BASIC DRUG IDENTIFICATION/CONFIRMATION BY GAS CHROMATOGRAPHY - MASS SPECTROMETRY/NITROGEN PHOSPHORUS DETECTION

14.1 METHOD

This test method may be used to identify and/or confirm the presence of select basic drugs in biological samples (see APPENDIX B). The targeted compounds and metycaine internal standard are isolated from biological matrices by the use of liquid-liquid extraction (LLE). Following LLE, the extracts are injected into a gas chromatograph (GC) coupled with both a nitrogen phosphorus detector (NPD) and a mass spectrometer (MS) detector equipped with an electron ionization source.

This test method may also be used to identify and/or confirm other basic drugs not included in the standard mixes described for this procedure (see 14.9), provided a traceable standard for the target compound is included in the contemporaneous batch.

14.2 SPECIMENS

The specimen volume is 1 mL. Specimens include, but are not limited to, whole blood, serum, plasma, urine, and tissue homogenate. Dilutions of specimens may be analyzed at the Forensic Scientist's discretion.

Matrix-matching of the full calibration curve and all positive control levels is required for quantitation in liver (tissue) homogenate and serum/plasma specimens (see 14.4.3.2). For analysis of urine specimens, refer to Appendix A.

14.3 REAGENTS, MATERIALS AND EQUIPMENT

14.3.1 REAGENTS

- Acetonitrile (ACN)
- Ammonium carbonate ($(NH_4)_2CO_3$), saturated
- Ammonium hydroxide (NH_4OH), concentrated
- N-butyl chloride
- Certified blank blood and/or other biological matrices
- Chloroform ($CHCl_3$)
- Deionized water (DI H_2O)
- Ethyl acetate (EtAC)
- Hydrochloric acid (HCl), concentrated
- 3N HCl

  Add 125 mL concentrated hydrochloric acid to 300mL DI H_2O in a glass flask. Dilute to 500 mL with DI H_2O. Store the acid in a glass bottle at room temperature for up to one year.

- Methanol (MeOH)
- Sodium borate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$)
- 0.13M sodium borate solution (saturated)

  In a glass flask, dissolve 4.9 g $Na_2B_4O_7 \cdot 10H_2O$ in approximately 75 mL DI H_2O. Dilute to 100mL with DI H_2O and mix thoroughly (may require low heating). The weighed contents may not go completely into solution. This is normal. Store the solution in a glass bottle at room temperature for up to six months.

NOTE: Adjustments to final volumes of prepared reagents are permitted as long as the proportions are maintained.

14.3.2 MATERIALS

- Disposable extraction tubes (16 x 100mm recommended) and screw-cap or centrifuge tubes with closures
- Disposable transfer pipettes
- GC Column (Agilent HP-5MS; 30 m x 0.250 mm i.d. x 0.250 µm film thickness, or equivalent)
- Glass autosampler vials with inserts and caps
- Laboratory glassware (graduated cylinders, flasks)

14.3.3 EQUIPMENT

- Agilent GC (6890 or equivalent) equipped with an NPD detector
- Agilent MS (5973 or equivalent)
- Calibrated, adjustable piston pipettes and verified, adjustable repeater-pipette with disposable pipette tips
- General-use equipment (centrifuge, rotary mixer, vacuum aspirator, vortex mixer)

14.4 STANDARDS, CALIBRATORS AND CONTROLS

14.4.1 STANDARDS

- Working standard (Group A): 10 ng/µL
- Working control standard (Group A): 10 ng/µL
- Working standard (Group B): 10 ng/µL*
- Working internal standard: 10 ng/µL
- Working standard – cocaine and metabolites 10 ng/µL
- Working standard – methadone 10 ng/µL

*alprazolam – 15 ng/µL, amitriptyline, nortriptyline, fluoxetine, verapamil – 20 ng/µL, trazodone – 33.3 ng/µL

NOTE: Metycaine HCl internal standard is purchased as a solid reference material and weighed at time of working internal standard preparation. Standards used in qualitative confirmation (retention time/diagnostic ion references) or SIM quantitation may include prepared standards (e.g., methadone WS, lidocaine WS) or direct dilutions from a certified reference material or reference material.

14.4.2 CALIBRATORS

For quantitative testing, calibrators are prepared in certified blank blood at the time of analysis, as detailed in 14.5 SAMPLE PREPARATION. Quantitation in liver (tissue) homogenate or serum/plasma specimens requires that a calibration curve be prepared in blank alternative matrix. If testing only an alternate matrix, a whole blood calibration curve is not required.

14.4.3 CONTROLS

14.4.3.1 For quantitative analysis, at least one negative whole blood control and two positive whole blood controls are tested with every batch, prepared as described in 14.5. If testing only an alternate matrix, whole blood controls are not required. For quantitative analysis of alternate matrices, matrix-matching of the full calibration curve and all positive control levels are required.

14.4.3.2 For qualitative screening analysis, all positive controls and one negative control must be included for each matrix type tested in the batch.

14.4.3.3 For qualitative confirmation analysis (Group A, Group B or other target compounds), one negative control and at least one positive control for the target compound must be included.

NOTE: If a batch is intended for qualitative confirmation of multiple compounds, a mix of positive controls may be used to bracket specimens. Where only one target compound is confirmed, two positive controls for that compound are necessary, in order to bracket specimens.

14.4.3.4 For both quantitative and qualitative analysis, controls (positive or negative) must make up at least 10% of the extracted batch (based on number of case specimen samples), with case specimens bracketed by positive controls. When the batch contains more than 20 specimens, a positive control (Group A, Group B or cocaine/ methadone) must be analyzed mid-run.

NOTE: When quantifying compounds in multiple matrices, controls must make up at least 10% of the extracted batch for each alternate matrix.

14.5 SAMPLE PREPARATION

14.5.1 Label a clean extraction tube for each member of the test batch. (i.e., calibrator, control, case sample).

14.5.2 Add 1 mL 0.13M sodium borate solution to each tube.

14.5.3 Using a calibrated pipette, add 1 mL of certified blank whole blood into each calibrator tube, the positive control tubes and the negative control tube(s).

14.5.4 If performing quantitative testing, prepare a 1:10 dilution of the Group A working standard. (1 ng/µL)

a. Using a calibrated pipette, combine 0.1 mL of the working standard with 0.9 mL of ACN or MeOH in a labeled tube.
b. Cap and vortex mix. This dilution shall be disposed of after calibrator preparation.

14.5.5 If performing quantitative testing: Using a calibrated pipette, spike the calibrators according to the following table, using the Group A working standard and the prepared dilution.

Calibrator Description	Volume (µL) Added	Standard Concentration	Dilution of WS (or WS)
Calibrator 1 – 100 ng/mL	100	1 ng/µL	1:10
Calibrator 2 – 250 ng/mL	25	10 ng/µL	WS
Calibrator 3 - 500 ng/mL	50	10 ng/µL	WS
Calibrator 4 - 1000 ng/mL	100	10 ng/µL	WS

If screening only:

Using a calibrated pipette, add 30µL of the Group A working standard and 30 µL of the Group B working standard to the respective positive control tubes.

Add 15 µL of the methadone working standard to the methadone positive control tube (target concentration is 150 ng/mL).

Where applicable, 15 µL of the cocaine working standard may also be added to the methadone positive control tube (e.g., if the batch is expected to contain cocaine positive specimens, as directed by immunoassay results or case history).

14.5.6 If performing quantitative testing: Using a calibrated pipette, spike the positive controls according to the following table, using the Group A control working standard.

Control Description	Volume (µL) Added	Standard Concentration	Dilution of QC (or QC)
Control 1 – 200 mg/L	20	10 ng/µL	QC
Control 2 - 800 mg/L	80	10 ng/µL	QC

If screening only: The Group A and Group B calibrators prepared in 14.5.5 serve as the positive controls for the batch.

14.5.7 Using a calibrated pipette, sample 1 mL of each case sample into its respective tube.

14.5.8 Using a calibrated pipette or verified repeater-pipette, add 50 µL of the working internal standard solution to each tube. Final concentration of the internal standard is 500 ng/mL.

14.5.9 Add 3 mL n-butyl chloride to each tube.

14.5.10 Cap the tubes and place on a rotary mixer for 20 minutes.

14.5.11 Centrifuge the tubes at 3500 rpm (recommended for 16 x 100 mm tubes) for 10 minutes.

14.5.12 Transfer the organic layer to clean, labeled 10 mL centrifuge or screw-cap tubes.

14.5.13 Add 200 µL 3N HCl to each tube.

14.5.14 Cap the tubes and place on a rotary mixer for 5 minutes.

14.5.15 Centrifuge the tubes for 5 minutes at 2000-2500 rpm.

14.5.16 Aspirate the organic layer to chemical waste.

14.5.17 Add 100 µL saturated ammonium carbonate to each tube.

14.5.18 Add 100 µL concentrated ammonium hydroxide to each tube and vortex-mix.

14.5.19 Add 150 µL chloroform to each tube and vortex mix for at least 30 seconds.

14.5.20 Cap tubes and centrifuge for 5 minutes at 2000 rpm.

14.5.21 Transfer the bottom (chloroform) layer to glass autosampler vials with inserts and cap.

14.6 INSTRUMENTAL PARAMETERS/DATA ANALYSIS

14.6.1 Group A (quantitative, scan)

- Acquisition method – BASIC (instrumental parameters in Appendix C)
- Calibration curve – linear, 1/a weighting factor, origin excluded
- Updating calibrator (retention times ±2%) – Cal 3
- Result comparisons – truncated whole integer values in units of ng/mL

14.6.2 Group A and B (qualitative, scan)

Same as 14.6.1 above, but using Group A and Group B positive controls to create two-point (positive control, origin) calibration curves (linear, 1/a weighting factor). Two-point calibration curves may also be created to obtain estimated concentrations when performing qualitative analysis of other compounds (e.g., methadone, cocaine/ cocaethylene).

14.6.3 Batch and case specimen acceptance criteria are found in the *General Requirements for Chromatographic Test Method Batch Analysis and Acceptance* (PQ12707), sections 7.3 and 7.4.

14.7 REPORTING

Quantitative results (Group A scan and Group A/other compounds SIM) are reported in units of milligrams per liter (mg/L), truncated to two significant figures. Group A quantitative results are reported from NPD data; however, quantitation based on MSD data is allowable if the reason is documented and approved. Qualitative results are reported as positive.

See PQ12707, section 7.4.4, for specific criteria for reporting qualitative and quantitative results in specimens.

14.8 METHOD PERFORMANCE

14.8.1 Group A (quantitative, scan) – tramadol, dextromethorphan, diphenhydramine

- Lower limit of quantitation: 0.1 mg/L
- Dynamic range: 0.1 – 1.0 mg/L
- Upper limit of quantitation: 1.0 mg/L

14.8.2 Group A (tramadol, dextromethorphan, diphenhydramine), lidocaine and methadone (quantitative, SIM)

Dynamic range listed in 14.9 below, with lowest and highest calibrator levels serving as the lower and upper limits of quantitation.

14.9 SIM ANALYSIS

14.9.1 Confirmation/quantitation of Group A compounds tramadol, dextromethorphan and diphenhydramine, and of lidocaine and methadone, may be performed in selected-ion mode (SIM), using the sample preparation procedure outlined in 14.5. Calibrator, positive control and internal standard concentrations, diagnostic ions and updating calibrators are listed below (bupropion, citalopram and venlafaxine ions included for qualitative identification).

Group A SIM

Calibrator Description	Volume (µL) Added	Standard Concentration	Dilution of WS (or WS)
Calibrator 1 – 50 ng/mL	50	1 ng/µL	1:10
Calibrator 2 – 100 ng/mL	100	1 ng/µL	1:10
Calibrator 3 – 150 ng/mL	150	1 ng/µL	1:10
Calibrator 4 - 200 ng/mL	20	10 ng/µL	WS
Calibrator 5 - 250 ng/mL	25	10 ng/µL	WS

Control Description	Volume (µL) Added	Standard Concentration	Dilution of QC (or QC)
Control 1 – 75 ng/mL	75	1 ng/µL	1:10
Control 2 - 200 ng/mL	20	10 ng/µL	QC

Internal Standard: Metycaine (200 ng/mL target), add 20 µL working IS
Diagnostic Ions (qual/target): Metycaine (246/112), bupropion (224/100, 139/100), citalopram (324/58, 238/58), dextromethorphan (150/271, 214/271), diphenhydramine (165/58, 73/58), tramadol (263/58, 135/58), venlafaxine (58/134, 179/134)
Updating Calibrator: Cal 3

LIDOCAINE SIM

Calibrator Description	Volume (µL) Added	Standard Concentration	Dilution of WS (or WS)
Calibrator 1 – 100 ng/mL	100	1 ng/µL	1:10
Calibrator 2 – 250 ng/mL	25	10 ng/µL	WS
Calibrator 3 – 500 ng/mL	50	10 ng/µL	WS
Calibrator 4 - 750 ng/mL	75	10 ng/µL	WS
Calibrator 5 - 1000 ng/mL	100	10 ng/µL	WS

Control Description	Volume (µL) Added	Standard Concentration	Dilution of QC (or QC)
Control 1 – 200 ng/mL	20	10 ng/µL	QC
Control 2 - 800 ng/mL	80	10 ng/µL	QC

Internal Standard: Metycaine (500 ng/mL target), add 50 µL working IS
Diagnostic Ions: Metycaine (246/112), lidocaine (120/86, 234/86)
Updating Calibrator: Cal 3

Methadone SIM

Calibrator Description	Volume (µL) Added	Standard Concentration	Dilution of WS (or WS)
Calibrator 1 – 25 ng/mL	25	1 ng/µL	1:10
Calibrator 2 – 50 ng/mL	50	1 ng/µL	1:10
Calibrator 3 – 100 ng/mL	100	1 ng/µL	1:10
Calibrator 4 - 250 ng/mL	25	10 ng/µL	WS
Calibrator 5 - 500 ng/mL	50	10 ng/µL	WS
Calibrator 6 – 1000 ng/mL	100	10 ng/µL	WS

Control Description	Volume (µL) Added	Standard Concentration	Dilution of QC (or QC)
Control 1 – 75 ng/mL	75	1 ng/µL	1:10
Control 2 - 800 ng/mL	80	10 ng/µL	QC

Internal Standard: Metycaine (500 ng/mL target), 50 µL working IS
Diagnostic Ions: Metycaine (246/112), Methadone (294/72, 223/72)
Updating Calibrator: Cal 4

14.9.2 Calibration curves, and result comparisons will be as described in 14.6.1. Retention times (±2% tolerance) and qualifier ion ratios (±20% tolerance) will be updated with the listed calibrator (14.9.1). A copy of the acquisition method will be included in the batch file.

APPENDIX A

Urine Matrix Basic Drug Screen - Instrument Analysis

- Analyze urine specimens in a batch separate from other matrices.
- Limit number of case specimens in the batch to 15.
- Empty syringe wash bottles and rinse thoroughly before refilling; check syringes for smooth movement.
- Run a solvent blank injection prior to the first case specimen and following each case specimen and control.
- Use a different vial for each solvent blank injection.
- When performing data analysis for the batch:
    - Do not use the QDel function to delete any compounds in matrix blanks, solvent blanks or negative controls
    - Generate data reports for all solvent blank injections. Blanks are filed with the case specimen they precede (see QA Principles 4.2.8.4).
    - Identify specimens with high concentrations (e.g., ≥ 1 mg/L, peak saturation) and evaluate subsequent solvent blanks and case specimens for possible carryover.
- Should carryover be suspected, refer to QA Principles section 4.11.
- Compounds identified in urine specimens using the basic drug screen test method must be confirmed using a different test method in order to report. For urine specimens with tramadol identified in the basic drug screen, the basic drug quantitation (by NPD or GC-MS SIM) may not be used as the confirmation analysis (must be performed by an external laboratory).

APPENDIX B

Group A – Screening control: positive control target 300 ng/mL

Bupropion
Citalopram
Dextromethorphan
Diphenhydramine
Tramadol
Venlafaxine

Group B – Screening control: positive control target 300 ng/mL**

Alprazolam
Amitriptyline
Chlorpheniramine
Chlorpromazine
Clonidine
Cocaethylene
Cocaine
Codeine
Cyclobenzaprine
Doxepin
Doxylamine
Fentanyl
Fluoxetine
Hydrocodone
Lidocaine
MDA
MDMA
Meperidine
Methamphetamine
Nordiazepam
Nortriptyline
Oxycodone
Propoxyphene
Sertraline
Trazodone
Verapamil
Zolpidem

**fluoxetine, amitriptyline, nortriptyline, verapamil – 600 ng/mL, alprazolam – 450 ng/mL, trazodone – 1000 ng/mL

Methadone (cocaine/cocaethylene) - Screening control: positive control target 150 ng/mL

APPENDIX C
BASIC TEST METHOD INSTRUMENTAL PARAMETERS

GAS CHROMATOGRAPH

Split/Splitless Inlet	
Mode	Pulsed Splitless
Inlet Liner	4mm gooseneck w/glass wool plug
Temperature	250˚C
Pulse Pressure	45.0 psi
Pulse Time	1.00 min
Purge Flow	3.0 mL/min
Purge Time	0.00 min
Autosampler	
Gas Type	Helium
Injection Volume	3.0 µL
Solvent Wash A	Ethyl acetate
Solvent Wash B	Ethyl acetate
Pre-injection Wash	4
Post-injection Wash	4
Sample Pumps	2

Oven/Column	
Carrier Gas Mode	Constant Flow
Carrier Gas Flow	1.5 mL/min
Initial Temperature	90˚C
Initial Time	1.00 min
Ramp Rate	15.00˚C/min
Final Temperature	180˚C
Final Time	0.00 min
Ramp Rate	10.00˚C/min
Final Temperature	300˚C
Final Time	10.00 min
Front Detector/NPD	
Temperature	320˚C
H_2 Flow	3.0 mL/min
Air Flow	50.0 mL/min
N_2 Flow (Makeup)	15.0 mL/min

MASS SPECTROMETER

Solvent Delay	3.00 min	MS Quad Temperature	150°C
EM Offset	200	MS Source Temperature	230°C
Mode	Scan	Scan Range	40-550
Transfer Line Temperature	280˚C		

LIST OF CHANGES

Revision Date	Description	Page Number
10/03/12	Method approved by Washington State Toxicologist. See DRA dated 09/28/12. Method released for use in evidentiary testing on 10/03/12.	All
04/22/13	References to “Group B positive control “ throughout were changed to “Group B calibrator.” Spiked positive controls were introduced in place of prepared, pooled, whole blood controls. See detailed changes in DRA dated 04/12/13.	3-10
10/28/13	Removed ketamine and methadone from Group A Working Standard and the list of compounds in 14.6.1.2. Added chlorpromazine to list of compounds in Group B Working Standard in 14.6.1.2. See detailed changes in DRA dated 10/17/13.	3-4
3/16/15	Amended wording for deviation approval by a member of TLD Management in 14.1. Reagent and sample preparation sections modified to reflect use of 0.13M sodium borate solution instead of pH9 phosphate buffer. See DRA dated 3/11/15.	1, 2, 6
9/30/16	Added note regarding CRM expiration dates in 14.6.1.3 and 14.6.1.4 and clarification to 14.6.3.2.c for use of same CRM in preparation of working standard and working control standard. Edited 14.12.1.3 to reflect that only two significant figures are used for reporting and added “Printed Copies are Uncontrolled” to the footer. Other minor edits throughout.	All
4/9/18	Removed policy, purpose and principle sections, summarizing under new section METHOD. Added specific wording regarding matrix-matching in 14.2 SPECIMENS. Specified use of calibrated pipettes for measurement of blank blood, specimens, and standards throughout section 14.5 SAMPLE PREPARATION. Edited STANDARDS section - this information is now included in APPENDIX A and in the revised Standard Solution Preparation procedure. Criteria for batch acceptance (calibrators, controls) and specimen acceptability criteria, and specific data analysis and reporting information are now included in the General Requirements for Chromatographic Test Method Batch Analysis and Acceptance. Section 14.9 details SIM analysis for Group A compounds, lidocaine and methadone. Test method parameters for BASIC moved to APPENDIX B. Formatting and minor edits throughout.	All
7/9/18	Admin correction - units in 14.9.1 control description tables corrected from mg/L to ng/mL. AB	6-7
7/5/22	Specified use of certified reference material for identification/ confirmation of drugs not included in standard mixes in 14.1. Added	1-3, 5-6, 9

	cocaine and methadone working standards to 14.4.1 and updated positive control information in 14.4.3, including all controls must be included for each matrix in the batch. Updated information in 14.8 and 14.9.1 to clarify use of method for tramadol, dextromethorphan and diphenhydramine quantitation, incorporating process change detailed in IOC dated 6/13/19. Added specific procedures for instrument analysis and data processing for urine specimens in Appendix A (referenced in 14.2). Compounds list moved to Appendix B and instrument parameters moved to Appendix C.	