatographic performance is improved with Trifluoroacetic anhydride (TFAA) derivatization. Specimens are analyzed using a gas chromatograph/mass spectrometer (GCMS) in full scan/selected ion monitoring (SIM) mode.

Qualitative identification is based on comparison of case sample and reference mass spectra supported by chromatographic retention time.

2 Safety

A. Universal Bloodborne Pathogen Precautions should be observed. Personal protective equipment during sample access should include: eye protection, lab coat, latex, nitrile, neoprene, or other non-porous polymer gloves, and a laminar flow hood. Proper disinfecting of all contaminated surfaces is recommended.

B. A hepatitis B inoculation is recommended for those who will be handling body fluid specimens.

C. Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Controls (TOX-01-04)

Target Compound Analysis (TOX-06-02)

Preparation of 1 M Acetic Acid (TOX-07-33)

Preparation of Acidified Methanol (TOX-07-34)

Preparation of Sodium Phosphate Buffer (TOX-07-35)

4 Equipment, Material, and Reagents

4.1 Equipment

- Pipette (0.1 - 1.0 mL) with disposable tips
- Micropipette with disposable tips
- Repeater pipette with appropriate size tips
- Bottle top dispenser
- Vortexer
- Centrifuge
- Sample evaporator
- Solid phase extraction manifold

4.2 Materials

- 16X100 mm tubes
- Polyethylene plug caps
- SPE Columns: SPEware Trace-B columns (711-335M, 35 mg/ 3mL)
- Autosampler vials with microvolume inserts
- Pasteur pipettes and bulbs

4.3 Chemicals – Different concentrations of stock solutions may be purchased if stated concentration is unavailable or pricing is different. Working solutions must be prepared to the stated concentration.

- H_2O (purified water)
- Hydrochloric acid (HCl), concentrated (ACS reagent grade)
- Methanol (high purity solvent suitable for trace analysis)
- Ethyl acetate (high purity solvent suitable for trace analysis)
- Trifluoroacetic anhydride (TFAA)
- Amine ISTD stock solutions:
  - 1.0 mg/mL (±)-Amphetamine-D_{11} stock solution
  - 1.0 mg/mL (±)-Methamphetamine-D_{14} stock solution
  - 1.0 mg/mL (±)-MDA-D_5 stock solution
  - 1.0 mg/mL (±)-MDMA-D_5 stock solution
- Amine stock solutions:
  - 1.0 mg/mL S-(+)-Methamphetamine stock solution
  - 1.0 mg/mL (±)-Amphetamine stock solution
  - 1.0 mg/mL (±)-3,4-MDMA stock solution
  - 1.0 mg/mL Phentermine stock solution
  - 1.0 mg/mL (±)-3,4-MDA stock solution
  - 1.0 mg/mL (±)-Phenylpropanolamine HCl stock solution
  - 1.0 mg/mL (-)-Ephedrine HCl stock solution
  - 1.0 mg/mL S,S-(+)-Pseudoephedrine stock solution

4.4 Solutions – Different volumes may be prepared in the same proportions as specified.

- Sodium Phosphate buffer
- 1 M Acetic Acid
- Elution Solvent – Ethyl acetate: ammonium hydroxide (49:1) then add 2 mL methanol per 50 mL elution solvent. (Prepare fresh)
- TFAA/ethyl acetate (1:1) (derivatizing agent):

  Add 1.5 mL ethyl acetate per 1.5 mL TFAA for 48 samples. (Prepare fresh)

- Amines ISTD working solution: 20 mg/L

  Add 1 mL of each of the amine internal standard stock solutions (see list under Chemicals) to a 50 mL volumetric flask. Dilute to 50 mL with methanol.

- 25 mg/L amine working solution

  Add 250 µL of each amine stock solution (see list under Chemicals) to a 10-mL volumetric flask and dilute to volume with methanol.

- 2.5 mg/L amine working solution

  Dilute 1.0 mL of 25 mg/L amine working solution to 10 mL with methanol in a volumetric flask.

- 100 mg/L ephedrine/phenylpropanolamine working solution

  Dilute 500 µL (-)-ephedrine HCl stock solution and 500 µL (±)-phenylpropanolamine HCl stock solution to 10 mL with methanol in a volumetric flask.

- 100 mg/L pseudoephedrine working solution

  Dilute 500 µL S,S-(+)-pseudoephedrine stock solution to 10 mL with methanol in a volumetric flask.

5 Standards, Controls, and Calibration

A. Calibrators

1. Drug standards: amphetamine, phentermine, phenylpropanolamine, ephedrine, methamphetamine, pseudoephedrine, MDA, and MDMA
2. Calibrator series: 0.1, 1.0, 2.5, 5.0 mg/L
3. Working solutions: 25 mg/L and 2.5 mg/L
4. Sample volume: 0.5 mL
5. Calibration plots for analytes are quadratic function with inverse weighting

B. Internal Standard

1. Amines ISTD working solution containing: (±)-amphetamine-D_{11}, (±)-methamphetamine-D_{14}, (±)-MDA-D_5, and (±)-MDMA-D_5
2. (±)-Amphetamine-D_{11} ISTD
   a) *For the qualitative assessment of amphetamine, phentermine, and phenylpropanolamine*
   b) *Final sample concentration of the (±)-amphetamine-D_{11} in the sample is 1.0 mg/L.*
3. (±)-Methamphetamine-D_{14} ISTD
   a) *For the qualitative assessment of methamphetamine, ephedrine, and pseudoephedrine*
   b) *Final sample concentration of the (±)-methamphetamine-D_{14} in the sample is 1.0 mg/L.*
4. (±)-MDA-D_5 ISTD
   a) *For the qualitative assessment of MDA*

b) *Final sample concentration of the (±)-MDA-D_5 in the sample is 1.0 mg/L.*

5. (±)-MDMA-D_5 ISTD

   a) *For the qualitative assessment of MDMA*

   b) *Final sample concentration of the (±)-MDMA-D_5 in the sample is 1.0 mg/L.*

C. Negative Control

1. Calibrator matrix blank - urine to match calibrator matrix
2. Solvent blank

D. Conversion control ephedrine/phenylpropanolamine

1. Drug standards: ephedrine/phenylpropanolamine
2. Concentration: 10 mg/L
3. Working control solution: 100 mg/L

E. Conversion control pseudoephedrine

1. Drug standards: pseudoephedrine
2. Concentration: 10 mg/L
3. Working control solution: 100 mg/L

6 Procedure

1. Allow specimens, matrix blank, and working solutions to equilibrate to room temperature.
2. Aliquot 0.5 mL of the respective case samples and matrix blank to clean, labeled 16x100 mm tubes.
3. Prepare calibrator samples by adding the specified amounts of calibrator working solution to 0.5 mL blank urine in clean, labeled 16x100 mm tubes.

   0.10 mg/L: 20 µL 2.5 mg/L working solution and 80 µL MeOH

   1.0 mg/L: 20 µL 25 mg/L working solution and 80 µL MeOH

   2.5 mg/L: 50 µL 25 mg/L working solution and 50 µL MeOH

   5.0 mg/L: 100 µL 25 mg/L working solution and no MeOH

4. Prepare conversion controls by adding the specified amounts of working solution to 0.5 mL blank urine in clean, labeled 16X100 mm tubes.

   10 mg/L ephedrine/phenylpropanolamine: 50 µL 100 mg/L ephedrine/phenylpropanolamine working solution and 50 µL MeOH

   10 mg/L pseudoephedrine: 50 µL 100 mg/L pseudoephedrine working solution and 50 µL MeOH

5. Add 100 µL methanol to each case sample and matrix blank.
6. Add to each tube 25 µL ISTD, vortex briefly, and allow mixture to equilibrate for approximately 15 minutes.
7. Add 2 mL sodium phosphate buffer and vortex briefly.

8. Centrifuge to concentrate any particulate in the sample to the bottom of the tube (12 minutes at 2629 x g).
9. Add samples to 3cc Trace B columns on SPEware manifold (or equivalent) with a waste reservoir below. Use sufficient vacuum/pressure to draw urine samples through the column.
10. Wash columns with 1 mL deionized H_2O.
11. Wash columns with 1 mL 1M acetic acid. Dry column on full vacuum/pressure for 5 minutes. Dispose of waste from reservoir.
12. Wash columns with 1 mL hexane.
13. Wash columns with 1 mL ethyl acetate.
14. Wash columns with 1 mL methanol. Dispose of waste from reservoir.
15. Place collection tubes below columns.
16. Elute drugs with 1 mL freshly prepared Elution Solvent (shake vigorously before using). No pressure is required to reach a steady flow of solvent through the column. Turn on pressure for a few seconds at the end to ensure all elution solvent has drained from the columns.
17. Add 100 µL 1% acidified methanol to each collection tube and vortex briefly.
18. Evaporate to dryness in SPEware concentrator at 40°C (~ 8-9 minutes).
19. Add 50 µL 1:1 TFAA:EtAc to each tube. Vortex briefly. Allow to derivatize at room temperature, uncapped, for 3 minutes.
20. Cap and centrifuge tubes (e.g. 1479 x g for 2 minutes) to drain the walls.
21. Evaporate to dryness in evaporator (e.g. 35°C at ~4 psi for ~2-3 minutes).

Note: Maintain low temperature and pressure as TFAA excess is killed. Remove immediately as sample reaches dryness.

22. Reconstitute in 50 µL ethyl acetate and vortex briefly.
23. Transfer solution to a microvolume insert in a labeled autosampler vial.

7 Interpretation

A. Proceed to “Target Compound Analysis” (TOX-06-02) with these additional steps:

1. Evaluate each sample containing reportable amphetamine to determine if the sample also contains phenylpropanolamine. If the response of the phenylpropanolamine exceeds the response from the batch 10 mg/L conversion control, then the amphetamine shall not be reported.
2. Evaluate each sample containing reportable methamphetamine to determine if the sample also contains ephedrine and/or pseudoephedrine. If the response of either drug or the combined response of these drugs exceeds the response from the batch 10 mg/L conversion control, then the methamphetamine shall not be reported.

B. Reporting: Administrative cut-offs:

1. 0.05 mg/L qualitative amphetamine, phentermine, phenylpropanolamine, ephedrine, methamphetamine, pseudoephedrine, MDA, and MDMA

8 Limitations

Samples containing high levels of ephedrine, pseudoephedrine, and/or phenylpropanolamine may have converted to detectable levels of methamphetamine and/or amphetamine during injection into GCMS. Analysis of a conversion control containing 10 mg/L ephedrine, pseudoephedrine, and phenylpropanolamine demonstrates that conversion is not occurring up to that level. High levels of ephedrine and phenylpropanolamine exceeding the level of the control may occur in some samples.

9 Literature and Supporting Documentation

Baselt RC. Disposition of Toxic Drugs and Chemicals in Man, 7th edition. 2004, or later edition.

Method developed by Sam Houston State University Regional Crime Laboratory: DRN TX-04-04, Version 03. Confirmation of Stimulants by Gas Chromatography/Mass Spectrometry (GC/MS).

Method developed by Arizona Department of Public Safety Scientific Analysis Bureau: TBP 2013.1, Revision 12. Sympathomimetic Amine Confirmation in Blood by GC/MS.

S. Valtier and J. Cody, Evaluation of Internal Standards for the Analysis of Amphetamine and Methamphetamine, J. Anal. Toxicol. 19: 375-380 (1995)

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Wu AHB, Wong SS, Johnson KG, Ballatore A, and Seifert Jr WE. The Conversion of Ephedrine to Methamphetamine and Methamphetamine-like Compounds During and Prior to Gas Chromatographic / Mass Spectrometric Analysis of CB and HFB Derivatives. Biol Mass Spectrometry. 1992. 21, pp. 278-284.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	05/01/2019	Original Issue

SCREENING BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

1 Scope

Extraction and purification of analytes is accomplished with the associated extraction. Specimens are analyzed using a gas chromatograph/mass spectrometer (GC/MS).

The presence of possible drugs is evaluated with a library search report. Qualitative identification is based on comparison of case sample and standard mass spectra supported by chromatographic retention time.

2 Related Documents

- Drug Standards, Calibrators, and Internal Standards (TOX-01-03)
- Controls (TOX-01-04)
- Report Writing Guidelines (TOX-01-08)
- Extraction for GC/MS Screen (TOX-05-06)
- GC/MS Instructions (TOX-INS-02)
- GC/MS Data Screen Worksheet (LAB-TOX-12)

3 Equipment / Materials / Reagents

3.1 Equipment

- Gas chromatograph/mass spectrometer
- Autosampler for batch injections

4 Instrument Calibration

4.1 GC/MS

A. The GC/MS shall be tuned on the initial day of instrumental processing for a batch of samples.

B. If tuning is not successful, the instrument should be taken out of service until corrective action is taken.

5 Procedure/Interpretation

5.1 GC/MS Analysis

A. Select instrument operating conditions for the batch.

B. Setup an acquisition sequence for the controls and case samples.

1. If 15 blood case samples or less, all blood case samples shall be followed by a re-injection of the positive blood control.
2. If greater than 15 blood case samples, approximately ½ the blood case samples shall be followed by a re-injection of the positive blood control, then the remainder of blood case samples shall be followed by another re-injection of the positive blood control.

3. If there are urine cases in the batch, the urine samples with appropriate controls would follow the same general layout of the sequence as blood samples.

C. Insert vials into the autosampler tray to match the sequence file.

D. Start the sequence program to inject and acquire data for each case sample, blank and control.

5.2 Detection of Drugs

A. A drug may be reported as detected if the chromatography is acceptable and retention time (RT) and mass spectrum criteria are met.

B. Chromatography is acceptable if distinct peaks are present for all plotted ions. At least two ions must be plotted for each drug.

C. The chromatographic peak is acceptable if it is within +/- 0.2 minutes of the expected retention time calculated from the relative retention time (RRT).

1. A RRT is calculated as the RT of a reference standard with respect to the RT of the internal standard run under the same operating conditions.

2. RRT = reference standard RT / internal standard RT

3. If the RT fails to match, examine the data for variation of RT with concentration. If noted, the case sample may be diluted to adjust the concentration and re-injected after the batch.

4. If a RT from a known reference standard is not available

   a) The standard will be injected with the internal standard to obtain a RT and RRT, and

   b) Reference spectrum obtained must be verified by an outside source before addition to library.

5. The mass spectrum is acceptable when the sample spectrum matches a reference spectrum from a library. The mass spectrum is acceptable if the following criteria are met:

   a) The sample spectrum will have a quality match with a reference spectrum from a library greater than or equal to 50.

   b) If a reference spectrum contains ions greater than 20% of the base peak, the sample spectrum should also contain those ions. Include chromatographic plots and/or spectrum before subtraction to document the absence of the ion(s).

   c) If a sample spectrum contains ions greater than 20% of the base peak, the reference spectrum should also contain those ions. Include chromatographic plots to document the interfering or co-eluting ion(s).

   d) If a reference spectrum contains a minimal number of ions with relative intensities greater than 20% of the base peak, compare prominent ions and/or groupings with the sample spectrum before determining a match.

5.3 GC/MS Data Processing of Matrix Blank

A. Evaluate a library search report. Record the libraries used on the GC/MS Data Screen Worksheet.

B. Print the extracted ion chromatogram (XIC) of the internal standard and mass spectra supporting the identification of the internal standard. Label and retain in the batch archive.

C. If a drug is detected in the negative control (matrix blank) due to contamination, that drug cannot be reported for any case samples in the batch.

D. If a reportable drug is detected in the negative control (matrix blank) due to carryover, the negative control (matrix blank) will be re-injected after the batch to demonstrate that no reportable drugs are detected. If re-injection is negative, that drug can be reported for any case samples in the batch.

E. Print, label, and retain the total ion chromatogram (TIC) in the batch archive.

5.4 GC/MS Data Processing of Control Data

A. Evaluate a library search report. Record the libraries used on the GC/MS Data Screen Worksheet.

B. For the internal standard, print, label and retain in the batch archive:

1. the XIC of at least two significant ions
2. the mass spectrum supporting the identification of the internal standard

C. For each analyte in the control detected, print, label, and retain in the batch archive:

1. the XIC of at least two significant ions with previous solvent blank
2. the sample spectrum
3. the sample spectrum compared to a reference spectrum supporting the detection of that drug

D. For any expected analyte(s) not detected print, label, and retain in the batch archive:

1. the XIC of at least two significant ions at its expected RT
2. a sample spectrum supporting exclusion of that analyte with “[drugname] not detected”, expected RT, and RRT
3. a reference spectrum

E. Print a TIC with peaks marked for the internal standard and detected drugs.

F. Complete GC/MS Data Screen Worksheet and retain in batch archive.

G. Case samples bracketed by acceptable positive controls may proceed to data processing for case samples.

H. If any injections of positive controls are not acceptable, determine if instrument maintenance or adjustment is necessary.

1. If necessary, document the actions performed and repeat GC/MS analysis of affected samples.
2. If not necessary, the Batch Archive Review Sheet will be labeled to indicate that the detection sensitivity may be impaired. Proceed to data analysis of

affected case samples with the restriction that negative results will not be sufficient to exclude that drug. Results for drugs excluded by another method and drugs detected meeting criteria are acceptable to report.

5.5 GC/MS Data Processing of Case Sample Data

A. Evaluate a library search report. Record the libraries used on the GC/MS Data Screen Worksheet.

B. Print the XIC of the internal standard and mass spectrum supporting the identification of the internal standard. Label and retain in the case record.

C. For each significant drug detected, print and retain in the case record:

1. the XIC of the analyte and previous solvent blank
2. the sample spectrum labeled with drug name, expected RT, and RRT
3. the sample spectrum compared to a reference spectrum supporting the detection of that drug labeled with case number

D. If an indication of carryover is observed for a reportable drug, it will be necessary to re-inject that sample to determine if it is carryover.

E. For each suspected drug not detected, and not already excluded or confirmed by other methods, print, label, and retain in the case record:

1. the XIC of at least two significant ions at its expected RT
2. the sample spectrum supporting exclusion of that drug labeled as “[drugname] not detected”, expected RT, and RRT
3. the reference spectrum

F. Print a TIC with peaks marked for the internal standard, identified drugs, and relevant unidentified compounds.

G. Complete GC/MS Data Screen Worksheet and retain in case record.

H. Additional considerations while evaluating the GC/MS screen data:

1. The analyst may perform appropriate derivatization to meet identification criteria to report an analyte.

I. Results may not meet all applicable criteria for reporting. On a case-by-case basis, additional analysis may be limited. Examples may include limiting additional testing for:

1. A drug when other drugs detected may be sufficient for an opinion on impairment.
2. A metabolite when the parent drug is detected.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
01	07/01/2003	Original Issue; Major Revision from previous GC/MS Data Screening SOP
02	10/20/2006	Major Revision – restructuring of documents and substantive changes to content
03	06/03/2009	Major Revision – Sections 5, 6, and 7 Minor Revision – Sections 4 and 9 Implementation of LCMS, additional practices and clarification of text
04	01/20/2010	Major Revision – Section 6.3 Minor Revision – Sections 4, 5, 6.1, 6.4, and 8 Advisory Board 01/11/2010
05	03/15/2017	Major Revision – Sections 3, 4, 5, and 6 (possibly 7)
06	04/02/2018	Minor Revision – Sections 5.1 and 5.2
07	07/20/2018	Major Revision – Version 05 restored

TARGET COMPOUND ANALYSIS

1 Scope

Drug content can be determined by analyzing biological specimens. This procedure is preceded by the extraction method appropriate for the target analyte and sample matrix, which specifies the preparation of controls, calibrators, and samples. Identification and/or quantitation are accomplished by establishing a calibration curve using a gas chromatograph/mass spectrometer (GCMS) or liquid chromatograph/mass spectrometer (LCMS). This procedure also lists the quality control for batch run acceptance.

2 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Related Documents

Drug Standards, Calibrators, and Internal Standards (TOX-01-03)

Controls (TOX-01-04)

Report Writing Guidelines (TOX-01-08)

Target extraction methods (TOX SOP, Section 5)

Screening by GCMS (TOX-06-01)

Screening by LCMS (TOX-06-03)

LCMS Instructions (TOX-INS-01)

GCMS Instructions (TOX-INS-02)

Zero Response Calculation page (LAB-TOX-15)

4 Equipment, Materials, and Reagents

4.1 Equipment

- Gas chromatograph/mass spectrometer
- Liquid chromatograph/mass spectrometer
- Autosampler for batch injections
- Mass spectral data system with software for control of sampling and data acquisition and for processing mass spectral data for identification and quantitation.

4.2 Samples, Standards, and Controls

- Defined by the extraction method
- Prepared and processed as prescribed by the extraction method
- Any deviation (e.g. spillage, breakage of tubes, sample confusion, equipment malfunction) for a sample or control during extraction is documented on the batch worklist for consideration when processing data.

5 Instrument Calibration

A. GCMS

1. The GCMS shall be tuned on the initial day of instrumental processing for a batch of samples.
2. If tuning is not successful, the instrument should be taken out of service until corrective action is taken.
3. After a batch analysis is started, the GCMS should not be retuned until it is determined that re-injection of samples is not necessary.

B. LCMS

1. The LCMS shall have its tune checked on the initial day of instrumental processing for a batch of samples.
2. If the tune check is not successful, the instrument should be tuned or taken out of service until corrective action is taken.
3. After a batch analysis is started, the LCMS should not be retuned until it is determined that re-injection of samples is not necessary.

6 Procedure

6.1 GCMS/LCMS Analysis

A. Select instrument operating conditions for the type of batch being run.

B. Setup an acquisition sequence (GCMS) or batch (LCMS) for the calibrators, controls, and case samples.

1. If 15 case samples or less, all case samples are followed by a re-injection of positive control(s), low calibrator, and mid-range calibrator
2. If greater than 15 case samples, approximately ½ the case samples are followed by a re-injection of positive control(s), low calibrator, and mid-range calibrator, then remainder of case samples followed by another re-injection of positive control(s), low calibrator, and mid-range calibrator

C. Insert vials into the autosampler tray to match the sequence file.

D. Start the sequence program to inject samples and acquire data for each calibrator, case sample, blank, and control.

6.2 GCMS/LCMS Data Processing of Calibrator Data

A. Ensure integrations for target analytes and internal standards are consistent in all calibrators.

B. Establish reference calibrator qualitative identification for internal standards and target analytes.

C. Prepare and/or evaluate calibration plot based on the respective calibration model for each target analyte.

D. An acceptable quantitative calibration plot will include all of the following criteria:

1. A calculated concentration within 20% of target value for the 2 lowest calibrators and within 10% of target value for the remaining calibrators

2. A least squares fit for the calibration plot is $R^2 \geq 0.990$ and $R^2 \leq 1.010$
3. The quadratic plot, if used, does not exhibit extreme curvature
4. The calibration plot is not forced through the origin
5. An absolute value of the concentration for the target analyte less than or equal to 25% of the low calibration point concentration when using the Zero Response Calculation page (LAB-TOX-15)
6. At least 4 calibration points in the calibration plot with no more than one calibrator excluded from within the calibration range.
   a) *The range may be modified by excluding one or both endpoint calibrators.*
   b) *Two calibrators may not be excluded if both points meet acceptable calculated concentration based on the calibration plot applied for casework.*
7. A calibration range that brackets the expected concentrations for all positive controls.

E. An acceptable qualitative calibration plot will include all of the following criteria:

1. At least 2 calibration points in the calibration plot for linear curves and at least 3 calibration points in the calibration plot for quadratic curves
2. A least squares fit for the calibration plot is $R^2 \geq 0.990$ and $R^2 \leq 1.010$
3. The quadratic plot, if used, does not exhibit extreme curvature
4. The calibration plot is not forced through the origin
5. A Zero Response Calculation page (LAB-TOX-15)

   If the result of the Zero Response Calculation is greater than 25%, the qualitative administrative cut-off becomes the value of the low calibration point.

6.3 Criteria for Qualitative Identification

A. The retention time must be within +/-0.2 minutes of the reference calibrator based on the quantitative ion/quantitative transition. If the retention time does not match:

1. Examine the data for the calibrators for variation of retention time with concentration. If noted, use the calibrator of closest concentration to match retention time.
2. The time may be calculated as the average of the times on each side of a peak at approximately half the peak height, if there is not an obvious apex
3. The match may be established by adjusting the retention time relative to the internal standard.

B. The ratios of qualifier ions to the quantitation ion must be within 30% for full scan, 20% for selected ion monitoring (SIM), and 25% for multiple reaction monitoring (MRM) of the ratio for the reference calibrator. If the ratio criteria is not met for a target analyte:

1. Manually adjust integration if the ion areas are not properly integrated.

2. Compare all ion ratios of the sample to a calibrator of similar concentration.
3. Dilute the prepared solution in the vial and inject (for case samples only)

C. If available, the mass spectrum must match the pattern of the reference calibrator.

6.4 GCMS/LCMS Data Processing of Matrix Blank Data

A. Verify integrations for each target analyte at its expected retention time.

B. Evaluate the matrix blank injected at the beginning of the sequence for the response area of the respective quantitative ion/quantitative transition for the target analytes.

1. If the response area in the initial injection of the matrix blank is < 10% of the response area for the low calibration point, the matrix material is acceptable for use in quantitation of the respective target analyte.
2. If the response area of the initial injection of the matrix blank is ≥ 10% of the response area for the low calibration point, the matrix material is unacceptable for use in reporting the respective target analyte.

C. Evaluate the three (3) sequential injections of the matrix blank injected for carryover of the respective target analytes.

1. All three sequential injections of the matrix blank should be similar to the matrix blank injected at the beginning of the sequence.
2. Carryover is indicated if a response of the quantitative ion/quantitative transition is greater than the response of the matrix blank injected at the beginning of the sequence and decreases in sequential injections. Qualitative ions/qualitative transition should also be present to indicate the possible presence of the target analyte.
3. If an indication of carryover is observed for a target analyte(s), it will be necessary to re-inject samples with a reportable concentration which follow samples of a high concentration.

   a) Sample re-injection must follow a matrix blank re-injection. Sample re-injection and matrix blank re-injection data will be placed in the case record.

6.5 GCMS/LCMS Data Processing of Control Data for Quantitative Analysis

A. Ensure integrations and internal standards are consistent with the calibrators.

B. Ensure that the target analytes of the controls meet their qualitative identification criteria.

C. The acceptance criterion for initial injections of controls is a calculated concentration within 20% of the verified value.

1. If the initial injection of a positive control does not meet acceptance criteria and the initial injection of the other positive control does, the calibration is acceptable if:

   a) the affected positive control has a calculated concentration within 30% of the verified value, or

   b) a later injection of the affected positive control meets the criteria for acceptance

D. The acceptance criteria for positive control injections not used for calibration acceptance is a calculated concentration within 20% of the verified value or within 15% of the calculated concentration for the injection used for calibration acceptance.

1. If one positive control injection not used for calibration acceptance does not meet acceptance criteria,

   a) *the adjacent positive control injection must meet acceptance criteria, and*

   b) *both adjacent calibrator re-injections must have a calculated concentration within 20% of the expected concentration or within 15% of the calculated concentration for the initial injection of the calibrator.*

E. Case samples must either be bracketed by acceptable control injections and/or calibrator re-injections to be evaluated quantitatively.

6.6 GCMS/LCMS Data Processing of Control Data for Qualitative Analysis

A. Ensure integrations and internal standards are consistent with the calibrators.

B. Ensure that the target analytes of the controls (if applicable) and calibrator re-injections meet their respective qualitative identification criteria.

If the qualitative identification criteria are not met, note in the batch archive how case samples may be affected.

6.7 GCMS/LCMS Data Processing of Case Sample Data

A. Ensure integrations and internal standards are consistent with the calibrators.

B. Ensure that all reportable analytes meet qualitative criteria and have been evaluated for potential carryover.

C. Results may not meet all applicable criteria for reporting. On a case-by-case basis, additional analysis may be limited. Examples may include limiting additional testing for:

1. A quantitative result of a metabolite.
2. An exact quantitative result of the drug detected at a level higher than the high calibrator.
3. A drug when other drugs detected may be sufficient for an opinion on impairment.

D. The following selection criteria will be used to determine the reported value for multiple extractions:

1. If a condition exists to give less weight to consideration of a sample result (e.g. degradation, extraction tube leakage), document the condition and exclude the sample result from comparisons.
2. If the reportable values (truncated) are equal then that value will be included on the report.
3. If the lowest concentration is <[low cal] and higher result (truncated) is equal to low cal, then report as <[low cal].

4. The lowest concentration is reported if higher results agree within 20% of the lower results.

   % difference is calculated:

$$\frac{(High\ Value - Low\ Value)}{Low\ Value} \times 100 = \%\ difference$$

   Values will not be truncated or rounded until reported.

5. If there are two or more analyses and agreement is not within 20%, the analyte may be reported qualitatively.

7 Limitations

Each method's extraction process and instrumentation parameters are targeted towards specific analytes. The presence or absence of a drug cannot be determined if the adequate method is not applied.

8 Records

GCMS Instrument logbook

LCMS Instrument logbook

GCMS Tune notebook or electronic equivalent

LCMS Tune notebook or electronic equivalent

Zero Response Calculation page (LAB-TOX-15)

9 Literature and Supporting Documentation

Baselt RC. Disposition of Toxic Drugs and Chemicals in Man, 7th edition. 2004.

Pierce WO, Lamoreaux TC, Urry FM, Kopjak L, and Finkle BS. A New, Rapid Gas Chromatography Method for the Detection of Basic Drugs in Postmortem Blood, Using a Nitrogen Phosphorus Detector. Part I. Qualitative Analysis. J Anal Tox. 1978. 2, pp. 26-31.

Pierce WO, Lamoreaux TC, Urry FM, Kopjak L, and Finkle BS. A New, Rapid Gas Chromatography Method for the Detection of Basic Drugs in Postmortem Blood, Using a Nitrogen Phosphorus Detector. Part II. Quantitative Analysis. J Anal Tox. 1979. 3, pp. 155-157.

Foerster EH, Hatchett D, and Garriott JC. A Rapid, Comprehensive Screening Procedure for Basic Drugs in Blood or Tissues by Gas Chromatography. J Anal Tox. 1978. 2, pp. 50-55.

Skoog DA. Principles of Instrumental Analysis, 3rd edition. Saunders College Publishing, New York. 1985, pp. 523-535, 554.

McLafferty FW, Interpretation of Mass Spectra, 3rd edition. University Science Books, Mill Valley, California. 1980.

Watson JT, Introduction to Mass Spectroscopy: Biomedical, Environmental, and Forensic Applications. Raven Press Books, New York. 1976.

Kames, Shiu, and Shah. Validation of Bioanalytical Methods. Pharm Res. 8(4), 1991.

Polettini A. Applications of LC-MS in Toxicology. Pharm Press. 2006.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00		Original Issue
…		Previous revision history included in archived documents.
04	11/12/2007	Major Revision including reference to calibration models specified in respective extraction methods, addition of specifications for qualitative identification criteria, addition of positive controls for quantitative analysis, addition of documentation of internal standards identification, addition of procedures concerning acceptance criteria of calibrator matrix blanks and determination of carryover.
05	06/03/2009	Major Revision – Title, Sections 1, and 4 through 11 Minor Revision – Section 3 Implementation of LCMS, additional practices and clarification of text
06	01/20/2010	Major Revision –Sections 7.1, 7.3, and 8 Minor Revision – Sections 4 and 6.1 Advisory Board 01/11/2010
07	10/26/2015	Major Revision – All sections; entire document reorganized
08	04/02/2018	Major Revision – Section 6
09	05/01/2019	Revision – Sections 3 and 8

SCREENING BY LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY (LC/MS)

1 Scope

Extraction and purification of analytes from blood specimens is accomplished with a protein precipitate extraction. Specimens are analyzed using a liquid chromatograph/mass spectrometer (LC/MS).

The presence of possible drugs is evaluated with information dependent acquisition (IDA) data. Qualitative identification is based on multiple reaction monitoring (MRM) data that is supported by an Enhanced Product Ion (EPI) spectrum and chromatographic retention time.

2 Related Documents

- Drug Standards, Calibrators, and Internal Standards (TOX-01-03)
- Controls (TOX-01-04)
- Report Writing Guidelines (TOX-01-08)
- Extraction for LC/MS Screen (TOX-05-20)
- LC/MS Instructions (TOX-INS-01)
- LC/MS Data Screen Worksheet (LAB-TOX-13)

3 Equipment, Materials, and Reagents

3.1 Equipment

- Liquid chromatograph/mass spectrometer
- Autosampler for batch injections

4 Instrument Calibration

4.1 LC/MS

A. The LC/MS shall have its tune checked on the initial day of instrumental processing for a batch of samples.

B. If the tune check is not successful, the instrument should be tuned. If the tune is not successful the instrument should be taken out of service until corrective action is taken.

5 Procedure/Interpretation

5.1 LC/MS Analysis

A. Select instrument operating conditions for the batch.

B. Setup an acquisition batch for the controls and case samples.

1. If 15 case samples or less, all case samples shall be followed by a re-injection of positive control.
2. If greater than 15 case samples, approximately ½ the case samples shall be followed by a re-injection of the positive control, then the remainder of case samples shall be followed by another re-injection of the positive control.

C. Insert vials into the autosampler tray to match the batch sequence.

D. Start the batch to inject and acquire data for each case sample and control.

5.2 Detection of Drugs

A. A drug may be reported as detected if the chromatography is acceptable and retention time (RT) and mass spectrum criteria are met.

B. Chromatography is acceptable if a distinct peak is present in the extracted ion chromatogram for the MRM.

C. The chromatographic peak is acceptable if it is within +/- 0.4 minutes of the expected retention time calculated from the relative retention time (RRT).

1. A RRT is calculated as the RT of a reference standard with respect to the RT of the internal standard run under the same operating conditions.

RRT = reference standard RT / internal standard RT

D. The mass spectrum is acceptable when the sample spectrum matches a reference spectrum from the library. The mass spectrum is acceptable if the following criteria are met:

1. The fit and reverse fit of the mass spectrum match must be greater than or equal to 50%.

2. When compared to the reference spectrum, the sample spectrum must contain strong similarities.

5.3 LC/MS Data Processing of Matrix Blank

A. Evaluate the negative control (matrix blank) for demonstration of no contamination of reagents.

B. Print and label the total ion chromatograms (TIC) of the MRMs, the extracted ion chromatograms (XIC) of the MRM, and EPI spectrum supporting the identification of the internal standard. Retain in the batch archive.

C. If a drug is detected in the negative control (matrix blank) due to contamination, that drug cannot be reported for any case samples in the batch.

D. If a reportable drug is detected in the negative control (matrix blank) due to carryover, the negative control (matrix blank) will be re-injected after the batch to demonstrate that no reportable drugs are detected. If re-injection is negative, that drug can be reported for any case samples in the batch.

5.4 LC/MS Data Processing of Control Data

A. Evaluate the positive control for proper detection of expected analytes.

1. Examine the EPI spectrum of the transitions for the expected analytes in the control.

2. For the internal standard and analytes detected in the injections of a control, print, label, and retain in the batch archive:

a) TIC of the MRMs,

b) XIC of the MRM

c) EPI scan supporting the identification of the analytes.

3. For any expected analyte(s) not detected in the initial injections of a control, print, label, and retain in the batch archive:
   a) *TIC of the MRMs,*
   b) *XIC of the MRM*
   c) *EPI spectrum (if available) with drug not detected, expected RT, and RRT*

B. Complete LC/MS Data Screen Worksheet and retain in batch archive.

C. Case samples bracketed by acceptable positive controls may proceed to data processing for case samples.

D. If any injections of positive controls are not acceptable, determine if instrument maintenance or adjustment is necessary.
   1. If necessary, document the actions performed and repeat LC/MS analysis of affected samples.
   2. If not necessary, the Batch Archive Review Sheet will be labeled to indicate that the detection sensitivity may be impaired. Proceed to data analysis of affected case samples with the restriction that negative results will not be sufficient to exclude that drug. Results for drugs excluded by another method and drugs detected meeting criteria are acceptable to report.

5.5 LC/MS Data Processing of Case Sample Data

A. Evaluate the EPI spectrums of the transitions detected for the possible presence of drugs.

B. For the internal standard, print, label, and retain in the case record:
   1. TIC of the MRMs,
   2. XIC of the MRM,
   3. Sample and reference EPI spectrum supporting the identification of the internal standard.

C. For each significant drug detected, print, label, and retain in the case record:
   1. TIC of the MRMs,
   2. XIC of the MRM,
   3. Sample and reference EPI spectrum labeled with drug, expected RT, and RRT, supporting the identification of the drugs.

D. Compare the sample spectrum to a reference spectrum.

E. If an indication of carryover is observed for a reportable drug, it will be necessary to re-inject that sample to determine if it is carryover.

F. For each suspected drug not detected, and not already excluded or confirmed by other methods, print, label, and retain in the case record:
   1. TIC of the MRMs,
   2. XIC of at its expected RT

3. Sample and reference EPI spectrum with drug not detected, expected RT, and RRT

G. Complete LC/MS Data Screen Worksheet and retain in case record.

H. Results may not meet all applicable criteria for reporting. On a case-by-case basis, additional analysis may be limited. Examples may include limiting additional testing for:

1. A drug when other drugs detected may be sufficient for an opinion on impairment.
2. A metabolite when the parent drug is detected.

Preparer

Megan Barton Date: 01/13/2017
Toxicology Advisory Board Chair

Concurrence

Katherine G. Sanchez Date: 02/15/2017
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	01/20/2010	Original Issue;
01	02/03/2014	Minor Revision- Sections 3, 4, 5, and 7
02	03/15/2017	Major Revision – Sections 5 and 6.1 Minor Revision – Section 6.4

PREPARATION OF BORATE BUFFER

1 Scope

This buffer is used for extraction of basic compounds in Drug Screens and protocols with similar extraction. It is also used in combination with 20% NaOH to boost the pH in the Amines extraction.

2 Specification

Borate Buffer (pH 11.0)

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Special Equipment and Supplies

- pH meter
- Balance

5 Reagents

- H_2O (purified water)
- Boric Acid (ACS reagent grade)
- Potassium Chloride (ACS reagent grade)
- Sodium Hydroxide (ACS reagent grade)

6 Instructions

1. Dissolve 30.92 g of Boric acid and 37.20 g KCl in 500 mL H_2O.
2. Dissolve 20 g NaOH in 500 mL H_2O.
3. Combine the two solutions in a container to allow pH checking.
4. Check pH for 11.0 (± 0.1). If necessary, increase pH with 20% Sodium Hydroxide.
5. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

- Minimum labeling includes specification above, initials, and date prepared.
- Verification can be accomplished by pH check after preparation equal to 11.0 (± 0.1) and inclusion with acceptable batch criteria.
- Store bulk solution refrigerated and shelf container at room temperature.
- Discard after 9 months or check that pH is 11 at use.

8 Literature and Supporting Documentation

Methods of Analysis. IRS Publication No. 341. Rev 12-66, pp. 107-108.

Stewart CP, and Stolman A. Toxicology Mechanisms and Analytical Methods. p. 492.

Official Methods of Analysis of AOAC, 11th edition. 1970, p. 873.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
01	07/01/2003	Minor Revision numbering Addition Section 7 literature citations
02	10/20/2006	Minor Revision numbering
03	06/03/2009	Major Revision – Section 6 Minor Revision – Section 8 Clarification of text and practice
04	02/03/2014	Minor Revision – Title Major Revision – Section 7
05	04/02/2018	Major Revision – Section 1

PREPARATION OF ACIDIFIED ETHANOL

1 Scope

This solution is added to organic extracts of samples to create the hydrochloride salt of basic drugs prior to evaporation. The salt is less volatile than the base and prevents loss of the drugs.

2 Specification

~2% HCl in Ethanol

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Amber glass container
- Pipette with disposable pipette tips
- Graduated Cylinder

5 Reagents

- Ethanol: 190 - 200 proof
- Hydrochloric acid

6 Instructions

1. Add 200 µL concentrated HCl to 10 mL ethanol.
2. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Verification can be accomplished by inclusion with acceptable batch criteria.

C. Store at room temperature in amber glass container to minimize light enhanced reaction products.

D. Avoid addition to old solution for storage.

E. Discard after 1 month due to possible creation of reaction products.

8 Literature and Supporting Documentation

Swallow, W. H. 1982. Decreases in yields of extracted drugs after exposure to 1% Hydrochloric Acid in Methanol. Journal of Forensic Science Society, 22:297-300.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
01	07/01/2003	Minor Revision numbering Addition Section 7 literature citations
02	10/20/2006	Minor Revision numbering
03	02/03/2014	Major Revision - Section 6, 7 Minor Revision – Title
04	05/01/2019	Revision – Sections 3, 4, and 7

PREPARATION OF 20% SODIUM HYDROXIDE

1 Scope

This basic solution is used to adjust pH of fresh Borate Buffer to 11.0.

2 Specification

20% Sodium Hydroxide

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Volumetric flask
- Balance

5 Reagents

- H_2O (purified water)
- Sodium Hydroxide (NaOH)

6 Instructions

1. Dissolve 20 g NaOH per 100 mL H_2O in a partially filled volumetric flask.
2. Dilute to total volume with H_2O.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Verification can be accomplished by inclusion with acceptable batch criteria.

C. Store at room temperature

D. Discard after 1 year.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
01	07/01/2003	Minor Revision – Numbering
02	10/20/2006	Minor Revision – Numbering
03	02/03/2014	Major Revision – Section 7
04	05/01/2019	Revision – Sections 1, 3, 4, and 7

PREPARATION OF 1 *M* POTASSIUM HYDROXIDE

1 Scope

This basic solution is used to adjust pH of 0.1 *M* Potassium phosphate buffer used in SPE for Opiate, Cocaine, and Cocaine Metabolites analysis and similar SPE methods.

2 Specification

1.0 *M* Potassium Hydroxide

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Volumetric flask
- Balance

5 Reagents

- H_2O (purified water)
- Potassium Hydroxide (KOH)

6 Instructions

1. Dissolve 5.6 g KOH (MW 56.11) per 100 mL H_2O in a partially filled volumetric flask.
2. Dilute to total volume with H_2O.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Store at room temperature.

C. Verification can be accomplished by inclusion with acceptable batch criteria.

D. Discard after 1 year.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
01	07/01/2003	Minor Revision – Numbering
02	10/20/2006	Minor Revision – Numbering
03	02/03/2014	Major Revision – Section 7
04	05/01/2019	Revision – Sections 1, 3, 4, and 7

PREPARATION OF 0.1*M* POTASSIUM PHOSPHATE BUFFER

1 Scope

This buffer is used to dilute blood samples and to prepare SPE columns in SPE for Opiates, Cocaine, and Cocaine Metabolites analysis and similar SPE methods.

2 Specification

0.1*M* Potassium Phosphate buffer (pH 6.0)

3 Related Documents

Preparation of 1*M* Potassium Hydroxide (TOX-07-04)

4 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

5 Equipment and Supplies

- pH meter
- Balance
- Volumetric flask

6 Reagents

- H_2O (purified water)
- Potassium di-Hydrogen Phosphate (KH_2PO_4)
- 1*M* Potassium Hydroxide (KOH)

7 Instructions

1. Add 13.61g KH_2PO_4 (MW 136.09) to a container to allow pH checking.
2. Dissolve in 900 mL H_2O.
3. With mixing, adjust pH to 6.0 ($\pm$ 0.1) with 1*M* potassium hydroxide (approximately 17 mL)
4. Quantitatively transfer to a 1.0 L volumetric flask and dilute to volume with H_2O.
5. A different volume may be prepared in the same proportion as specified.

8 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Verification can be accomplished by pH check after preparation equal to 6.0 (± 0.1) and inclusion with acceptable batch criteria.

C. Store at room temperature.

D. Discard after 4 months or check pH at use.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
01	07/01/2003	Minor Revision numbering
02	10/20/2006	Minor Revision numbering
03	06/03/2009	Major revision – Section 6 Clarification of text and practice
04	02/03/2014	Major revision- Sections 1, 7
05	05/01/2019	Revision – Sections 1, 2, 3 (now 4), 4 (now 5), and 7 (now 8) Added – Section 3 Related Documents

PREPARATION OF SODIUM ACETATE BUFFER

1 Scope

This buffer is used to adjust the pH of blood samples in the extraction of barbiturates, and other SPE methods.

2 Specification

0.1 *M* Sodium Acetate buffer (pH 4.5)

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Special Equipment and Supplies

- pH meter
- Balance
- Volumetric flask

5 Reagents

- H_2O (purified water)
- Sodium Acetate trihydrate ($NaC_2H_3O_2 \bullet 3H_2O$) (ACS grade)
- Glacial Acetic Acid (ACS grade)
- If needed: 1 *M* Sodium Acetate:

  Dissolve 13.6 g Sodium Acetate trihydrate per 100 mL H_2O. (Prepare fresh)
- If needed: 0.1 M Acetic Acid:

  Dilute 572 µL Glacial Acetic Acid per 100 mL H_2O. (Prepare fresh)

6 Instructions

1. Add 5.86 g $NaC_2H_3O_2 \bullet 3H_2O$ to a container to allow access for pH checking.
2. Add 900mL H_2O and mix to dissolve.
3. Add 3.24 mL Glacial Acetic Acid and mix.
4. With mixing, adjust pH to 4.5 ($\pm$ 0.1) with 1 M Sodium Acetate (approximately 25 mL) (raises pH) or 0.1 M Acetic Acid (lowers pH), if needed.
5. Quantitatively transfer solution to a 1.0 L volumetric flask and dilute to volume with H_2O.
6. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

1. Minimum labeling includes specification above, initials, and date prepared
2. Verification can be accomplished by pH check after preparation equal to 4.5 (± 0.1) and inclusion with acceptable batch criteria.

3. Store refrigerated
4. Discard after 6 months or check that pH is 4.5 at use.

8 Reference

United Chemical Technologies Inc. Application Manual. Solutions. Instruction SSMRS100491.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	09/01/2001	Original Issue
01	07/01/2003	Minor Revision numbering
02	10/20/2006	Minor Revision numbering
03	06/03/2009	Major revision – Section 6 Minor revision – Section 8 Clarification of text and practice
04	02/03/2014	Major revision- Section 7
05	04/02/2018	Major Revision – Sections 1 and 5

PREPARATION OF MALEIC ACID SOLUTION

1 Scope

This acidic solution is used to adjust pH of a sample in 9-Carboxy THC in urine analysis after hydrolysis to give an acidic pH for extraction.

2 Specification

2.5 *M* Maleic Acid Solution

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Balance
- Volumetric flask

5 Reagents

- H_2O (purified water)
- Maleic Acid (Assay 99% min)

6 Instructions

1. Dissolve 28.6 g maleic acid per 100 mL H_2O in a partially filled volumetric flask.
2. Dilute to total volume with H_2O.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Verification can be accomplished by inclusion with acceptable batch criteria.

C. Store at room temperature.

D. Discard after 6 months.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	10/20/2006	Original Issue
01	02/03/2014	Major revision- Section 7, Title
02	05/01/2019	Revision – Sections 3 and 4

PREPARATION OF DILUTE PHOSPHORIC ACID

1 Scope

This acidic solution is used to adjust pH, if necessary, of urine samples prior to analysis to give a more acidic pH. This solution is near 10% concentration.

2 Specification

Dilute Phosphoric Acid (approximately 10%)

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Special Equipment and Supplies

- Volumetric flask

5 Reagents

- H_2O (purified water)
- Phosphoric Acid (Concentrated -approximately 85%)

6 Instructions

1. Add (slowly) 6 mL concentrated phosphoric acid per 50 mL H_2O in a partially filled (approximately ¾) volumetric flask.
2. Allow solution to cool to room temperature.
3. Dilute to total volume with H_2O.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Verification can be accomplished by inclusion with acceptable batch criteria.

C. Store at room temperature

D. Discard after 1 year.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	10/20/2006	Original Issue
01	02/03/2014	Major revision- Section 2, 7 and Title
02	05/01/2019	Revision – Sections 3 and 7

VERIFICATION OF EMIT REAGENTS

1 Scope

These solutions are used in the Enzyme Multiplied Immunoassay Technique (EMIT) analysis to determine the presence of a category of drug (amphetamines, barbiturates, benzodiazepines, carisoprodol/meprobamate, cocaine and its metabolites, opiates, phencyclidine, THC and its metabolite, oxycodone, and zolpidem) in blood and urine. These are manufactured reagents ready for use.

2 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3 Equipment and Supplies

- EMIT sample cups and tubes
- EMIT reagent containers
- Immunoassay Analyzer

4 Reagents

- DRI® Amphetamine (AMPH) Antibody/Substrate Reagent A
- DRI® Amphetamine (AMPH) Enzyme Conjugate Reagent E
- DRI® Barbiturate (BARB) Antibody/Substrate Reagent A
- DRI® Barbiturate (BARB) Enzyme Conjugate Reagent E
- DRI® Benzodiazepine (BENZ) Antibody/Substrate Reagent A
- DRI® Benzodiazepine (BENZ) Enzyme Conjugate Reagent E
- Immunalysis Carisoprodol (SOMA) Antibody/Substrate Reagent A
- Immunalysis Carisoprodol (SOMA) Enzyme Conjugate Reagent E
- DRI® Cocaine (COC) Antibody/Substrate Reagent A
- DRI® Cocaine (COC) Enzyme Conjugate Reagent E
- DRI® Opiate (OPI) Antibody/Substrate Reagent A
- DRI® Opiate (OPI) Enzyme Conjugate Reagent E
- DRI® Phencyclidine (PCP) Antibody/Substrate Reagent A
- DRI® Phencyclidine (PCP) Enzyme Conjugate Reagent E
- DRI® Cannabinoid (THC) Antibody/Substrate Reagent A
- DRI® Cannabinoid (THC) Enzyme Conjugate Reagent E
- DRI® Oxycodone (OXY) Antibody/Substrate Reagent A
- DRI® Oxycodone (OXY) Enzyme Conjugate Reagent E
- Immunalysis Zolpidem (ZOLP) Antibody/Substrate Reagent A
- Immunalysis Zolpidem (ZOLP) Enzyme Conjugate Reagent E

5 Instructions

Reagent A and Reagent E are purchased ready to use. No reagent preparation is required.

6 Storage, Expiration, and Disposal

A. Minimum labeling includes reagent name, initials, date opened, and expiration date.

B. Store refrigerated in brown reagent bottles in compartment on instrument or refrigerated in the original plastic bottles from manufacturer.

C. Avoid addition to old solution for storage. (Note: The EMIT reagents are provided as matched sets. They should not be interchanged with components of kits with different lot numbers.)

D. Discard after the expiration date listed on the bottle.

7 Quality Control

A. All verification analyses must be reviewed and signed by the Toxicology Section Technical Leader, Toxicology Section Supervisor, or designee prior to first use in processing case samples.

B. Verification of Reagents in Blood

1. To verify the difference between the negative and cutoff calibrator:

   a) *Prepare and run a negative and cutoff calibrator with the new reagent. This can be done separately or extracted in a batch with casework.*

   b) *If the difference between the negative and cutoff calibrator meets historical standards if available (see table below), proceed using the minimum acceptable difference from the table. If the difference between the negative and cutoff calibrator is not acceptable relative to historical standards, proceed to establish a minimum acceptable difference for the current reagent lot number.*

   c) *Historical standards*

amphetamines	≥ 10 ΔA/min
barbiturates	≥ 158 ΔA/min
benzodiazepines	≥ 9 ΔA/min
carisoprodol/meprobamate	≥ 12 ΔA/min
cocaine/metabolites	≥ 13 ΔA/min
opiates	≥ 13 ΔA/min
phencyclidine	≥ 14 ΔA/min
THC/metabolite	≥ 14 ΔA/min

   d) *Record on Blood Immunoassay Reagent Log (LAB-TOX-17).*

2. To establish a minimum acceptable difference between the negative and cutoff calibrator for the lot number of reagent:

   a) *Data will be obtained from 5 replicate preparations of the negative calibrator, 5 replicate preparations of the high negative calibrator, and 5*

replicate preparations of the cutoff calibrator run with the new lot of reagent.

b) If outliers exist, a maximum of two per calibrator can be discarded. There must be at least 3 replicates of each calibrator level remaining. An outlier would be considered any data point more than one standard deviation away from the average, when the average and standard deviation are computed using all data points.

c) The minimum acceptable difference for batch acceptance will be truncated and is calculated using the sum of: 3 standard deviations of the negative calibrator, two times 3 standard deviations of the high negative calibrator, and 3 standard deviations of the cutoff calibrator.This will be completed by utilizing the EMIT Reagent Validation Form (LAB-TOX-36).

d) The reagent is acceptable for use if the cutoff average minus 2 standard deviations does not overlap the negative average plus 2 standard deviations.

e) Record on Blood Immunoassay Reagent Log (LAB-TOX-17).

f) This documentation including the EMIT log will be retained in a log book after review.

C. Verification of Reagents for Urine

For the reagent needing to be verified, run the assay for that drug category on the negative, calibrators, and controls using the new reagents.

a) Evaluate data from controls. Results for the kit are acceptable if the control values are within acceptable ranges established for the current lot.

b) Record on Urine Immunoassay Reagent Log (LAB-TOX-10).

c) This documentation including the EMIT log will be retained in a log book after review.

8 Records

A. Urine Immunoassay Reagent Log (LAB-TOX-10)

B. Blood Immunoassay Reagent Log (LAB-TOX-17)

C. EMIT Reagent Validation Form (LAB-TOX-36)

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	08/10/2007	Original Issue
01	08/19/2009	Major revision – Sections 4, 5, 7, and 8 Implementation of procedures used with MGC240 EMIT Immunoassay instrument and clarification of text
02	02/03/2014	Major revision- Sections1, 2, 5, 7, 8 and Title
03	04/14/2014	Major revision – Section 1 Minor revision – Sections 2, 7
04	04/02/2018	Major Revision – Sections 1, 6, and 7
05	05/01/2019	Revision – Sections 1, 3, 4, and 7 Added – Section 8 Records

PREPARATION OF AMMONIUM FORMATE

1 Scope

This solution is used in the preparation of eluents for LCMS analysis.

2 Specification

1.0 *M* Ammonium Formate

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Volumetric flask
- Balance

5 Reagents

- H_2O (HPLC grade)
- Ammonium formate, for mass spectrometry

6 Instructions

1. Dissolve 63 g of ammonium formate per 1 L H_2O.
2. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Store in the refrigerator.

C. Verification can be accomplished by inclusion with acceptable batch criteria.

D. Discard after 6 months.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue
01	02/03/2014	Major revision- Section 7 and Title
02	05/01/2019	Revision – Sections 1 and 3

PREPARATION OF PPG DILUTION SOLUTION

1 Scope

This solution is used to dilute PPG (Polypropylene Glycol) standard solution to tune the LCMS.

2 Specification

PPG Dilution Solution

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Volumetric flask
- Balance
- Pipette with disposable tips

5 Reagents

- H_2O (HPLC grade)
- Methanol (HPLC grade)
- Acetonitrile (HPLC grade)
- Ammonium acetate, for mass spectrometry
- Formic acid, ACS grade

6 Instructions

1. Dissolve 15.4 mg of ammonium acetate in 49.9 mL of H_2O in a glass vial.
2. In a separate glass vial, mix 49.9 mL methanol, 0.1 mL formic acid, and 0.1 mL acetonitrile.
3. Mix the contents from the two vials together to make the final PPG dilution solution.
4. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Store in the refrigerator.

C. Verification can be accomplished by successful tune of LCMS.

D. Discard after 6 months.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue
01	02/03/2014	Major revision- Title and Section 7
02	05/01/2019	Revision – Sections 3 and 4

PREPARATION OF POTASSIUM CARBONATE BUFFER

1 Scope

This basic solution is used to rinse the SPE column used in the Benzodiazepine Mix analysis and similar SPE methods.

2 Specification

Potassium Carbonate Buffer (pH 9.0)

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Volumetric flask
- Balance
- pH Meter

5 Reagents

- H_2O (purified water)
- Potassium Carbonate (K_2CO_3)
- Potassium Bicarbonate ($KHCO_3$)
- HCl, if needed

6 Instructions

1. Dissolve 20 g of $KHCO_3$ and 10 g of K_2CO_3 in a 1 L volumetric flask partially filled with 500 mL of H_2O.
2. Adjust the pH to 9.0 ($\pm$ 0.1) (e.g. with HCl).
3. Bring to volume with H_2O.
4. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Verification can be accomplished by pH check after preparation equal to 9.0 (± 0.1) and inclusion with acceptable batch criteria.

C. Store at room temperature.

D. Discard after 6 months or verify pH at use.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue
01	02/03/2014	Major revision- Section 7 and Title
02	05/01/2019	Revision – Sections 2, 3, 4, 5, and 7

PREPARATION OF MOBILE PHASE FOR LCMS

1 Scope

These solutions are used in the analysis of analytes using the LCMS.

2 Specification

Eluent A, Eluent B, and THC Eluent B

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

Graduated cylinder

5 Reagents

- H_2O (HPLC grade or greater)
- Acetonitrile (HPLC grade or greater)
- Methanol (HPLC grade or greater)
- 1*M* Ammonium formate (TOX-07-24)
- Formic acid, ACS grade

6 Instructions

1. Eluent A: 2 mL of 1*M* ammonium formate solution and 2 mL formic acid per 996 mL of H_2O.
2. Eluent B: 2 mL of 1*M* ammonium formate solution and 2 mL formic acid per 996 mL of acetonitrile.
3. THC Eluent B: 1 mL formic acid per 999 mL of methanol.
4. Sonicate the eluents before use. Ensure there is no precipitate in the eluents.
5. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Store at room temperature.

C. Verification can be accomplished by inclusion with acceptable batch criteria.

D. Discard Eluent A after 1 month. Discard Eluent B and THC Eluent B after 6 months.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue
01	01/20/2010	Major Revision – Section 6 Minor Revision – Section 1 Advisory Board 01/11/2010
02	02/03/2014	Major Revision- All sections
03	04/20/2015	Minor Revision – Section 7
04	05/01/2019	Revision – Sections 3, 4, 5, and 6

PREPARATION OF 0.1 *M* HYDROCHLORIC ACID

1 Scope

This solution is used to rinse SPE columns in the SPE for Opiates, Cocaine, and Cocaine Metabolites method.

2 Specification

0.1 *M* Hydrochloric Acid

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Volumetric flask
- Pipette with disposable tips

5 Reagents

- H_2O (purified water)
- Hydrochloric acid (ACS reagent grade)

6 Instructions

1. Add 8.3 mL HCl to a 1.0 L volumetric flask partially filled with about 800 mL of H_2O.
2. Bring to volume with H_2O.
3. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Verification can be accomplished by inclusion with acceptable batch criteria.

C. Store at room temperature.

D. Discard after 6 months.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	01/20/2010	Original Issue
01	02/03/2014	Major revision- Sections 1 and 7 Minor revision- Title
02	05/01/2019	Revision – Sections 1, 3, 4, and 7

PREPARATION HEXANE:ETHYL ACETATE EXTRACTION SOLVENT

1 Scope

This solvent is used in liquid-liquid extraction of drugs in the THC analysis in blood and other similar extractions.

2 Specification

Hexane:Ethyl Acetate (80:20) Extraction Solvent

3 Safety

A. Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Graduated cylinder

5 Reagents

- Ethyl acetate (high purity solvent suitable for trace analysis)
- Hexane (high purity solvent suitable for trace analysis)

6 Instructions

1. Mix 800 mL of hexane per 200 mL of ethyl acetate.
2. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Verification can be accomplished by inclusion with acceptable batch criteria.

C. Store at room temperature.

D. Discard after 6 months.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	04/14/2014	Original Issue
01	05/01/2019	Revision – Sections 1, 3, 4, and 7

SILANIZATION OF GLASSWARE

1 Scope

The use of silanized glassware may be necessary for some toxicological procedures. A silanizing reagent is used to deactivate the surface of the glass and reduce adsorptive losses that may occur with polar drugs (and/or metabolites), particularly those that may be present at very low concentrations.

2 Safety

A. This procedure must be conducted with the Safety Manual and the Chemical Hygiene Plan. The silanizing reagent is extremely toxic and corrosive. Wear gloves and personal protective equipment at all times. Handle under a functioning chemical hood.

B. Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

3. Related Documents

A. Thermo Scientific Vacuum Oven (TOX-INS-13)

4 Equipment / Materials /Reagents

4.1 Equipment

- Thermo Scientific Vacuum Oven Model 3608. Refer to the Operator's Manual for further instructions.
- Hamilton 1.0 mL syringe
- Dessicator
- Bimetallic thermometer
- Test Tube Basket
- Chemical Hood

4.2 Materials

- Test tubes
- Inserts
- Beakers
- GC or LC vial
- Dessicant

4.3 Reagent

- Silanizing reagent: Hexamethyldislazane CAUTION: <u>Flammable liquid,extremely harmful when inhaled and ingested,extremely corrosive to skin,mucous membranes, and respiratory tract. Causes eye burns.</u>
- Silanizing reagent must be kept in dessicator and handled under the hood at all times.

5 Procedure

5.1 Silanizing Glassware

1. Place glassware upside vertically in test tube baskets. Place inserts in a beaker. Place glassware items to be silanized into the Thermo Scientific Vacuum Oven.
2. UNDER THE HOOD. Prepare 100 µL of Hexamethyldisilazane with the syringe and dispense into the GC vial. Place vial in the oven.
3. Ensure the door is closed and evacuate/vent valves are closed.
4. Turn on the oven and turn the temperature dial to ~160°C marked by the red line.
5. As the oven heats it will automatically vacuum. DO NOT ABORT THIS PROCESS.
6. Silanize glassware for at least 1 hour. Turn down the temperature dial and turn off the oven.
7. Allow the oven to completely cool before venting.

5.2 Venting the Oven

1. On the hood, open the vacuum valve. Ensure that the hood is shut.
2. Ensure that the temperature dial is turned off. Turn on the oven.
3. Open the evacuate valve on the oven. Allow the pressure to stabilize ~ 20 in.Hg – 25 in.Hg.
4. Open the vent valve so that the pressure starts to fall gradually to zero.
5. Repeat steps 3 – 4 until the evacuate pressure is ~10 in.Hg. Close the vacuum valve on the hood so the pressure falls to zero.
6. Turn off the oven. Open the door.
7. Remove the glassware. Keep vial in the oven. Close the door.

6 Literature and Supporting Documentation

Operator Manual, Thermo Scientific Vacuum Oven, Thermo Scientific; Manual LT1495X2 Rev. 3

Preparer

Anna Mudd Date: 02/28/2014
Advisory Board Chair

Concurrence

Katherine G. Sanchez Date: 03/20/2014
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	04/14/2014	Original Issue

PREPARATION OF 1 M ACETIC ACID

1 Scope

This solution is used to wash SPE columns in the Blood and Urine Amines methods.

2 Specification

1 M Acetic Acid

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Volumetric flask

5 Reagents

- H_2O (purified water)
- Glacial Acetic Acid, ACS grade

6 Instructions

1. Add 28.6 mL glacial acetic acid to a 500 mL volumetric flask partially filled with approximately 400 mL of H_2O.
2. Bring to volume with H_2O.
3. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

1. Minimum labeling includes specification above, initials, and date prepared.
2. Verification can be accomplished by inclusion with acceptable batch criteria.
3. Store at room temperature.
4. Discard after 12 months.

8 Literature and Supporting Documentation

Method developed by Sam Houston State University Regional Crime Laboratory: DRN TX-04-04, Version 03. *Confirmation of Stimulants by Gas Chromatography/Mass Spectrometry (GC/MS).*

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	05/19/2014	Original Issue
01	04/02/2018	Major Revision – Sections 5 and 6

PREPARATION OF ACIDIFIED METHANOL

1 Scope

This solution is added to organic extracts of samples to create the hydrochloride salt of amine drugs prior to evaporation. The salt is less volatile than the base and prevents loss of the drugs.

2 Specification

~1% HCl in Methanol

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- Amber glass container
- Pipette with disposable tips
- Volumetric flask

5 Reagents

- Methanol (High Purity Solvent suitable for trace analysis)
- Hydrochloric acid, ACS grade

6 Instructions

1. Pipette 1 mL concentrated HCl into a 100 mL volumetric flask ¾ filled with methanol. Bring to volume with methanol.
2. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Verification can be accomplished by inclusion with acceptable batch criteria.

C. Store at room temperature in amber glass container to minimize light enhanced reaction products.

D. Avoid addition to old solution for storage.

E. Discard after 3 months due to possible creation of reaction products.

8 Literature and Supporting Documentation

Method developed by Sam Houston State University Regional Crime Laboratory: DRN TX-04-04, Version 03. Confirmation of Stimulants by Gas Chromatography/Mass Spectrometry (GC/MS).

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	05/19/2014	Original Issue
01	05/01/2019	Revision – Sections 3 and 4

PREPARATION OF SODIUM PHOSPHATE BUFFER (pH 6.0)

1 Scope

This buffer is used to dilute organic extracts of blood/urine samples and to prepare SPE columns in Amines analysis and similar SPE methods.

2 Specification

Sodium Phosphate buffer (pH 6.0)

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Equipment and Supplies

- pH meter
- Balance
- Volumetric flask
- Beaker

5 Reagents

- H_2O (purified water)
- Dibasic Sodium Phosphate, ACS grade
- Monobasic Sodium Phosphate, ACS grade

6 Instructions

1. Add 7.1 g of dibasic sodium phosphate to a 500 mL volumetric flask and fill to volume with deionized water while mixing.
2. Add 6.0 g of monobasic sodium phosphate to a second 500 mL volumetric flask and bring to volume with deionized water while mixing.
3. Pour monobasic sodium phosphate solution into beaker. With mixing, adjust pH to 6.0 (± 0.1) with dibasic sodium phosphate solution (approximately 63 mL).
4. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

A. Minimum labeling includes specification above, initials, and date prepared.

B. Verification can be accomplished by pH check after preparation equal to 6.0 (± 0.1) and inclusion with acceptable batch criteria.

C. Store at room temperature.

D. Discard after 6 months or verify pH at use.

8 Literature and Supporting Documentation

Method developed by Sam Houston State University Regional Crime Laboratory: DRN TX-04-04, Version 03. *Confirmation of Stimulants by Gas Chromatography/Mass Spectrometry (GC/MS).*

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	05/19/2014	Original Issue
01	05/01/2019	Revision – Sections 3 and 7

PREPARATION OF TRIS BUFFER

1 Scope

This solution is used in Blood EMIT immunoassay and LCMS screen analyses.

2 Specification

0.5M Tris Buffer

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Special Equipment and Supplies

- Graduated glass cylinder (sufficient volume to contain 500 mL)

5 Reagents

- H_2O (LCMS Grade Purified Water)
- 1 M Tris-HCl pH 8.0
- TritonX

6 Instructions

1. Measure 500 mL 1 M Tris-HCl and pour into a clean storage container.
2. Measure 500 mL LCMS grade purified H2O and pour into the same storage container.
3. Pipette 100 µL TritonX into the storage container.
4. Mix until dissolved.
5. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

1. Minimum labeling includes specification above, initials, and date prepared.
2. Verification can be accomplished by inclusion with acceptable batch criteria.
3. Store refrigerated.
4. Avoid addition to old solution for storage.
5. Discard after 3 months.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	04/02/2018	Original Issue

PREPARATION OF TRIS BUFFER IN METHANOL

1 Scope

This solution is used in Blood EMIT immunoassay and LCMS screen analyses to reconstitute samples after evaporation from blood extracted for analysis.

2 Specification

Tris Buffer:MeOH

3 Safety

Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the SDS for additional safety information for specific chemicals.

4 Special Equipment and Supplies

- Graduated glass cylinder

5 Reagents

- 0.5 M Tris Buffer (TOX-07-09)
- Methanol (High Purity Solvent suitable for trace analysis)

6 Instructions

1. Measure 200 mL 0.5 M Tris Buffer in a graduated cylinder and pour into a clean storage container.
2. Measure 200 mL methanol in the graduated cylinder and pour into the same storage container.
3. Swirl to mix.
4. A different volume may be prepared in the same proportion as specified.

7 Testing, Storage, Expiration, and Disposal

1. Minimum labeling includes specification above, initials, date prepared, and expiration date.
2. Verification can be accomplished by inclusion with acceptable batch criteria.
3. Store refrigerated.
4. Avoid addition to old solution for storage.
5. Discard after 3 months.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	04/02/2018	Original Issue

LCMS INSTRUCTIONS

1 Scope

The instructions for the day to day operation of the 3200 QTRAP Applied Biosystems tandem MS and Shimadzu Prominence HPLC for use in identification and quantitation of drugs in biological specimens. The menu may vary slightly between instruments depending on software.

2 Related Documents

Preparation of PPG Dilution Solution (TOX-07-25)

3 Instructions

3.1 Perform Q1 and Q3 Tune Check

Before performing analysis using the LCMS with the ion trap mode off, perform a manual tune check of the Q1 and Q3 quadrupoles in positive mode using the PPG (Polypropylene Glycol) standard solution to check the instrument for optimization and verification of the performance of the MS. This tune check must be performed on the day of usage for individual samples or on the initial day of usage for a batch of samples.

1. Set up the syringe that is loaded with the manufacturer recommended PPG calibration solution (in the infusion holder and attach to the mass spectrometer.
2. From the main menu, select the “API Instrument” project folder and double click Hardware Configuration. Activate the Mass Spec Only profile and close the window.
3. Click on the Tune and Calibrate tab and double click Manual Tune. Open the file Q1 Pos PPGs.dam.
4. Click Start Syringe Pump (flow rate 10 µL/min for 1.0 mL syringe with 4.61 mm diameter).
5. Click Start and wait for the scanning to complete.
6. Right click the mass spectrum pane and select Open File. Right click on a window and select List Data. Click the Calibration Peak List Data tab to review the results.
7. For Q3, open file Q3 Pos PPGs.dam, and repeat steps 4-6.
8. Compare the results with Laboratory recommendations. Print this window. Initial the report and signify if the tune check is “acceptable” or “unacceptable” (alternatives: “Pass”, “P”, “Fail”, or “F”).”. Recommendations for acceptable tune check:
   a) *The mass shift is within +/- 0.2 amu of the target mass.*
   b) *The peak widths are between 0.60 to 0.80 amu wide.*
   c) *The Q1 intensity for nominal mass 906 exceeds 8 e^6.*
   d) *The Q3 intensity for nominal mass 906 exceeds 6 e^6.*

9. If necessary, adjust the peak widths by changing the offset values found under the Resolution tab and Advanced button.
   a) *Uncheck the MCA box, and start the scanning for 5 minutes.*
   b) *While the system is scanning, adjust the offset values by no more than 0.005 units at a time.*
   c) *Increasing the offsets will narrow the peaks and decreasing the offsets will make the peaks wider.*
10. If necessary, correct the mass shift by calibrating the mass range.
   a) *Click on one of the six individual spectra and click the Calibrate from Spectrum button in the toolbar.*
   b) *A Mass Calibration Option dialog will open. Choose the appropriate Standard – PPG Pos.*
   c) *Click the Start button to begin the calibration procedure.*
   d) *A Mass Calibration Report appears. Print this window. Select the Replace Calibration button in the upper left corner.*
   e) *A Mass Calibration Results text page will appear. Check that the slope variation is equal to 1.000 ± 0.004 and print this window.*
11. If the peak widths or mass shift had to be corrected, repeat steps 5-6.
12. If a successful tune cannot be obtained, perform automated Resolution Optimization.
   a) *Start the syringe pump and set to 5 µL per minute. Click Resolution Optimization on the toolbar. Choose the appropriate calibration standard.*
   b) *Select either Q1 or Q3.*
   c) *Select Unit resolution.*
   d) *Select Mass Calibration on Success.*
   e) *Select Update Mass Dependent Parameters.*
   f) *Click Start. When the Auto Resolution Dialog message appears, click OK.*
   g) *Click Yes when the Save Resolution Table message appears.*
13. Perform the manual tune check again and if a successful tune check still cannot be obtained, perform maintenance.
14. Record the date, type of tune check, and intensity of nominal mass 906 in the instrument logbook and the printouts in the instrument tune notebook or electronic equivalent.

3.2 Perform LIT Tune Check

Before performing analysis using the LCMS with the ion trap mode on, perform a manual tune check of the linear ion trap (LIT) in positive mode using the PPG 3000 (Polypropylene Glycol) standard solution to check the instrument for optimization and verification of the

performance of the LIT. This tune check must be performed on the day of usage for individual samples or on the initial day of usage for a batch of samples.

1. Set up the syringe that is loaded with PPG 3000 solution in the infusion holder and attach to the mass spectrometer.
2. From the main menu, select the "API Instrument" project folder and double click Hardware Configuration. Activate the Mass Spec Only profile and close the window.
3. Click on the Tune and Calibrate tab and double click Manual Tune. Open the file Pos Enh Res.dam.
4. Select the MS Tab and select the Scan Rate to verify (verify the scan speed that will be used for analysis).
5. Set the number of cycles to 50 and check the MCA box.
6. Click Start Syringe Pump (flow rate 10 µL/min for 1.0 mL syringe with 4.61 mm diameter).
7. Click Start and wait for the scanning to complete.
8. Right click the mass spectrum pane and select Open File. Right click on a window and select List Data. Click the Calibration Peak List Data tab to review the results.
9. Compare the results with Laboratory recommendations. Print this window. Initial the report and signify if the tune is "acceptable" or "unacceptable" (alternatives: "Pass", "P", "Fail", or "F"). Recommendations for acceptable tune:

Scan Speed	m/z 115 *intensity*	m/z 115 *peak width*	m/z 1661 *intensity*	m/z 1661 *peak width*
4000	1.8×10^7	≤ 0.50	9.0×10^6	≤ 0.70

10. If a successful tune check cannot be obtained, consult a service representative.
11. Record the date, type of tune check, and intensity of nominal mass 115 for the scan speed that will be used for analysis in the instrument logbook and the printouts in the instrument tune notebook or electronic equivalent.

3.3 Data Acquisition of Samples

1. From the main menu, select the appropriate project folder and double click Hardware Configuration. Activate the appropriate profile and close the window.
2. Click Build Acquisition Batch under the Acquire tab and open the batch sequence template file for the appropriate method.
3. Change the data file name on the sequence.

4. Fill in the sample name and comments on the sequence. Verify the vial positions are correct.
5. Save the sequence as the data file name.
6. Click the Submit tab, then the Submit button.
7. From the Queue screen, equilibrate the instrument, and then click the Start icon.

3.4 Data Processing

1. Under the Explorer tab, choose Open Data File.
2. Click the desired .wiff file and then the desired sample.
3. The MRM (multiple reaction monitoring) transitions and IDA (information dependent acquisition) fragmentation spectrum can be evaluated depending on the type of data acquired.

3.5 Quantitation

1. Under the Quantitation tab, click the Quantitation Method and open the method.
2. Select the data file of the reference calibrator and update the retention time of each analyte.
3. Save the quantitation method.
4. Double click Quantitation Wizard to choose the samples to quantitate, the results table setting, and the quantitation method.
5. The quantitation table will appear.
6. Click the Peak Review icon to manually check the peak integrations to verify accurate calculated concentrations.

3.6 Data Analysis

Refer to Toxicology SOP TOX-06-01 and TOX-06-02 for data analysis methods.

3.7 Troubleshooting

Consult the 3200 Q TRAP LC/MS/MS User manual or the manufacturer website.

3.8 Instrument Maintenance

A. Check the mobile phase and wash bottles and replace with freshly prepared solutions if level is low or if solutions are expired.
B. Replace the in-line frit if necessary.
C. Clean the curtain plate if significantly dirty.
D. Replace the solvent filters if back flow pressure increases.
E. Replace the analytical column or guard column, when necessary, to improve sensitivity or chromatography.
F. Perform front end cleaning of the mass spectrometer, when necessary, to improve sensitivity.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue
01	02/03/2014	Minor Revision – Sections 3.1 and 3.2
02	10/26/2015	Minor Revision – Sections 1 and 3.8
03	04/02/2018	Major Revision – Sections 1 and 3

GCMS INSTRUCTIONS

1 Scope

Instructions for the day-to-day operation of Agilent GCMS series for use in identification and quantitation of drugs in biological specimens. The menu may vary slightly for instruments depending on software. Follow the instructions below appropriate to the version of the software being used.

2 Instructions

2.1 Tune

Before performing analysis using the GCMS, tune the instrument for optimization and verification of the performance of the MSD. Calibrate the MSD with PFTBA (perfluorotributylamine) using the standard spectra tune (STUNE). This tune must be performed on the day of usage for individual samples or on the initial day of usage for a batch of samples.

1. In Instrument Control window:

   a) *Menu: Instrument >> Perform MS Autotune*
   Select Tune Type "Standard Spectra Tune" (stune.u)

   b) *Menu: Instrument >> Tune MSD <u>OR</u> select Icon*

   Select "Tune MSD" -loads current tune (stune.u)

2. Compare printed tune report with laboratory criteria. Initial the report and signify if the tune is "acceptable" or "unacceptable" (alternatives: "Pass", "P", "Fail", or "F"). Criteria for acceptable tune:

   a) *The mass assignments for 69, 219, and 502 should be within +/- 0.2 amu*

   b) *The peak widths (PW) of these three peaks should be <0.63*

   c) *The Electron Multiplier Voltage recorded in the instrument logbook should be monitored for trends as an indication of a need for source cleaning.*

   d) *There should be <200 peaks to signify that there is not excessive noise.*

   e) *Relative ratios for prominent masses are typically:*

      i. *m/z 69 100% (base peak)*

      ii. *219/69 ≥40% but ≤85%*

      iii. *502/69 ≥2.5% but ≤5%*

   f) *Peaks lower than 60 amu should be less than 10% relative abundance.*

B. Record the date, type of tune, Electron Multiplier Voltage, and relative abundance of ion ratios in the instrument logbook.

2.2 Sample Injection

A. Autosampler injection of batch of samples

1. Sequence Setup in Menu Guide-From Instrument Control Window:

   a) *Load Sequence*

      i. *Menu: Sequence >> Load Sequence OR select sequence option from icons*

      ii. *Select sequence template for appropriate method*

   b) *Edit sequence*

      i. *Menu: Sequence >> Edit Sequence OR select sequence option from icons*

      ii. *Enter the samples and blanks in the order in which they are to be tested.*

      iii. *Include information as specified in the SOP regarding sample name, misc. information, vial number, data file number, method, and type of sample*

   c) *Simulate and Save sequence*

      i. *Menu: Sequence >> Simulate Sequence OR select sequence option from icons.*

      ii. *Fill in correct data file directory path, operator name, and sequence comment information.*

      iii. *Select Run Sequence in dialog box which only runs a sequence check.*

      iv. *Menu: Sequence >> Save Sequence As OR select sequence option from icons*

2. Acquisition of Data

   a) *Load vials into autosampler tray positions corresponding with vial number in sequence table*

   b) *Fill solvent wash vials A and B with solvents appropriate for the specific analysis*

   c) *Run Sequence*

      i. *Menu: Sequence >> Run Sequence OR select sequence option from icons*

3. Record the sequence name and information in the Instrument Log book

B. Autosampler injection from a single sample vial

1. Setup instrument for acquisition in Menu Guide-From Instrument Control Window

   i. *Menu: Method >> Load Method*

   ii. *Select the appropriate method*

2. Acquisition of Data

   a) *Place vial in position in the autosampler tray*

b) *Fill solvent wash vials A and B with solvents appropriate for the specific analysis*

c) *Click on Sample Name box (a vial and large green arrow)*

i. *Enter "Data File Name"*

ii. *Enter vial number for tray position*

iii. *Enter your initials in "Operator Name"*

iv. *Complete "Sample Name"*

v. *Enter sample prep information in "Misc. Info"*

vi. *Click "Start Run"/"Ok and Run Method"*

3. Record the file name and information in the Instrument Log Book

2.3 Analysis of Data

Refer to Toxicology SOP TOX-06-01 and TOX-06-02 for data analysis methods

2.4 Troubleshooting

A. Consult the Agilent Technologies Mass Selective Detector User Information CD or the manufacturer's website.

B. If peaks lower than 60 amu are above 10%, resolve the issue and perform another tune.

1. Water (18 amu) at higher abundance may indicate a need to continue allowing the vacuum to "dry" the system after the vacuum chamber has been open before evaluation with another tune.

2. Nitrogen (28 amu), and oxygen (32 amu) at approximately a 3:1 ratio indicating an air leak at a septum, column connection, or elsewhere that needs to be located and eliminated.

3. Nitrogen (28 amu) with very little oxygen (32 amu) indicates air contamination of the helium supply (bottle or plumbing leak) prior to the oxygen scrubber in the helium supply room. Verification of this problem can be made by observing other instruments on the system. If it is determined that there are no leaks (air in supply bottle), and nitrogen does not exceed 50% of PFTBA (69 amu), it is acceptable to run. If bottle changes have been made, assure time for equilibration of system. All laboratory GC and GCMS users should be made aware of the situation.

4. Argon (40amu) indicates contamination of the helium supply bottle. If Argon does not exceed 50%, it is acceptable to run.

C. If peak shape has deteriorated and/or the multiplier voltage trend indicates a dirty source, clean the source.

2.5 Instrument maintenance

A. Clean the injection port and trim the front end of the GC column to maintain sensitivity required for the method.

B. Replace injection port liners and septum as needed.

C. Clean autosampler syringe as needed.

D. Trim or replace guard column as needed.

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue
01	02/03/2014	Minor revision- Section 2.4
02	04/02/2018	Major Revision – Sections 1 and 2

CEREX PRESSURE PROCESSOR INSTRUCTIONS

1 Scope

Instructions for the operation of the SPE Ware Cerex Pressure Processor System 48 and 48 II for the use in the extraction and purification of analytes utilizing a solid phase extraction (SPE) technique.

2 Instructions

2.1 Gas Source

Turn on the gas flow to the processors. The gas supply should be set at 40-50 psi by a regulator positioned between the gas source and the processor.

2.2 Flow Control

A. Turn middle knob to either *Adjust Flow* or *Max Flow*.

B. *Adjust Flow* – In this position gas flow is controlled from the rotometer located on the left side of the instrument panel. This allows for a precise control of low volume flow rates.

C. *Max Flow* – This selector switch position chooses a preset maximum flow rate. This is controlled by adjusting the regulator located below the pressure gauge on the right side of the instrument panel.

2.3 Processor Loading

A. Install the platform by placing the polymer-coated runners onto the track that is on the deck of the processor. The platform should be placed with the handgrip facing towards the front of the unit.

B. Stack the SPE Column Rack onto the Collection Tube Rack or the Waste Container Rack and set the stack onto the platform with the black bands in the back.

C. Flip the switches on top of the manifold for the corresponding rows being used to the “on” position.

D. When the platform is pushed to the back end of the slide, the toggle switches at both sides of the processor are pressed simultaneously to connect the manifold and sample rack which pressurizes the system.

2.4 Maintenance and Service

Gasket Cleaning – After each sample batch is run it is recommended to wipe the gasket clean with a lint free paper towel moistened with methanol followed by wiping with a dry paper towel.

Preparer

Kathy Jean Erwin Date: 03/18/2009
Advisory Board Chair

Concurrence

Zoë M. Smith Date: 03/18/2009
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue

TURBOVAP EVAPORATOR INSTRUCTIONS

1 Scope

Instructions for the use of the Turbovap evaporator for evaporating samples to dryness

2 Instructions for Turbovap

1. Turn the gas supply and unit ON
2. Check the water bath level
3. Set the water bath temperature, and the evaporation time as indicated in the extraction method
4. Load the evaporator with the sample tubes
5. Press the start button
6. Adjust the gas pressure to the pressure indicated in the extraction method
7. Remove the sample tubes promptly after evaporation
8. Turn OFF the unit and gas supply
9. Set the gas supply regulator to zero psi
10. Lift the cover to prevent moisture from accumulating on the inside cover, and oxidizing the gas manifold

3 Literature and/or Supporting Documentation

TurboVap LV Evaporator Operator's Manual

Preparer

Kathy Jean Erwin Date: 03/18/2009
Advisory Board Chair

Concurrence

Zoë M. Smith Date: 03/18/2009
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue

CEREX EVAPORATOR

1 Scope

Instructions for the use of the Cerex evaporator for evaporating samples to dryness

2 Instructions

1. Turn on the concentrator
2. Adjust the rack level by using the ‘up’ and ‘down’ arrows to a distance of ½” to 1” between the liquid level and the dry down tips
3. Turn on the gas supply and adjust the rotameter to the desired gas flow level
4. Set the run time and oven temperature
5. The evaporation process is controlled by a timer and will shut off the heat and gas flow at the end of the run
6. Turn off the gas supply

3 Literature and/or Supporting Documentation

Cerex Sample Concentration Manual

Preparer

Kathy Jean Erwin Date: 03/18/2009
Advisory Board Chair

Concurrence

Zoë M. Smith Date: 03/18/2009
Quality Assurance

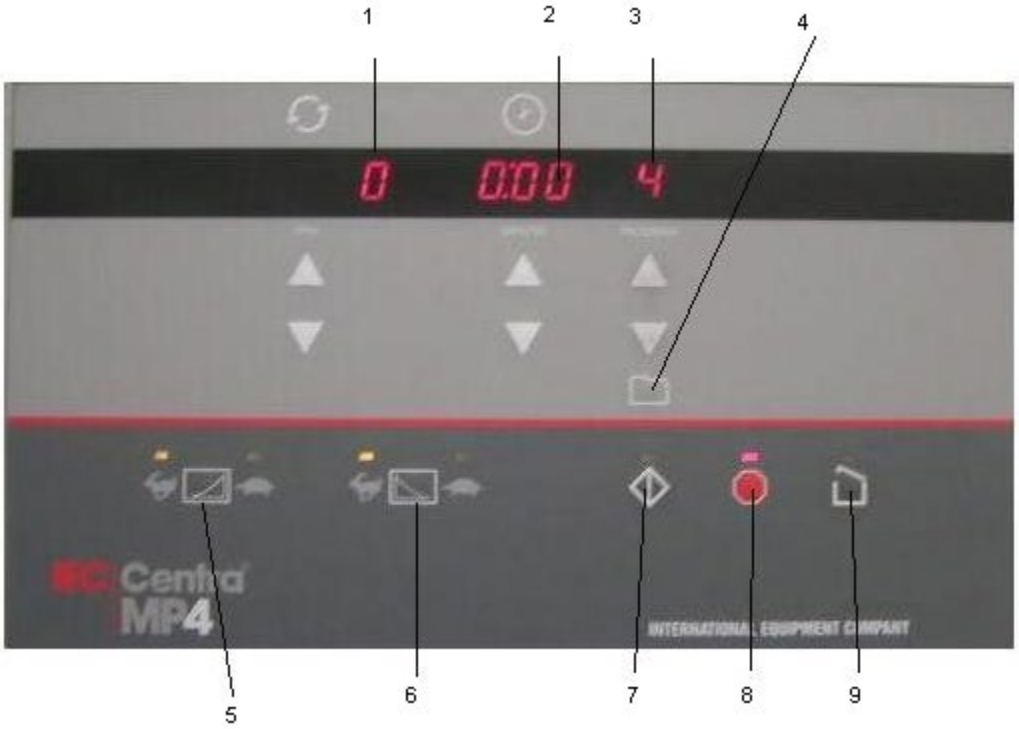
Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue

CENTRA MP4 CENTRIFUGE INSTRUCTIONS

1 Scope

Instructions for the use of the Centra MP4 centrifuge to separate layers in the extraction procedures.

2 Instructions



2.1 Operation

1. Turn off with button (8) shaped like a stop sign. If unit is off, push any button to restore display on front panel.
2. Open cover by pressing cover unlock button (9) on lower right front panel (power must be on to release cover).
3. Place the sample tubes into the proper swinging bucket for the size of tubes used.
4. If the sample tubes contain solvents or biological fluids, attach the bucket cover.
5. A balanced load is essential. Ensure that the rotor is loaded symmetrically with a paired set of tubes.

6. Choose one of the following stored program settings to match settings indicated in the extraction method:

   a) *Program 1 – 6 minutes at 4000 rpm: 2629 x g (within 2%)*

      Acceleration: Slow *Deceleration: Slow*

   b) *Program 2 – 2 minute at 3000 rpm: 1479 x g (within 2%)*

      Acceleration: Fast *Deceleration: Slow*

   c) *Program 3 – 12 minutes at 4000 rpm: 2629 x g (within 2%)*

      Acceleration: Slow *Deceleration: Slow*

7. Close the cover and press the Start button. (7).
8. The spin stops automatically at the end of the specified time.
9. Press the Open button (9) and remove the sample tubes.

2.2 Centrifugal Force

The relative centrifugal force may be calculated:

1. Ascertain the spinning radius by measuring the distance from the center of the drive shaft to the most outer part of the centrifuge tube when it is in spinning position. The spinning radius for the Centra with the 224 rotor, 4224 buckets, and 7225 tube adapters is 14.7 centimeters.
2. Calculate the relative centrifugal force (RCF): $g = 1.118 \times 10^{-5} \times r \times n^2$ (r = spinning radius in centimeters; n = revolutions per minute (RPM))
3. The RPM necessary to produce a specified RCF value (i.e., *g* values) can be calculated from this relationship.

2.3 Programming

A. Set Timing Mode

1. Press the time down arrow button and scroll below zero to display ACC or SPd.
2. Press again if needed to select the other mode.
3. Options: ACC to start time counting down when start key is pressed or SPd to start the time counting down when the rotor has reached 95% of set speed. Select ACC for programs to match programs on the IEC CL40 and the Jouan C3i V1 centrifuges.

B. Set speed

1. The left LED display (1) next to “RPM” shows speed values.
2. To increase/decrease the speed values in increments of 100 RPM, press on the corresponding up/down arrows.

C. Set run time

1. The middle LED display (2) next to “Minutes” shows time values (*minutes:seconds*).
2. To increase/decrease the run time values in increments of 15 seconds (1 minute when greater than 5 minutes), press on the corresponding up/down arrows.

D. Set acceleration profile

1. The acceleration button (5) on the lower midsection of the front panel selects the acceleration profile.
2. The LED is illuminated to mark the current profile of slow (turtle) or fast (rabbit).
3. To change the value, press the button (5) to cycle through the choices of slow or fast.

E. Set deceleration profile

1. The deceleration button (6) on the lower midsection of the front panel selects the deceleration profile.
2. The LED is illuminated to mark the current profile of slow (turtle) or fast (rabbit).
3. To change the value, press the button (6) to cycle through the choices of off (coast), slow, or fast.

F. To pre-program the centrifuge

1. There are nine available memory locations for pre-programmed programs.
2. The right LED display (3) next to “Program” displays the program number.
3. To enter/save a program into one of the locations, press on the corresponding up/down arrows to select a program.
4. Enter the desired parameter values.
5. Press the folder button (4) to save parameters in memory (program number stops flashing).

G. To lock a program

1. Programs are not locked.
2. If parameters are changed from saved settings, the program number will flash to indicate an unsaved program.
3. Press the up/down arrows to select the unchanged program (no flashing) or press the folder button to save the new parameters under the program number.

3 Literature and/or Supporting Documentation

Centra-MP4 Centrifuge Operation Manual

Preparer

Kathy Jean Erwin Date: 03/18/2009
Advisory Board Chair

Concurrence

Zoë M. Smith Date: 05/19/2009
Quality Assurance

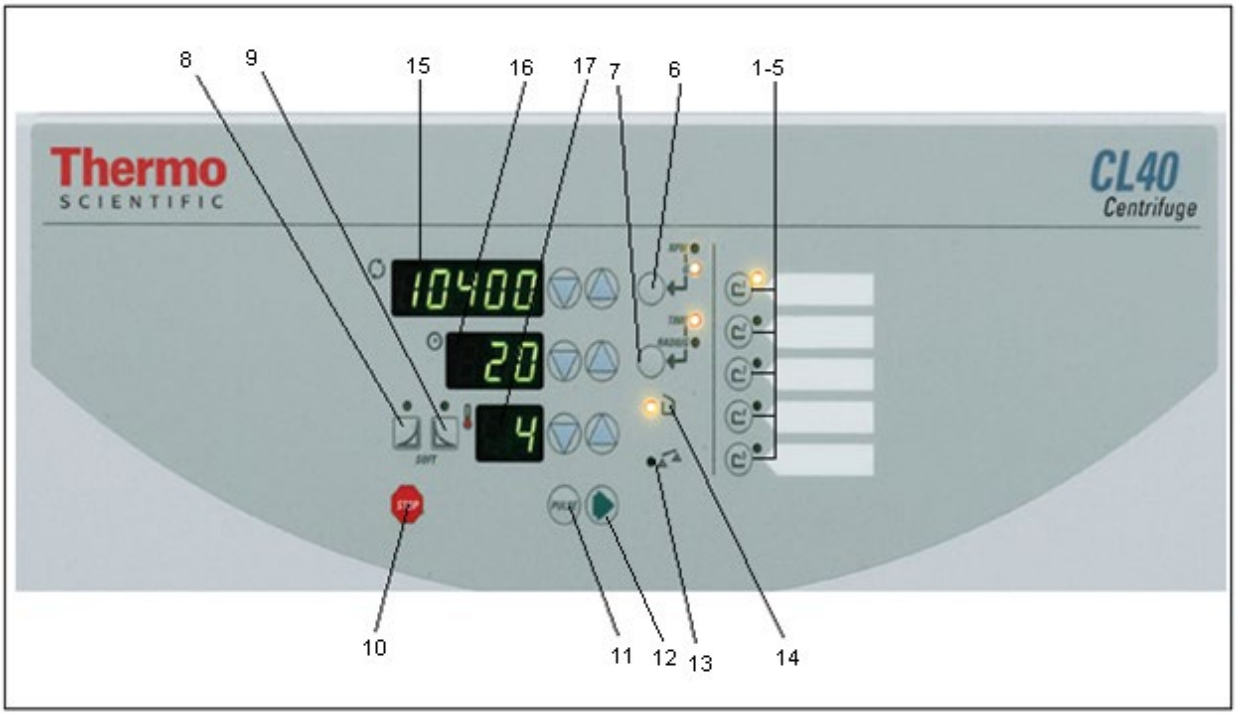
Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue

IEC CL40 CENTRIFUGE INSTRUCTIONS

1 Scope

Instructions for the use of the IEC CL40 centrifuge to separate layers in the extraction procedures

2 Instructions



2.1 Operation

1. The power switch on located on the front at bottom right.
2. Open cover by pulling release on upper right side panel (power must be on to release cover).
3. Place the sample tubes into the proper swinging bucket for the size of tubes used.
4. If the sample tubes contain solvents or biological fluids, attach the bucket cover.
5. A balanced load is essential. Ensure that the rotor is loaded symmetrically with a paired set of tubes.

6. Choose one of the following stored program settings to match settings indicated in the extraction method:

   a) *Program 1 – 6 minutes at 2629 x g: 3575 rpm (within 2%)*

      Acceleration: Slow *Deceleration: Slow*

   b) *Program 2 – 2 minute at 1479 x g: 2680 rpm (within 2%)*

      Acceleration: Fast *Deceleration: Slow*

   c) *Program 3 – 12 minutes at 2629 x g: 3575 rpm (within 2%)*

      Acceleration: Slow *Deceleration: Slow*

7. Close the cover and press the Start button (12).
8. The spin stops automatically at the end of the specified time.
9. Press the Open button and remove the sample tubes.

2.2 Centrifugal Force

The relative centrifugal force (RCF) may be displayed in place of the RPM with the spinning radius entered in the parameters or it may be calculated using the spinning radius and RPM:

1. Ascertain the spinning radius by measuring the distance from the center of the drive shaft to the most outer part of the centrifuge tube when it is in spinning position. The spinning radius for the IEC CL40 with the M4 rotor, 11174218 buckets, and 11174190 tube adapters is 18.4 centimeters.
2. Calculate the relative centrifugal force (RCF): $g = 1.118 \times 10^{-5} \times r \times n^2$ (r = spinning radius in centimeters; n = revolutions per minute (RPM))
3. The RPM necessary to produce a specified RCF value (i.e., *g* values) can be calculated from this relationship.

2.3 Programming

A. Timing Mode

1. Time counting down begins when the start key is pressed (no alternative selection).

B. Selecting speed or RCF on upper display (15)

1. Standard program settings for the CL40 will include RCF set to match the Centra.
2. Press the button (6) to the right of the display.

C. Set speed

1. When the LED next to “RPM” (18) is illuminated, the upper display (15) shows speed values.
2. To increase/decrease the speed values in increments of 10, press on the corresponding up/down arrows.

D. Set RCF

1. When the LED next to “G” (19) is illuminated, the upper display (15) shows RCF values.
2. To increase/decrease the RCF values, press on the corresponding up/down arrows.

3. When using RCF as a control/display value, make sure the correct centrifugal radius is entered into the control system (see below).
4. If an incorrect radius value is in the control system, the RCF value displayed will not be accurate.

E. Selecting run time or radius on middle display (16)

Press the button (7) to the right of the display.

F. Set run time

When the LED next to “TIME” (20) is illuminated, the middle display (16) shows time values (*minutes.seconds*). To increase/decrease the run time values in increments of 30 seconds, press on the correspon*ding up/down ar*rows.

G. Set centrifugal radius

1. When the LED next to “RADIUS” (21) is illuminated, the middle display (16) shows the value of the centrifugal radius in millimeters.
2. To increase/decrease the centrifugal radius, press on the corresponding up/down arrows.
3. This parameter can only be viewed and set while the centrifuge is at rest.
4. It is essential that this value be correct for the RCF control/display function to be accurate.
5. The correct value of the centrifugal radius depends on the combination of rotor/buckets/adapters being used.

H. Set acceleration profile

1. When the button (8) is pressed to illuminate the LED, the lower display (17) shows the number corresponding to the acceleration profile (1 to 5: with 5 being the quickest).
2. To change the value, press the corresponding up/down arrows.

I. Set deceleration profile

1. When the button (9) is pressed to illuminate the LED, the lower display (17) shows the number corresponding to the deceleration profile (1 to 5 with 5 being the quickest).
2. To change the value, press the corresponding up/down arrows.

J. To pre-program the centrifuge

1. There are five available memory locations for pre-programmed programs.
2. Each corresponds to one of the five memory buttons (1-5).
3. To enter/save a program into one of the locations, press the memory button corresponding to the desired program position.
4. The corresponding LED (1a-5a) will be illuminated. While the LED is illuminated, enter the desired parameter values.
5. These values are automatically saved in memory.

K. To lock a program

1. To lock a program, press and hold the corresponding button for several seconds until the corresponding LED begins to flash.
2. Release the button, and the program is locked. Parameters of the program cannot be changed.

L. To unlock a program

1. To unlock a program (when a program is locked, the associated LED flashes), press and hold the corresponding button for several seconds until the associated LED stops flashing.
2. Release the button, and the program is unlocked.

3 Literature and/or Supporting Documentation

IEC CL40 Centrifuge User's Manual

Preparer

Kathy Jean Erwin Date: 03/18/2009
Advisory Board Chair

Concurrence

Zoë M. Smith Date: 05/19/2009
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	06/03/2009	Original Issue

IMMUNOASSAY INSTRUCTIONS

1 Scope

Instructions for the operation of the MGC240, an EMIT testing instrument

2 Instructions

2.1 Instrument Start-Up

A. If the first run of the day, restart the computer.

B. Load MGC240 software on Instrument computer.

C. Turn on power to instrument.

D. Allow instrument lamp to warm up before processing any samples.

2.2 Daily Maintenance

A. Check and/or fill deionized water reservoir.

B. Check and/or fill 2% alkaline wash reservoir.

C. Check and/or fill 1% acid wash reservoir.

D. Check that reagent compartment is cool to the touch.

E. Check and/or replace barcode paper.

F. Complete daily log checklist.

2.3 Preparing Samples and Instrument for Tests

A. Ready the instrument after the lamp has warmed up for approximately 30 minutes.

B. Fill reagent bottles if necessary.

C. Set the current round (blood or urine) and check the number of tests remaining per assay as estimated by the software. Click update to save any changes.

2.4 Instrument Cleaning and Shutdown

A. Exit MGC240 software and QuickLab software.

B. Turn off power to instrument.

C. Wipe reagent and sample probes with deionized water.

3 Monthly Maintenance of Instrument

A. Check reagent fan screen.

B. Perform cuvette and lamp life check.

C. Inspect reagent and sampling pumps and inspect probe wash pump unit by priming.

D. Check/exchange water reservoir filter.

E. Check/exchange wash reservoir filters.

F. Perform sample probe wash with 10% bleach solution.

4 Literature and/or Supporting Documentation

Consult manufacturer's information as necessary

Preparer

Megan Barton Date: 01/27/2014
Advisory Board Chair

Concurrence

Heather Greco Date: 01/27/2014
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	08/19/2009	Original Issue
01	02/03/2014	Major revision- Section 2 Minor revision- Section 3

ANALYTICAL BALANCE OPERATION

1 Scope

Instructions for the operation of an analytical balance, the Scientech ZSA120. The primary use for this balance will be weighing material that needs 0.1 mg resolution (e.g., verifying pipette performance, weighing powder drug standards in mg quantities).

2 Instructions

2.1 Precautions Before Use

For the optimum operation of the balance, follow these conditions as closely as possible:

A. The best operating temperature is about 20°C / 68°F at about 50% relative humidity.

B. The weighing area should be clean and free of dust.

C. The weighing table should be solid and free from vibration, drafts (such as frequently opening doors or windows) and as level as possible. Ensure that the balance is used on the vibration damping mount on the laboratory bench.

D. If the balance needs to be moved, consider the following for placement: 1) Corners of rooms are best as they are less prone to vibrations; 2) Do not install the balance near heaters or air conditioners; 3) Do not install the balance in direct sunlight; 4) Do not use the balance near other equipment which produces magnetic fields; 5) **Do not place or use the balance where there is flammable or corrosive gas present.**

E. Calibrate the balance after installation prior to initial use and verify the calibration after moving it to another location.

F. Plug in the balance and allow at least one hour warm-up before measurements.

2.2 Precautions During Use

Note the following items to get accurate weighing data:

A. Discharge static electricity from the weighing material. When the weighing sample (plastics, insulator, etc.) has a static charge, the weighing value is influenced. Static problems are minimized if the ambient humidity is above 45%RH.

B. This balance uses a strong magnet as part of the balance assembly. Use caution when weighing magnetic materials. If there is a problem, use the underhook (on the bottom of the balance) to suspend the material away from the influence of the magnet.

C. Avoid temperature differences between the weighed sample and the environment. When a sample is warmer (cooler) than the ambient temperature, the sample will appear lighter (heavier) than the true mass. This error is due to the rising (falling) draft next to the sample. If you touch the sample, the same type of error will occur. Do not touch the sample directly with your hand. Use tweezers or other tools.

D. Make each weighing gently and quickly to avoid errors due to changes in the environmental conditions. Shorten the operation time as much as possible (opening and closing door, placing and removing sample). Use a pair of tweezers to avoid

temperature changes due to heat from inserting your hand into the weighing chamber.

E. Inspect the balance to ensure that the air bubble is centered and the balance appears level and free from vibration.

F. Do not drop things upon the weighing pan, or put a weight beyond the range of the balance on the weighing pan. Place item to be weighed on the center of the weighing pan.

G. Do not use a sharp instrument (such as a pencil or ball point pen) to press the keys, use your finger only.

H. Press either of the two ZERO buttons (tare buttons) before each weighing to prevent possible errors.

I. Calibrate the balance periodically so as to eliminate possible errors and to maintain weighing accuracy. This calibration will be performed by an outside calibration service and is done to an external calibration weight. Performance of the balance will be verified periodically with a certified calibration weight(s) and recorded in a logbook.

J. Take into consideration the affect of air buoyancy on a sample when more accuracy is required.

K. Avoid allowing foreign matter (dust, liquid or metal fragments) to invade the inside of the balance.

2.3 Precautions After Use

A. Avoid mechanical shock to your balance.

B. Do not use solvents to clean the balance. For best cleaning, wipe with a dry lint free cloth or a lint free cloth that is moistened with warm water and a mild detergent.

C. Avoid allowing foreign matter (dust, liquid or metal fragments) to invade the inside of the balance. Close all doors to avoid dust accumulation.

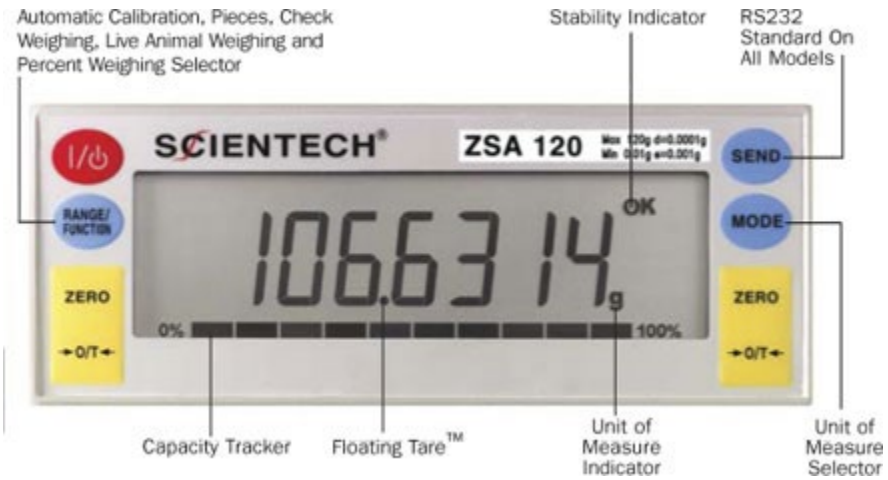
D. Remove any containers from the pan when done and clean up any spills immediately.

2.4 Power Supply Precautions

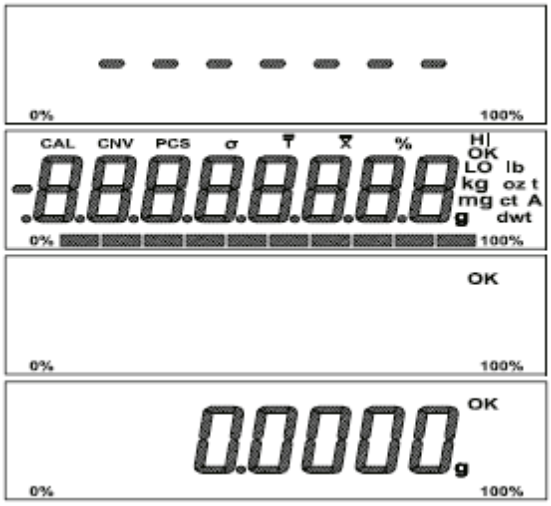
A. The proper sequence for connecting the power supply to the balance is to connect the round DIN connector on the power supply cable to the balance then plug the power supply into the wall outlet. If this sequence is not followed, the balance may remain in the turn on sequence or weird characters may appear on the display.

B. It is recommended that the balance remain plugged into an electrical outlet at all times. This insures the balance is always warmed up and ready for use. For accurate weighing, plug in the balance at least an hour before use so it can warm up if it has been unplugged.

2.5 Front Panel Controls



A. **The ON/OFF (I/O) Button:** When the balance is off, pressing the ON/OFF button will start the turn on sequence shown below. During this sequence the balance is doing an automatic systems checkout to insure it is functioning properly. No objects should be on the weighing pan during the turn on sequence. Once the balance goes through its checkout procedure and the stability indicator OK appears, the balance may be zeroed and weighing operations may start.



B. When the balance is on, pressing the ON/OFF button will turn off the display.

C. **The MODE Button:** Pressing the MODE button starts the unit of weight cycle as follows: grams (g), carats (ct), pennyweights (dwt), troy ounces (ozt), ounces (oz), pounds (lb), kilograms (kg) and milligrams (mg). When the desired unit of weight appears, press the MODE button a second time to select that unit of weight.

D. **The SEND Button:** Pressing the SEND button sends the information on the balance's display to an external device via the RS-232 interface.

E. **The ZERO Buttons:** There are two (2) ZERO buttons. Pressing either button at any time returns the display to zeros. When a weighing that has been zeroed out is removed from the weighing pan, a negative reading is displayed. To return the display to zeroes, press one of the ZERO buttons.

F. **The Capacity Tracker:** The capacity tracker provides a graphic display of the used and unused portions of the weighing range. Each segment represents 10% of the balance's total capacity. As 10% of the balance's capacity is used the first segment will illuminate. As 20% of the balance's capacity is reached, the second segment will light and so on.

2.6 Basic Weighing Operation

1. Check the level indicator near the front of the balance to verify that the balance is level. Make appropriate adjustments with the front leveling feet, if necessary.
2. Clean the weighing pan, if necessary.
3. Turn on the balance display using the ON/OFF button. Wait until the balance completes the turn on sequence and a zero readout appears. Then proceed to the next step.
4. Select a preset unit (g or mg) using the MODE key.
5. Put a weighing container or weighing paper on the weighing pan, unless weighing solid articles. Press the ZERO button to tare the weight. Then zero is displayed.
6. Place the item to be weighed on the pan or in the container.
7. Wait for the stability indicator OK to appear and read or record the weight.
8. Remove the sample and container from the pan.

3 Maintenance

Keep the area around the balance clean and free of dust and debris. Clean up any spills in the balance weighing chamber immediately, especially on the weighing pan and around it. Some chemicals will react with stainless steel and corrode it, so it is important to keep the weighing chamber clean.

4 Literature and/or Supporting Documentation

Consult the manufacturer's information, Scientech® Zeta Rev-E & Series 12000 Rev-E Electronic Balance Setup and Operating Procedures, as necessary.

Refer to the balance logbook for calibration, periodic checks of performance, and maintenance documentation.

Preparer

Kathy Jean Erwin Date: 01/11/2010
Advisory Board Chair

Concurrence

Zoë M. Smith Date: 01/11/2010
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	01/20/2010	Original Issue

SEMI-MICRO BALANCE OPERATION

1 Scope

Instructions for the operation of a semi-micro balance, the A&D GH-202. The primary use for this balance will be weighing material that needs 0.01 mg resolution (e.g., verifying pipette performance, weighing powder drug standards in mg quantities).

2 Instructions

2.1 Precautions Before Use

A. For the optimum operation of the balance, follow these conditions as closely as possible:

B. The best operating temperature is about 20°C / 68°F at about 50% relative humidity.

C. The weighing area should be clean and free of dust.

D. The weighing table should be solid and free from vibration, drafts (such as frequently opening doors or windows) and as level as possible. Ensure that the balance is used on the vibration damping mount on the laboratory bench.

E. If the balance needs to be moved, consider the following for placement: 1) Corners of rooms are best as they are less prone to vibrations; 2) Do not install the balance near heaters or air conditioners; 3) Do not install the balance in direct sunlight; 4) Do not use the balance near other equipment which produces magnetic fields; 5) **Do not place or use the balance where there is flammable or corrosive gas present.**

F. Calibrate the balance after installation prior to initial use and after moving it to another location.

G. Plug in the balance and allow at least one hour warm-up before measurements.

2.2 Precautions During Use

A. Note the following items to get accurate weighing data:

B. Discharge static electricity from the weighing material. When the weighing sample (plastics, insulator, etc.) could have a static charge, the weighing value is influenced. Static problems are minimized if the ambient humidity is above 45%RH.

C. This balance uses a strong magnet as part of the balance assembly. Use caution when weighing magnetic materials. If there is a problem, use the underhook (on the bottom of the balance) to suspend the material away from the influence of the magnet.

D. Avoid temperature differences between the weighed sample and the environment. When a sample is warmer (cooler) than the ambient temperature, the sample will appear lighter (heavier) than the true mass. This error is due to the rising (falling) draft next to the sample. If you touch the sample, the same type of error will occur. Do not touch the sample directly with your hand. Use tweezers or other tools.

E. Make each weighing gently and quickly to avoid errors due to changes in the environmental conditions. Shorten the operation time as much as possible (opening and closing door, placing and removing sample). Use a pair of tweezers to avoid temperature changes due to heat from inserting your hand into the weighing chamber.

F. Before a weighing for which the minimum display is 0.01 mg, the "fine range breeze break ring" can be installed in place of the "breeze break ring" to avoid errors caused by draft.

G.

H. Inspect the balance to ensure that the air bubble is centered and the balance appears level and free from vibration.

I. Do not drop things upon the weighing pan, or put a weight beyond the range of the balance on the weighing pan. Place item to be weighed on the center of the weighing pan.

J. Do not use a sharp instrument (such as a pencil or ball point pen) to press the keys, use your finger only.

K. Press the RE-ZERO key (tare) before each weighing to prevent possible errors.

L. Calibrate the balance periodically so as to eliminate possible errors and to maintain weighing accuracy. This function will normally be inhibited to prevent accidental changes to calibration parameters. Calibration is performed according to the A&D GH Series Analytical Balance Instruction Manual using the internal mass (200 g) and recorded in the equipment logbook. Immediately after performing a calibration, a calibration test will be performed according to the A&D GH Series Analytical Balance Instruction Manual using the internal mass (200 g) and recorded in the equipment logbook. A performance verification of the balance will be performed periodically with an external 200 mg weight and recorded in the equipment logbook.

M. Take into consideration the effect of air buoyancy on a sample when more accuracy is required.

N. Avoid allowing foreign matter (dust, liquid or metal fragments) to invade the inside of the balance.

2.3 Precautions After Use

A. Avoid mechanical shock to your balance.

B. Do not use solvents to clean the balance. For best cleaning, wipe with a dry lint free cloth or a lint free cloth that is moistened with warm water and a mild detergent.

C. Avoid allowing foreign matter (dust, liquid or metal fragments) to invade the inside of the balance. Close all doors to avoid dust accumulation.

D. Remove any containers from the pan when done and clean up any spills.

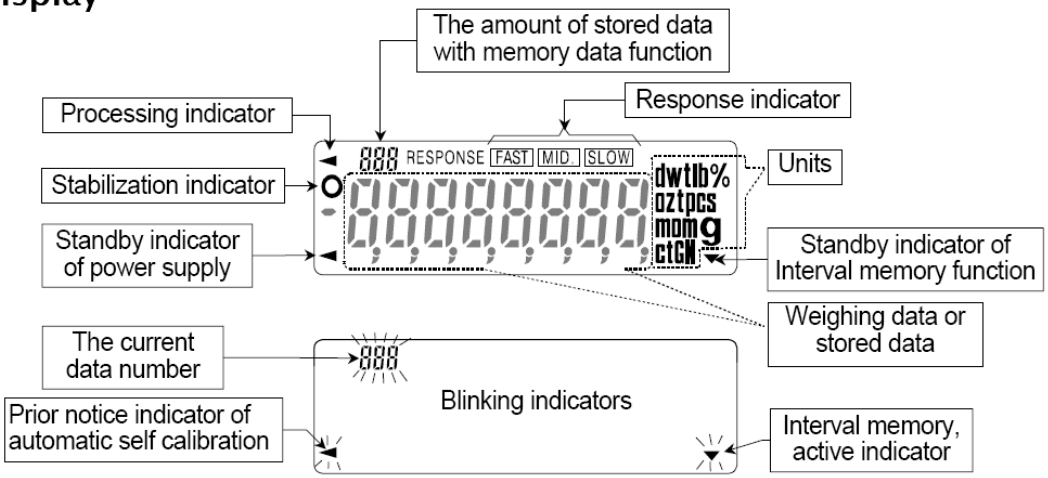
2.4 Power Supply Precautions

A. Do not remove the AC adapter while the internal mass is in motion, for example, right after the AC adapter is connected, or during calibration using the internal mass. If the AC adapter is removed under the conditions described above, the internal mass will be left unsecured; that may cause mechanical damage when the balance is moved. Before removing the AC adapter, press the ON:OFF key and confirm that zero is displayed in weighing mode.

B. When the AC adapter is connected, the balance is in the standby mode if the standby indicator is on. This is a normal state and does not harm the balance. For accurate weighing, plug in the balance at least an hour before use so it can warm up if it has been unplugged.

2.5 Display Symbols and Key Operation

Display



Key Operation

- Press and release the key immediately" or "Click the key" .....

- Press and hold the key .....

Key	When pressed and released	When pressed and held
I/⏻ ON:OFF	The key to turn the display ON and OFF. The standby indicator is displayed when the display is turned off. The weighing mode is enabled when the display is turned on. This key is available anytime. Pressing the key during operation will interrupt the operation and turn the display OFF.	
1/10d RANGE	▫ In the weighing mode, the key to turn the minimum weighing value ON and OFF. ▫ In the counting or percent mode, the key to enter the sampling mode.	The key to enter the function table mode. Refer to "10. Function Table".
MODE	The key to switch the preset weighing units stored in the function table. Refer to "5. Weighing Units".	The key to perform automatic response adjustment.
CAL	The key to perform calibration using the internal mass.	The key to display other items of the calibration menu.
PRINT	The key to store the weighing data in memory or outputs to a printer or personal computer depending on the function table settings. (Factory setting = output)	No function at the factory setting. By changing the function table: ▫ "Title block" and "End block" for GLP report are outputted. ▫ The data memory menu is displayed.
→0/T← RE-ZERO	The key to set the display to zero.	

2.6 Basic Weighing Operation

A. Turn on the balance display using the ON:OFF key.

B. Select a preset unit (g or mg) using the MODE key.

C. Put a weighing container or weighing paper on the weighing pan, unless weighing solid articles. Press the RE-ZERO key to tare the weight. Then zero is displayed.

D. Place the item to be weighed on the pan or in the container.

E. Wait for the stabilization indicator “**o**” and read the value.

F. Remove the sample and container from the pan.

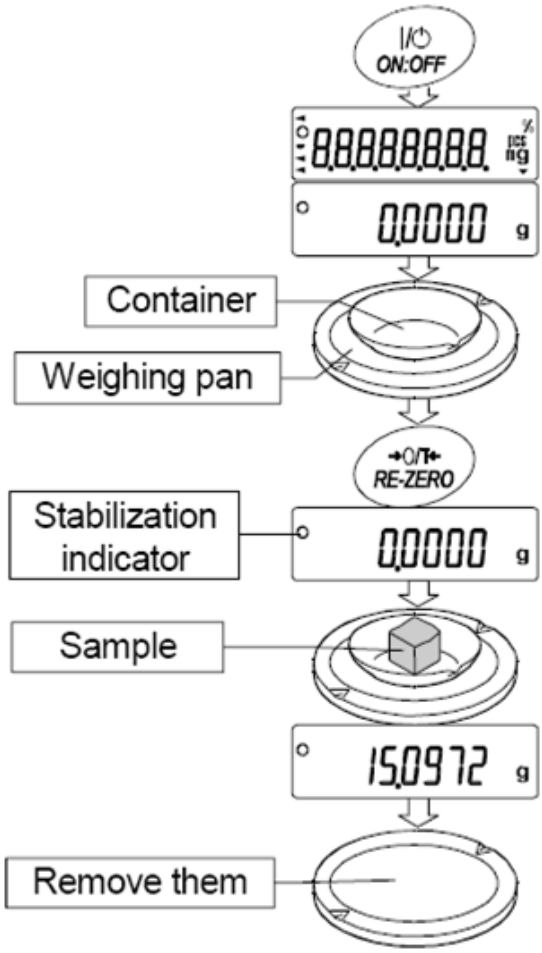
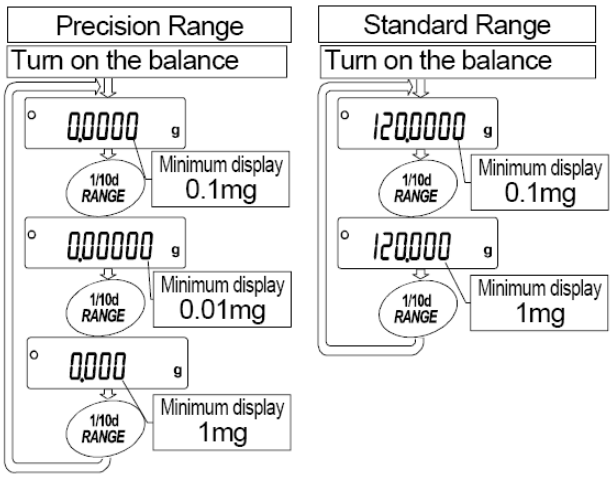


Figure. 1 (steps 1-6)

2.7 Weighing Using the Dual Range Features

Model GH-202 is equipped with two ranges, “precision range” and “standard range”.

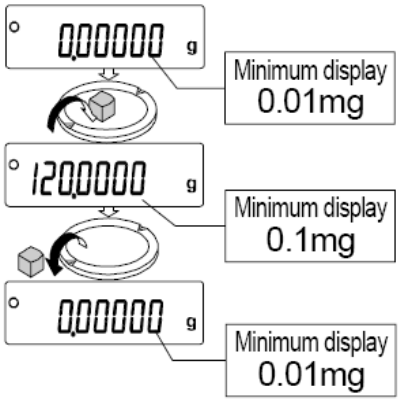
	Weighing range	Available minimum displays		
Precision range	0 g to 51 g	0.01 mg	0.1 mg	1 mg
Standard range	51 g to 220 g		0.1 mg	1 mg



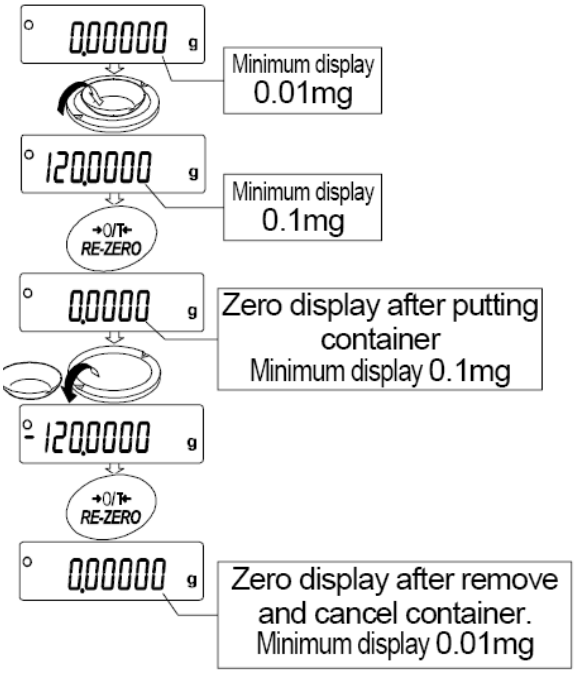
The range is switched automatically depending on the value displayed. Pressing the RE-ZERO key allows weighing in the precision range, regardless of the tare value.

Pressing the RANGE key, the display can be switched back and forth between ranges.

When placing and weighing a sample that exceeds the precision weight range with the precision range currently on the minimum display of 0.01 mg, the minimum display is changed to 0.1 mg of the standard range. When removing the sample, the minimum display is changed back to 0.01 mg of the precision range automatically. (see below).



When a tare weight exceeds the precision range, even if the net weight is within the precision range, 0.01 mg of the precision range cannot be selected as the minimum display. In order to select 0.01 mg of precision range, remove tare weight and press the RE-ZERO key to cancel it. (see below)



When a minimum display of 0.1 mg or 1 mg is selected by the RANGE key, even if changing range, the minimum display is maintained.

3 Literature and/or Supporting Documentation

Consult the manufacturer's information, GH Series Analytical Balance Instruction Manual by A&D Company, as necessary.

Refer to the balance logbook for calibration, periodic checks of performance, and maintenance documentation.

Preparer

Megan Barton Date: 01/27/2014
Advisory Board Chair

Concurrence

Heather Greco Date: 01/27/2014
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
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00a	02/03/2014	Formatting changes only

TOP-LOADER BALANCE OPERATION

1 Scope

Instructions for the operation of top-loader balances (eg. Sartorius BP 310 P)

2 Practice

2.1 Pre-weighing

A. Inspect the balance to ensure that the air bubble in the level indicator is centered and the balance appears level and free from vibration. Make appropriate adjustments with the leveling feet to level the balance, if necessary.

B. Ensure that the balance and the area around it is clean and free of objects which may interfere with the operation of the balance.

C. Turn on the balance display by pressing the I/O button.

2.2 Weighing

A. Clean the weighing platform and/or place a protective sheet, glassine weighing paper, or other weighing boat on the pan.

B. Tare the balance to zero, if necessary, by pressing the TARE button.

C. Place the item to be weighed on the scale or in the appropriate tared weighing container.

D. Record the weight.

2.3 Troubleshooting

A. If the weight of the item to be weighed exceeds the capacity of the balance, use a different balance.

B. If different units are required, press the unit button or equivalent until the appropriate units are displayed.

C. If the reading does not stabilize, use a wind shield to protect the item from air currents.

3 Literature and/or Supporting Documentation

Consult manufacturer's information as necessary (for Sartorius BP 310 P balance, use manual titled: Sartorius Basic^{plus} Installation and Operating Instructions Standard Balances and Verified Balances, Manual # 98648-004-59).

Refer to balance logbook for calibration, periodic checks of performance, and maintenance and documentation.

Preparer

Kathy Jean Erwin Date: 01/11/2010
Advisory Board Chair

Concurrence

Zoë M. Smith Date: 01/11/2010
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	01/20/2010	Original Issue

SORVALL ST40 CENTRIFUGE INSTRUCTIONS

1 Scope

Instructions for the use of the Sorvall ST40 centrifuge to separate layers in the extraction procedures

2 Instructions

See end for diagram of display.

2.1 Operation

1. The power switch is located on the back at bottom right.
2. Open cover by pushing the OPEN key on front at upper right(power must be on to release cover).
3. Place the sample tubes into the proper swinging bucket for the size of tubes used.
4. If the sample tubes contain solvents or biological fluids, attach the bucket cover.
5. A balanced load is essential. Ensure that the rotor is loaded symmetrically with a paired set of tubes.
6. Choose one of the following stored program settings to match settings indicated in the extraction method:
   a) *Program 1 – 6 minutes at 2600 x g: 3489rpm (within 2%)*
      Acceleration: 7 Deceleration: 7 Bucket Code: 3608
   b) *Program 2 – 2 minute at 1500 x g: 2650 rpm (within 2%)*
      Acceleration: 9 Deceleration: 7 Bucket Code: 3608
   c) *Program 3 – 12 minutes at 2600 x g: 3489 rpm (within 2%)*
      Acceleration: 7 Deceleration: 7 Bucket Code: 3608
7. Close the cover and press the Start key.
8. The spin stops automatically at the end of the specified time.
9. Press the OPEN key and remove the sample tubes.

2.2 Centrifugal Force

The relative centrifugal force (RCF) may be displayed in place of the RPM with the spinning radius entered in the parameters or it may be calculated using the spinning radius and RPM:

1. Ascertain the spinning radius by measuring the distance from the center of the drive shaft to the most outer part of the centrifuge tube when it is in spinning position. The spinning radius for the IEC CL40 with the TX 750 rotor, 3608 buckets, and 3719 tube adapters is 19.1 centimeters.
2. Calculate the relative centrifugal force (RCF): $g = 1.118 \times 10^{-5} \times r \times n^2$ (r = spinning radius in centimeters; n = revolutions per minute (RPM))
3. The RPM necessary to produce a specified RCF value (i.e., *g* values) can be calculated from this relationship.

2.3 Programming

A. Timing Mode

1. Time counting down begins when the start key is pressed (no alternative selection).

B. Selecting speed or RCF on display

1. Standard program settings for the ST40 will include RCF set to match the other centrifuges.
2. Press the button Speed/RCF-value key to the left of the display to display speed or RCF value.

C. Set speed

1. When the LED next to “RPM” is illuminated, the display shows speed values.
2. To increase/decrease the speed values, press on the corresponding up/down arrows.

D. Set RCF

1. When the LED next to “X G” is illuminated, the display shows RCF values.
2. To increase/decrease the RCF values, press on the corresponding up/down arrows.

E. Set run time

1. Press the corresponding up/down arrows. The display will show Set time H.mm.
2. To enter the desired run time in hh:mm format, press the corresponding up/down arrows until the desired time is selected.

F. Set acceleration profile

1. Press the corresponding up/down arrows to set acceleration profile. The display will show Set acceleration and the selected acceleration curve (1 to 9: with 9 being the quickest).
2. To adjust the acceleration curve, press the corresponding up/down arrows until the desired curve is selected.

G. Set deceleration profile

1. Press the corresponding up/down arrows to set deceleration profile. The display will show Set deceleration and the selected deceleration curve (0 to 9: with 9 being the quickest).
2. To adjust the deceleration curve, press the corresponding up/down arrows until the desired curve is selected.

H. Set bucket code

1. Press the Bucket selection key. The display will show Set bucket code and the code for the currently selected bucket.
2. To adjust the Bucket code, press the Bucket selection key repeatedly until the code for the bucket currently in use is displayed.

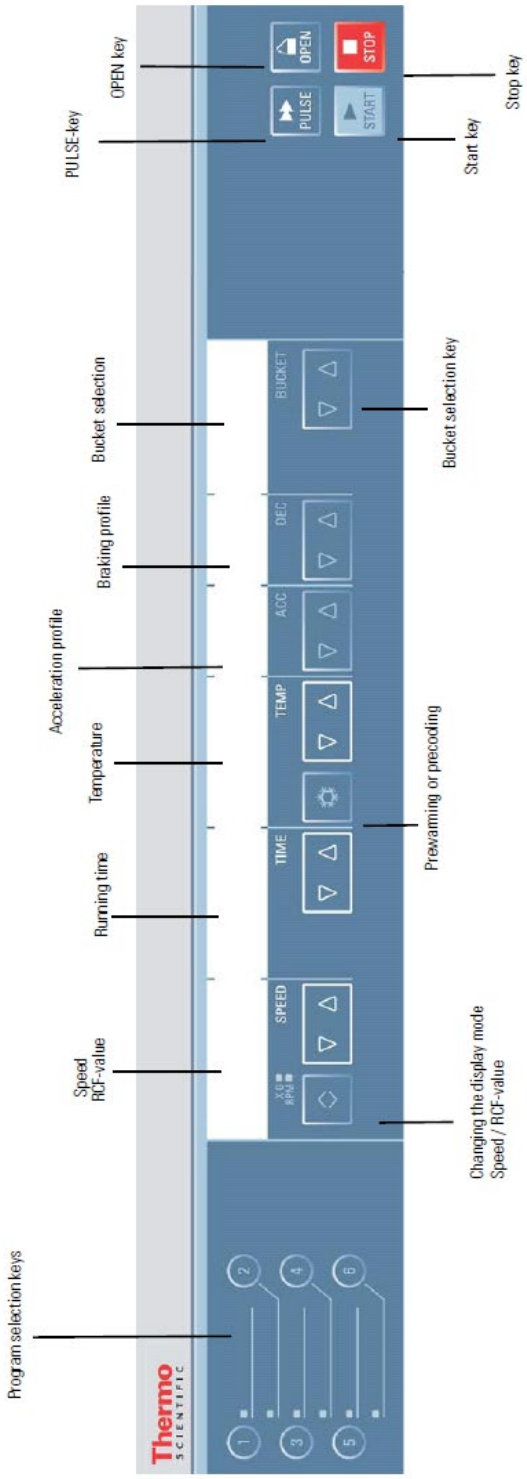
I. To pre-program the centrifuge

1. There are six available memory locations for pre-programmed programs.
2. Each corresponds to one of the six memory buttons (1-6).
3. To enter/save a program into one of the locations, enter the desired program parameters.
4. Press the memory button corresponding to the desired program position and hold for 5 seconds.

3 Literature and/or Supporting Documentation

IEC CL40 Centrifuge User's Manual

Diagram of Display


Program selection keys
Speed
RCF-value
Running time
Temperature
Acceleration profile
Braking profile
Bucket selection
PULSE-key
OPEN key
Changing the display mode
Speed / RCF-value
Prewarming or precooling
Bucket selection key
Start key
Stop key
Thermo
SCIENTIFIC
SPEED
TIME
TEMP
ACC
DEC
BUCKET
PULSE
OPEN
START
STOP

Preparer

Megan Barton Date: 01/27/2014
Advisory Board Chair

Concurrence

Heather Greco Date: 01/27/2014
Quality Assurance

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VACUUM OVEN INSTRUCTIONS

1 Scope

Instructions for the operation of the Thermo Scientific Vacuum Oven 3608 for the use in the silanization of glassware.

2 Instructions

2.1 Hose Connections

Connect a ¼-inch vacuum hose to the "EVACUATE" hose connector on the control panel. Connect the other end to the vacuum connector in the chemical hood.

Connect a ¼-inch vacuum hose to the "Vent" hose connector. The other end should be set in the chemical hood.

2.2 Temperature and Thermometer

Rotate the thermostat knob clockwise to increase the set point or counterclockwise to decrease the set point. Temperature should be set at zero before operating the oven.

A. During silanization, the temperature dial should be set at ~160°C indicated by the red mark.

B. During venting, the temperature should be set at zero.

The thermometer should be hanging from the oven tray so the temperature gauge can be read through the oven door.

2.3 Venting the Oven

Allow the oven to cool before venting the oven. Ensure the thermostat knob is set at zero.

1. Turn the middle knob on the control panel to either Evacuate or Vent.
   - *a) Evacuate - In this position the vacuum level will decrease. This allows for the door to open.*
   - *b) Vent - In this position the oven is purging the chemical out of the oven chamber.*
2. On the hood, open the vacuum valve. Ensure that the hood is shut.
3. Turn on the oven.
4. Open the evacuate valve on the oven. Allow the pressure to stabilize ~20 in.Hg – 25 in.Hg.
5. Open the vent valve so that the pressure starts to fall gradually to zero.
6. Turn on the oven.
7. Open the evacuate valve on the oven until the evacuate pressure is ~10 in.Hg. Close the vacuum valve on the hood so the pressure falls to zero.
8. Turn off the oven. Open the door.
9. Remove the glassware. Keep vial in the oven. Close the door.

2.4 Maintenance and Service

Clean up spills inside the chamber according to the SDS as soon as possible to prevent them from being baked on.

Service must be performed by qualified service personnel only.

Troubleshooting instructions can be found in the Vacuum Oven Manual.

3 Literature and Supporting Documentation

Operator Manual, Thermo Scientific Vacuum Oven, Thermo Scientific; Manual LT1495X2 Rev. 3

Preparer

Anna Mudd Date: 02/28/2014
Advisory Board Chair

Concurrence

Katherine G. Sanchez Date: 03/20/2014
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	04/14/2014	Original Issue

CEREX ALD-III PRESSURE PROCESSOR INSTRUCTIONS

1 Scope

Instructions for the operation of the SPE Ware Cerex Automated Liquid Dispensing (ALD) Pressure Processor System for the use in the extraction and purification of analytes utilizing a solid phase extraction (SPE) technique.

2 Instructions

2.1 Gas Source

Turn on the gas flow to the processors. The gas supply should be set at approximately 80 psi by a regulator positioned between the gas source and the processor.

2.2 Flow Control

Flow control is regulated by pre-set pressure profiles generated during processor set-up. These pressure profiles are specific to each extraction type performed.

2.3 Processor Loading

A. Stack the SPE Column Rack onto the Waste Container Rack or the Collection Tube Rack and set the stack onto the platform with the black bands in the back.

B. Turn on computer and open ALD software.

2.4 Maintenance and Service

A. Daily checks before use

1. Verify pressure flow into instrument is approximately 80 psi.
2. Verify liquid level in waste container and make sure the waste tubings are not submerged in the liquid.
3. Verify solvent/reagent levels and replenish if needed.
   - *a) Reset level of each replenished solvent as a % to the bottle size of solvent.*
   - *i. Levels → reset button next to solution that was replaced → set level*
   - *b) Prime tubing for each solvent/reagent replenished.*
   - *i. Prime → select solution that was replaced → select amount to prime (e.g. 5 mL) → prime → prime system with methanol*
4. Inspect pump for leaks, manifold gasket for detachment, and solvent lines for any air pockets.

B. Daily maintenance after use

1. It is recommended to rinse waste tray with MeOH and/or DI water.
2. It is recommended to shut down computer and turn processor off at end of day.

3 Literature and Supporting Documentation

SPEWare Automated Liquid Dispensing Cerex 48/96/192 ALD-II/III User Manual

Preparer

Megan Barton Date: 09/08/2014
Advisory Board Chair

Concurrence

Heather Greco Date: 09/08/2014
Quality Assurance

Version #	Effective Date	Brief Description of Change(s)
00	09/19/2014	Original Issue

XCELVAP EVAPORATOR SYSTEM INSTRUCTIONS

1 Scope

Instructions for the use of the XcelVap evaporator for evaporating samples to dryness

2 Instructions for XcelVap Evaporator

1. Turn the gas supply and unit ON.
2. Check the water bath level.
3. Set the water bath temperature, the automatic pressure profile, and the evaporation time as indicated in the extraction method.
4. Load the evaporator with the sample tubes.
5. Press the start button.
6. Remove the sample tubes promptly after evaporation.
7. Turn OFF the unit and gas supply.
8. Lift the cover to prevent moisture from accumulating on the inside cover and oxidizing the gas manifold.

3 Literature and Supporting Documentation

XcelVap Evaporator System Operator's Manual

Preparer

Megan Barton Date: 04/02/2015
Toxicology Advisory Board Chair

Concurrence

Katherine G. Sanchez Date: 04/02/2015
Quality Assurance Specialist

Version #	Effective Date	Brief Description of Change(s)
00	04/20/2015	Original Issue

INSTRUCTIONS FOR pH METER

1 Scope

Instructions for the operation of the pH meter in the Toxicology Section for use in measuring and controlling the acidity or alkalinity of a solution.

2 Instructions

A. Remove the electrode from the storage solution.

B. Twist the plastic cover open to expose the filling hole.

C. Rinse the electrode with deionized water and blot dry, repeat this step after each measurement.

D. For all readings, immerse the electrode in the solution and stir.

E. Record all readings on the pH Meter Calibration form (LAB-TOX-27).

F. Measure and record the buffer solution before standardization cleared (pH 4 or 7, depending on curve). Tolerance should be +/- 0.10.

G. Standardize the pH meter and electrode and record the standardized slope for the two pH ranges. Tolerance should be 90%-105%.

H. Measure and record the buffer solution at the completion of standardization. Tolerance should be +/- 0.10.

NOTE: Standardization curve must be performed to bracket the pH of solution being prepared. If a pH lower than 10 is being prepared use pH 4, 7, 10 solutions. If a pH above 10 is being prepared use pH 7, 10, and 12 solutions.

I. The pH meter is now ready for use on the desired solution.

J. Record the solution temperature indicated on the pH meter.

K. Close the filling hole and return the electrode to the storage solution.

L. Change the electrode storage solution monthly.

3 Supporting Documentation

Accumet Basic pH Meter User's Guide

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	04/02/2018	Original Issue, formerly AUS-INS-402

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Urine Immunoassay Reagent Log

LAB-TOX-10 Rev.02 (10/2015) p.1 Issued by: QAC

Assay Class: _____

Kit Lot #	Reagent A Lot #	Reagent E Lot #	Expiration Date	Low Control		High Control		Date Verified	Acceptable (Y/N)	Initials	Date First Used in Casework	Initials
				Lot #	Response ng/mL	Lot #	Response ng/mL					

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Working Solution Log

LAB-TOX-11 Rev.03 (04/2016) p.1 Issued by: QAC

Pipette(s) _____
Target _____
Actual _____
Balance _____

☐ Calibrator (pink)
☐ Control (yellow)
☐ ISTD (blue)

Working Solution (WS) (Name/Concentration) _____ Lot # _____ Exp. Date _____

Preparation

Preparer _____ Date Prepared _____ ☐ Reconstituted Diluted to _____ with _____

Component	Conc.	Supplier/Lot #	Exp. Date	Amount Used

WS Dilution (Name/Concentration) _____ Lot # _____
Amount WS used _____ diluted to _____ with _____

Quality Control

WS Calibrators				**ISTD Verification**	
☐EMIT		☐ Confirmation	☐ Comparison New to Previous ☐ Comparison New to Verified Control	Batch ID:	
Batch ID:		Batch IDs:		Analyst:	
Analyst:		Analysts:			
☐ Pass	☐ Fail	☐ Pass	☐ Fail		

WS Controls			
☐ Manufacturer Verified Value	☐ Lab Verified Value	Batch IDs:	Analysts:

Analyte	**Casework Value**	**Analyte**	**Casework Value**

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

GCMS Data Screen Worksheet

LAB-TOX-12 Rev.00 (01/2010) p.1 Issued by: QAC

Lab Case # _____
Analyst _____
Batch Date _____
Data Analysis Date _____

Internal Standard			Specimen Type	
Ions	Drug	Detected?		
86, 99	SKF-525A ISTD		☐ Blood	☐ Negative Screen
207, 242	Medazepam ISTD		☐ Urine	

Commonly Reported Drugs			Additional Drugs		
Ions	Drug	Detected?	Ions	Drug	Detected?
193, 236	Carbamazepine				
58, 158	Carisoprodol				
58, 238	Citalopram				
162, 299	Codeine				
58, 215	Cyclobenzaprine				
150, 271	Dextromethorphan				
58, 73	Diphenhydramine				
58, 165	Doxepin				
242, 299	Hydrocodone				
83, 114	Meprobamate				
72, 165	Methadone				
44, 220	Norpropoxyphene				
44, 234	Norpropoxyphene (amide)				
44, 202	Nortriptyline				
200, 242	Phencyclidine				
180, 252	Phenytoin				
58, 208	Propoxyphene				
43, 324	Topiramate				
58, 263	Tramadol				
176, 205	Trazodone				
219, 235	Zolpidem				

IF A SPECIFIC ION PLOT WAS PERFORMED, MARK ON THE "DETECTED?" LINE (+) FOR DETECTED DRUGS AND NEG OR N.D. FOR NOT DETECTED (A BLANK LINE INDICATES NO PLOT MADE)

Search Report Library

- ☐ Screen
  (selected spectra of drugs of interest)
- ☐ TMS
  (spectra of TMS derivatized drugs)
- ☐ AAFS
  (spectra from the American Academy of Forensic Sciences)
- ☐ NIST
  (spectra from the National Institute of Standards)
- ☐ GBI
  (spectra from the Georgia Bureau of Investigation)

◆

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Lab Case # _____
Analyst _____
Batch Date _____

LC/MS Data Screen Worksheet

LAB-TOX-13 Rev.02 (03/2017) p.1 Issued by: QAC

Internal Standard Detected?		Negative Screen?	

Common Screen Drugs	Plot Response	Common Additional Drugs	Plot Response
7-Aminoflunitrazepam		7-aminoclonazepam	
Amitriptyline		Alprazolam	
Baclofen		Amphetamine	
Carbamazepine		Benzoylecgonine	
Chlorpheniramine		Carisoprodol	
Citalopram		Chlordiazepoxide	
Cyclobenzaprine		Clonazepam	
Dextromethorphan		Cocaethylene	
Dihydrocodeine		Cocaine	
Diphenhydramine		Codeine	
Doxepin		Diazepam	
Doxylamine		Hydrocodone	
Etizolam		Lorazepam	
Fentanyl		Meprobamate	
Gabapentin		Methamphetamine	
Guaifenesin		Nordiazepam	
Hydroxyzine		Oxycodone	
Ketamine		Phencyclidine	
Lamotrigine		Phentermine	
Lidocaine		Pseudoephedrine	
Methadone		Temazepam	
Methocarbamol			
Mirtazapine		**Additional Drugs**	**Plot Response**
Modafinil			
Nortriptyline			
O-desmethylvenlafaxine			
Oxazepam			
Oxcarbazepine			
Phenazepam			
Phenytoin			
Pregabalin			
Promethazine			
Quetiapine			
Sertraline		**Plot Response: +, ND, Ind., #, or Possible**	
Tramadol			
Trazodone		**# Drug identified but not documented, as the drug has**	

Venlafaxine		been confirmed or excluded (negative or below cut-off) through other analyses.
Zolpidem		

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Lab Case # _____
Date _____

Toxicology Report Uncertainty Calculation Sheet

LAB-TOX-14 Rev.02 (04/2016) p.1 Issued by: QAC

Drug	Test Result	Units	Sig Fig	k=2 (%)	Uncertainty Estimate	Testing Analyst Initials

Reported Conclusion(s):

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Zero Response Calculation

LAB-TOX-15 Rev. 02 (05/2019) p.1 Issued by: QAC

Drug: Batch ID:

Equation: $Y = a X^2 + b X + c$

Conc ISTD= mg/L where X = Conc Unk/Conc ISTD
Y = Area Unk/Area ISTD

Low Calib= mg/L

Absolute value of concentration for zero area relative to the low calibration point: #N/A

The area for an unknown is calculated for a range of concentrations from -0.5 to 0.5. The table of calculated values is used for locating the smallest area above zero and its corresponding concentration.

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Blood Immunoassay Reagent Log

LAB-TOX-17 Rev.00 (10/2015) p.1 Issued by: QAC

Assay Class: _____

Kit Lot #	Reagent A Lot #	Reagent E Lot #	Expiration Date	ΔA	ΔA Acceptance Criteria	Date Verified	Acceptable (Y/N)	Initials	Date First Used in Casework	Initials

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Urine Immunoassay Calibrators and Controls Form

LAB-TOX-18 Rev.00 (10/2015) p.1 Issued by: QAC

Analyst: _____

Batch Date: _____

Calibrators

Position	ID	Assay(s)	Lot Number	Expiration Date
B1	Negative Urine Cal	AMPH, BARB, BENZ, COC, OPI, PCP, THC, SOMA		
S1	Multi Drug Cal 1	BARB, BENZ, COC, PCP		
S2	Multi Drug Cal 2	BARB, BENZ, COC, PCP		
S3	Multi Drug Cal 3	BARB, BENZ, COC, PCP		
S4	Multi Drug Cal 4	BARB, BENZ, COC, PCP		
S5	THC Urine 20 Cal	THC		
S6	THC Urine 50 Cal	THC		
S7	THC Urine 100 Cal	THC		
S8	Opiate Cal 1	OPI		
S9	Low Urine Cal	OPI		
S10	Opiate Cal 3	OPI		
S11	High Urine Cal	OPI		
S12	Mep/Meth Level 2 Cal	AMPH, SOMA		
S13	Mep/Meth Level 3 Cal	AMPH, SOMA		
S14	Mep/Meth Level 4 Cal	AMPH, SOMA		
S15	Mep/Meth Level 5 Cal	AMPH, SOMA		

Controls

Specimen ID #	ID	Assay(s)	Lot Number	Expiration Date
1	Primary Low	BARB, BENZ, COC, PCP		
2	Primary High	BARB, BENZ, COC, PCP		
3	Clinical Low	OPI		
4	Clinical High	OPI		
5	Negative Urine Cal	THC		
6	THC 40 Control	THC		
13	Mep/Meth Low Control	AMPH, SOMA		
14	Mep/Meth High Control	AMPH, SOMA		

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Urine Immunoassay Reagents Form

LAB-TOX-19 Rev.00 (10/2015) p.1 Issued by: QAC

Analyst: _____

Batch Date: _____

Position	Assay	Lot Number (Reagent A only)	Expiration Date
1	Amphetamines		
2	Barbiturates		
3	Benzodiazepines		
4	Cannabinoids		
5	Cocaine		
6	Opiates		
7	Phencyclidine		
8	Soma		

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Urine Immunoassay Control Verification

LAB-TOX-20 (Rev.00 (10/2015) p.1 Issued by: QAC

Control: _____

Lot # _____

	Result			
Drug Name(s) →				
Target Concentration (ng/mL) →				
1st Batch				
2nd Batch				
3rd Batch				

Mean (Lab Est. Value)				
Std Dev				
% Difference from Expected				

For Casework:

Plus 30%				
Minus 30%				

Notes:

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CRIME LABORATORY

Urine Immunoassay Control Log

LAB-TOX-21 Rev.00 (10/2015) p.1 Issued by: QAC

Name of Control: _____

*See Working Solution Log for verification of each lot number

Lot Number	Expiration Date	Drug Category and Acceptable Range	Initials	Date Verified	Date First Used in Casework	Initials

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Urine Immunoassay Calibrator Log

LAB-TOX-22 Rev.00 (10/2015) p.1 Issued by: QAC

Name of Calibrator: _____

Lot Number	Expiration Date	Calibration comparable to previous? (Yes/No)	Initials	Date Verified

Date First Used in Casework	Initials

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

MGC240 Maintenance Log

LAB-TOX-23 Rev.00 (10/2015) p.1 Issued by: QAC

Month/Year: _____

DAILY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Beginning of Day																															
Check and/or Fill DI Water Reservoir																															
Check and/or Fill 2% Alkaline Wash Reservoir																															
Check and/or Fill 1% Acidic Wash Reservoir																															
Check and/or Replace Printer Paper																															
Check Reagent Compartment Temp (by touch)																															
Operator's Initials																															
End of Day																															
Wipe Probes with DI Water (5.2.1, 5.2.2)																															
Operator's Initials																															

MONTHLY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Clean Reagent Fan Screen																															
Perform a Lamp Life Check																															
Perform a Cuvette Check (4.12.4)*																															
Inspect Reagent Pump Unit & Sampling (5.2.8, 5.2.9)																															
Inspect Probe Wash Pump Unit (5.2.10)																															
Check/Replace Water Reservoir Filter (5.2.4)																															
Check/Replace Wash Reservoir Filters (5.2.4)																															
Perform Sample Probe Wash																															
Operator's Initials																															

AS NEEDED	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Replace Lamps																															
Replace Cuvette(s)																															
Other:																															
Operator's Initials																															

1st Yearly Preventative Maintenance Date Performed: _____ **2nd Yearly Preventative Maintenance** Date Performed: _____

** Check cuvettes by using all assay wavelengths currently used.*

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Reagent Log

LAB-TOX-24 Rev.01 (05/2019) p.1 Issued by: QAC

Reagent Name:_____

Related SOP: _____ Effective Date: _____

Deviation: _____

Preparation Date	Verification Batch	Reagent Acceptable? (Y/N)	Lot #	Notes (e.g. pH, bottle #s)	Expiration Date (if applicable)	Initials

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Vacuum Pump Oil Level Log

LAB-TOX-25 Rev.00 (10/2015) p.1 Issued by: QAC

Instrument: _____ **Year:** _____

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
1												
2												
3												
4												
5												
6												
7												
8												
9												
10												
11												
12												
13												
14												
15												
16												
17												
18												
19												
20												
21												
22												
23												
24												
25												
26												
27												
28												
29												
30												
31												

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Toxicology Matrix Blank Verification

LAB-TOX-26 Rev.00 (10/2015) p.1 Issued by: QAC

Manufacturer: _____

Lot Number: _____(attach Certificate of Analysis, if available)

Matrix: ☐ Blood ☐ Urine

Source: ☐ Bovine ☐ Sheep ☐ Human ☐ Other _____

Screening Results:

EMIT	Batch ID: _____	☐ Negative	☐ Positive _____
GCMS Screen	Batch ID: _____	☐ Negative	☐ Positive _____
LCMS Screen	Batch ID: _____	☐ Negative	☐ Positive _____
GCMS Amphetamines	Batch ID: _____	☐ Negative	☐ Positive _____
_____	Batch ID: _____	☐ Negative	☐ Positive _____
_____	Batch ID: _____	☐ Negative	☐ Positive _____
_____	Batch ID: _____	☐ Negative	☐ Positive _____

Acceptable for Casework in the Following:

☐ EMIT*†
☐ GCMS Screen*†
☐ GCMS Barbiturates
☐ GCMS Amphetamines*†
☐ GCMS Urine THC
☐ LCMS Screen*
☐ LCMS Target Qualitative Analysis
☐ LCMS Opiates-Cocaine†
☐ LCMS Benzodiazepines†
☐ LCMS Blood THC

*Blood screening required before use in casework for the category
†Urine screening required before use in casework for the category

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

pH Meter Calibration

LAB-TOX-27 Rev.00 (10/2015) p.1 Issued by: QAC

Serial Number: _____

Calibration range must cover expected pH of reagent tested.

Date Calibrated	Buffer Solution Lot Numbers Options: pH 4, 7, 10, & 12		pH measured before Standardization cleared Tolerance (±0.10) Example: pH 4	Standardization Slope Tolerance (90%-105%) Example: pH 4-7	Standardization Slope Tolerance (90%-105%) Example: pH 7-10	pH measured at completion of Standardization Tolerance(±0.10) Example: pH 10	Temperature (°C)	Electrode Storage Solution Changed	Acceptable (Y/N)	Analyst Initials
	pH	Lot Number								
			Buffer pH ___	Buffer pH ___ - ___	Buffer pH ___ - ___	Buffer pH ___				
			Buffer pH ___	Buffer pH ___ - ___	Buffer pH ___ - ___	Buffer pH ___				
			Buffer pH ___	Buffer pH ___ - ___	Buffer pH ___ - ___	Buffer pH ___				
			Buffer pH ___	Buffer pH ___ - ___	Buffer pH ___ - ___	Buffer pH ___				
			Buffer pH ___	Buffer pH ___ - ___	Buffer pH ___ - ___	Buffer pH ___				
			Buffer pH ___	Buffer pH ___ - ___	Buffer pH ___ - ___	Buffer pH ___				

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Instrument Log

LAB-TOX-28 Rev.00 (10/2015)p.1 Issued by: QAC

Date	Description e.g. maintenance (maint), shot number, tune type	Notes e.g. specific maintenance, tune information, batch file/sequence name	Initials

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Control Verification

LAB-TOX-29 Rev.01 (07/2017) p.2 Issued by:QAC

Control:
Lot #: **Initials:** **Date:**
All units are in

DATA	Batch 1			Batch 2			Batch 3		
Analyte	Batch ID:			Batch ID:			Batch ID:		

	Manufacture verified value (<10%)	**Batch 1 Acceptable cannot be "NO"**
	Lab prepared value (<5%)	
x	**Lab established value (YES/NO based on <5%)**	

	Expected	**Average of Batch1**	**% Diff. of Batch 1**	**Batch 1 Acceptable**	**Average of all batches**	**-20% range**	**+20% range**	**% Diff. from Expected**	**St. Dev**

% Difference from Expected or Average

	Batch 1			**Batch 2**			**Batch 3**		
	Batch ID:			**Batch ID:**			**Batch ID:**		

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Control Verification

LAB-TOX-29 Rev.01 (07/2017) p.2 Issued by:QAC

Control: 0
Lot #: 0

Initials: 0 **Date:** 1/0/1900

	Batch 4			Batch 5			Batch 6		
	Batch ID:			Batch ID:			Batch ID:		

	Batch 4			**Batch 5**			**Batch 6**		
	Batch ID:			**Batch ID:**			**Batch ID:**		

		-20% range	+20% range	Average of all batches
	#VALUE!			
	#VALUE!			
	#VALUE!			
	#VALUE!			
	#VALUE!			
	#VALUE!			
	#VALUE!			
	#VALUE!			

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Drug Standard Log

LAB-TOX-30 Rev. 00 (03/2016) p.1 Issued by: QAC

Date Received	Standard	Supplier	Lot #	Exp. Date	CofA Received?	Initials

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Drug Standard Log

LAB-TOX-30 Rev. 00 (03/2016) p.1 Issued by: QAC

Date Received	Standard	Supplier	Lot #	Exp. Date	CofA Received?	Initials

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Post-Calibration Pipette Check Report

LAB-TOX-31 Rev.01 (03/2017) p.1 Issued by: QAC

Pipette ID:		**Analyst:**	
Brand:			
Model:		**Date:**	
Serial Number:			
Range:		**Status:**	**#DIV/0!**

Balance used:

Water Temp (°C) **Room Temp (°C)** **Density of Water** mg/µL

Pipetting Data

Volume Setting (µL)						
Weighings (g)						
Results	**Actual**	**Specifications**	**Actual**	**Specifications**	**Actual**	**Specifications**
Mean (g)	#DIV/0!		#DIV/0!		#DIV/0!	
Mean (µL)*	#DIV/0!		#DIV/0!		#DIV/0!	
%Dev	#DIV/0!	±	#DIV/0!	±	#DIV/0!	±
Stdev	#DIV/0!		#DIV/0!		#DIV/0!	
%CV	#DIV/0!	≤	#DIV/0!	≤	#DIV/0!	≤

*An adjustment was made for the density of the water.

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Pipette Performance Verification Report

LAB-TOX-32 Rev.01 (03/2017) p.1 Issued by: QAC

Pipette ID:		**Analyst:**	
Brand:			
Model:		**Date:**	
Serial Number:			
Range:		**Status:**	**#DIV/0!**

Balance used:

Water Temp (°C) **Room Temp (°C)** **Density of Water** mg/µL

Pipetting Data

Volume Setting (µL)						
Weighings (g)						
Results	**Actual**	**Specifications**	**Actual**	**Specifications**	**Actual**	**Specifications**
Mean (g)	#DIV/0!		#DIV/0!		#DIV/0!	
Mean (µL)*	#DIV/0!		#DIV/0!		#DIV/0!	
%Dev	#DIV/0!	±	#DIV/0!	±	#DIV/0!	±
Stdev	#DIV/0!		#DIV/0!		#DIV/0!	
%CV	#DIV/0!	≤	#DIV/0!	≤	#DIV/0!	≤

*An adjustment was made for the density of the water.

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Unconfirmed EMIT Positives Log

LAB-TOX-33 Rev.00 (03/2017) p.1 Issued by: QAC

Category

			EMIT		Confirmation		
	Case Number	Analyst	Date	EMIT Level	Analyst	Date	Other Positive Drugs
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Toxicology Pipette Log

LAB-TOX-34 Rev. 00 (03/2017) p.1 Issued by: QAC

Pipette ID:	
Pipette Range:	
Pipette Model & Type:	
Pipette Serial Number:	

Date	Analyst	Notes

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

Blood EMIT Results Workbook

Contains:
EMIT Results – LAB-TOX-35
EMIT Results (individual page) – LAB-TOX-35a
Batch Archive Review Sheet

Revision History

Version #	Effective Date	Brief Description of Change(s)
00	07/2017	Original Issue
01	05/2019	Added: macro to insert data from instrument, individual results page (LAB-TOX-35a) and macro to create it, conditional formatting based on change in absorbance values

TEXAS DEPARTMENT OF PUBLIC SAFETY
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EMIT Results

LAB-TOX-35 Rev. 01 (05/2019) p.3 Issued by: QAC

BATCH DATE:
ANALYST:

Matrix: Blood Units: (mg/L)

STDS	AMPH	RANGE	BARB	RANGE	BENZ	RANGE	COC	RANGE	OPI	RANGE	OXY	RANGE	PCP	RANGE	SOMA	RANGE	THC	RANGE	ZOLP	RANGE
< NEG	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N
B1	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N
OPI-Hydrocodone Cutoff PCP-Calculated Cutoff									0	*ELEV			0	*ELEV						
B2	0	*HI NEG	0	HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	HI NEG	0	HI NEG	0	HI NEG
B3	0	0.1-0.4	0	1.0-2.0	0	0.02-0.1	0	0.1-0.3	0	0.1-0.3	0	0.02-0.1	0	0.04-0.05	0	2.0-4.0	0	0.015-0.025	0	0.02-0.06
B4	0	0.4-1.2	0	2.0-6.0	0	0.1-0.3	0	0.3-0.9	0	0.3-0.9	0	0.1-0.3	0	0.05-0.15	0	4.0-12.0	0	0.025-0.075	0	0.06-0.18
B5	0	>1.2	0	>6.0	0	>0.3	0	>0.9	0	>0.9	0	>0.3	0	>0.15	0	>12.0	0	>0.075	0	>0.18

B3 - B1	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**
Reagent A Lot #	73235160		73349645		73312834		73308531		73339921		73110167		73261889		E33604		73272560		E33150	
Criteria	10		158		9		13		13		14		14		12		14		38	

CAL 2ND INJ	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5
(% Diff from 1st Inj)	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**

CASES	AMPH	CONC	BARB	CONC	BENZ	CONC	COC	CONC	OPI	CONC	OXY	CONC	PCP	CONC	SOMA	CONC	THC	CONC	ZOLP	CONC

* Further Analysis Needed

DATE PRINTED: 4/28/2019

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

BATCH DATE:
ANALYST:

EMIT Results

Matrix: Blood Units: (mg/L)

LAB-TOX-35 Rev. 01 (05/2019) p.3 Issued by: QAC

STDS	AMPH	RANGE	BARB	RANGE	BENZ	RANGE	COC	RANGE	OPI	RANGE	OXY	RANGE	PCP	RANGE	SOMA	RANGE	THC	RANGE	ZOLP	RANGE
< NEG	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N
B1	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N
OPI-Hydrocodone Cutoff PCP-Calculated Cutoff									0	*ELEV			0	*ELEV						
B2	0	*HI NEG	0	HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	HI NEG	0	HI NEG	0	HI NEG
B3	0	0.1-0.4	0	1.0-2.0	0	0.02-0.1	0	0.1-0.3	0	0.1-0.3	0	0.02-0.1	0	0.04-0.05	0	2.0-4.0	0	0.015-0.025	0	0.02-0.06
B4	0	0.4-1.2	0	2.0-6.0	0	0.1-0.3	0	0.3-0.9	0	0.3-0.9	0	0.1-0.3	0	0.05-0.15	0	4.0-12.0	0	0.025-0.075	0	0.06-0.18
B5	0	>1.2	0	>6.0	0	>0.3	0	>0.9	0	>0.9	0	>0.3	0	>0.15	0	>12.0	0	>0.075	0	>0.18

B3 - B1	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**
Reagent A Lot #	73235160		73349645		73312834		73308531		73339921		73110167		73261889		E33604		73272560		E33150	
Criteria	10		158		9		13		13		14		14		12		14		38	

CAL 2ND INJ	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5
(% Diff from 1st Inj)	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**

CASES	AMPH	CONC	BARB	CONC	BENZ	CONC	COC	CONC	OPI	CONC	OXY	CONC	PCP	CONC	SOMA	CONC	THC	CONC	ZOLP	CONC

* Further Analysis Needed

DATE PRINTED: 4/28/2019

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

BATCH DATE:
ANALYST:

EMIT Results

Matrix: Blood Units: (mg/L)

LAB-TOX-35 Rev. 01 (05/2019) p.3 Issued by: QAC

STDS	AMPH	RANGE	BARB	RANGE	BENZ	RANGE	COC	RANGE	OPI	RANGE	OXY	RANGE	PCP	RANGE	SOMA	RANGE	THC	RANGE	ZOLP	RANGE
< NEG	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N
B1	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N
OPI-Hydrocodone Cutoff PCP-Calculated Cutoff									0	*ELEV			0	*ELEV						
B2	0	*HI NEG	0	HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	HI NEG	0	HI NEG	0	HI NEG
B3	0	0.1-0.4	0	1.0-2.0	0	0.02-0.1	0	0.1-0.3	0	0.1-0.3	0	0.02-0.1	0	0.04-0.05	0	2.0-4.0	0	0.015-0.025	0	0.02-0.06
B4	0	0.4-1.2	0	2.0-6.0	0	0.1-0.3	0	0.3-0.9	0	0.3-0.9	0	0.1-0.3	0	0.05-0.15	0	4.0-12.0	0	0.025-0.075	0	0.06-0.18
B5	0	>1.2	0	>6.0	0	>0.3	0	>0.9	0	>0.9	0	>0.3	0	>0.15	0	>12.0	0	>0.075	0	>0.18

B3 - B1	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**
Reagent A Lot #	73235160		73349645		73312834		73308531		73339921		73110167		73261889		E33604		73272560		E33150	
Criteria	10		158		9		13		13		14		14		12		14		38	

CAL 2ND INJ	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5
(% Diff from 1st Inj)	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**

CASES	AMPH	CONC	BARB	CONC	BENZ	CONC	COC	CONC	OPI	CONC	OXY	CONC	PCP	CONC	SOMA	CONC	THC	CONC	ZOLP	CONC

* Further Analysis Needed

DATE PRINTED: 4/28/2019

TEXAS DEPARTMENT OF PUBLIC SAFETY
CRIME LABORATORY

EMIT Results

LAB-TOX-35a Rev. 00 (05/2019) p.1 Issued by: QAC

BATCH DATE: 01/00/00
ANALYST: 1/0/1900

Matrix: Blood Units: (mg/L)

STDS	AMPH	RANGE	BARB	RANGE	BENZ	RANGE	COC	RANGE	OPI	RANGE	OXY	RANGE	PCP	RANGE	SOMA	RANGE	THC	RANGE	ZOLP	RANGE
< NEG	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N	0	< N
B1	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N	0	N
OPI-Hydrocodone Cutoff PCP-Calculated Cutoff									0	*ELEV			0	*ELEV						
B2	0	*HI NEG	0	HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	*HI NEG	0	HI NEG	0	HI NEG	0	HI NEG
B3	0	0.1-0.4	0	1.0-2.0	0	0.02-0.1	0	0.1-0.3	0	0.1-0.3	0	0.02-0.1	0	0.04-0.05	0	2.0-4.0	0	0.015-0.025	0	0.02-0.06
B4	0	0.4-1.2	0	2.0-6.0	0	0.1-0.3	0	0.3-0.9	0	0.3-0.9	0	0.1-0.3	0	0.05-0.15	0	4.0-12.0	0	0.025-0.075	0	0.06-0.18
B5	0	>1.2	0	>6.0	0	>0.3	0	>0.9	0	>0.9	0	>0.3	0	>0.15	0	>12.0	0	>0.075	0	>0.18

B3 - B1	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**	**0**	**FAIL**
Reagent A Lot #	73235160		73349645		73312834		73308531		73339921		73110167		73261889		E33604		73272560		E33150	
Criteria	10		158		9		13		13		14		14		12		14		38	

CAL 2ND INJ	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5	0	B5
(% Diff from 1st Inj)	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**	#####	**#DIV/0!**

CASES	AMPH	CONC	BARB	CONC	BENZ	CONC	COC	CONC	OPI	CONC	OXY	CONC	PCP	CONC	SOMA	CONC	THC	CONC	ZOLP	CONC

EMIT Dilution: ____

Ownership of Data: _____

* Further Analysis Needed

DATE PRINTED: 4/28/2019

TEXAS DEPARTMENT OF PUBLIC SAFETY
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Batch Archive Review Sheet

Immunoassay Screening (TOX-03-01)

Batch Date:
Run Date:
Analyst:

Cal WS Lots: LOW121718 HIGH121718 **HC WS Lot:** HC083117 **Blank Lot:** S-R7301

Instrument: MGC240

- **Was B3-B1 criteria met during initial injections of calibrators for all categories?** **Yes**
- **If not acceptable, what action was taken?**

- **Was the B5 reinjection criteria met for all categories?** **Yes**
- **If not acceptable, what action was taken?**

- **Archive Components:**
  - Batch Worklist
  - Paracelsus Worklist
  - Results Summary Table (LAB-TOX-35)
  - Log results from MGC240 for entire batch

- **Additional Comments:**

Dev-SYS-TOX-03-01-2018-1001 was used for this batch.
For LAB-TOX-35, Dev-SYS-LAB-TOX-35-2018-1001 was used.
#VALUE! = Oxy and Zolp not run due to submission date of case.

Reviewed by: _____ Date: _____

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EMIT Reagent Validation Form

LAB-TOX-36 Rev.01 (05/2019) p.1 Issued by: QAC

Reagent Assay	0	0	0	0	0	0	0
A Lot #:							
E Lot #:							
Exp. Date							
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Neg. Avg.							
SD							
Avg + (2*SD)							
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
High Neg. Avg.							
SD							
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cutoff Avg.							
SD							
Avg - (2*SD)							

	0	0	0	0	0	0	0
>ΔA Criteria							

Reagent acceptable if negative calibrator plus two standard deviations does not overlap with the cut-off minus two standard deviations.

Outliers = +/- 2*SD from average
To exclude outliers, print original form then delete outliers, print, and include all forms for verification purposes.

ANALYST: _____

REVIEWER (INITIALS/DATE): _____

TEXAS DEPARTMENT OF PUBLIC SAFETY
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EMIT Solution Verification Checklists – Blood/Urine

LAB-TOX-37 Rev.00 (07/2017) p.2 Issued by: QAC

New Reagent Lot Number Historical Acceptance Criteria - Blood

- ☐ Prepare reagent bottles for instrument with reagent name, lot number, reagent type (A/E) and expiration date
- ☐ Prepare negative and cutoff calibrator to extract and run on instrument with new reagent
- ☐ Verify that ΔA meets historical criteria
- ☐ Record in Immunoassay Reagent Log (LAB-TOX-17) lot numbers, ΔA observed and ΔA criteria (historical value)
- ☐ Record date verified on reagent kit boxes
- ☐ Technical Leader or Section Supervisor will review data

First use

- ☐ Record first use date in Immunoassay Reagent Log (LAB-TOX-17) – Blood section
- ☐ Change lot number on Blood EMIT Results sheet (LAB-TOX-35) on instrument computer. Save template.
- ☐ Change lot number on EMIT Reagents Currently in Use sheet next to instrument.

Working Solution (Calibrator) Verification - Blood

- ☐ Process spiked calibrators with new working solutions
- ☐ Compare new lot to current lot
- ☐ Results acceptable if B3-B1 meet criteria
- ☐ Record preparation and pass/fail on Working Solution Log (LAB-TOX-11)
- ☐ Technical Leader or Section Supervisor will review data

New Reagent Lot Number Establish Minimum Acceptance Criteria - Blood

- ☐ Prepare reagent bottles for instrument with reagent name, lot number, and expiration date
- ☐ Prepare 5 replicates of negative (B1), high negative (B2), and cutoff (B3) calibrators and extract and run on instrument
- ☐ Prepare Reagent Validation sheet (LAB-TOX-36). See SOP TOX-07-18 and reagent log book for examples. Be sure to document all lot numbers and expiration dates.
- ☐ Record in Immunoassay Reagent Log (LAB-TOX-17) lot numbers and newly established ΔA criteria
- ☐ Record date verified on reagent kit boxes
- ☐ Technical Leader or Section Supervisor will review data

First use

- ☐ Record first use date in Immunoassay Reagent Log (LAB-TOX-17)
- ☐ Change lot number <u>and</u> ΔA on Blood EMIT Results sheet (LAB-TOX-35) on instrument computer. Save template.
- ☐ Change lot number <u>and</u> ΔA on EMIT Reagents Currently in Use sheet next to instrument.

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EMIT Solution Verification Checklists – Blood/Urine

LAB-TOX-37 Rev.00 (07/2017) p.2 Issued by: QAC

New Reagent Lot Number Acceptance Criteria – Urine

- ☐ Run the assay for that drug category on the negative, calibrators, and controls using new reagent.
- ☐ Verify that control values are within acceptable ranges
- ☐ Record in Immunoassay Reagent, Calibrators, and Controls Log (LAB-TOX-10) the lot numbers and responses for control
- ☐ Record date verified on reagent kit boxes
- ☐ Technical Leader or Section manager will review data

First use

- ☐ Record first use date in Immunoassay Reagent, Calibrators, and Controls Log – Urine section

New Calibrator Verification - Urine

- ☐ Run new lot in place of current lot on calibration curve
- ☐ Compare new curve to previous curve
- ☐ New lot acceptable if comparable to previous curve
- ☐ Record new lot numbers and acceptable ranges in Urine Immunoassay Calibrator Log (LAB-TOX-22)

First use

- ☐ Record first use date in Urine Immunoassay Calibrator Log (LAB-TOX-22)

New Control Verification – Urine

- ☐ Determine lab established value by processing 3 batches as 3 prepared samples in each batch
- ☐ Fill out Urine Immunoassay Control Verification form (LAB-TOX-20) for each control verified
- ☐ Create batch archive for data to be reviewed by Technical Leader or Section Manager.
- ☐ Record new lot numbers and acceptable ranges in Urine Immunoassay Control Log (LAB-TOX-21)

First use

- ☐ Update values and ranges in QuikLab software.
- ☐ Record first use date in Urine Immunoassay Control Log (LAB-TOX-21)
- ☐ Batch archive review sheet should contain note about control's first use. Batch reviewer will check lot numbers and ranges in Quiklab software.

TEXAS DEPARTMENT OF PUBLIC SAFETY
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Balance Check Log

LAB-TOX-38 Rev.00 (03/2017) p.1 Issued by: QAC

Balance ID:	
Manufacturer:	
Model:	
Serial Number:	

Weights

Weight Range	Specification

Date	Weight	Reading	Pass/Fail	Initials	Weight Set SN