ANALYSIS OF BIOLOGICAL SPECIMENS FOR THE PRESENCE OF ETHYLENE GLYCOL BY GAS CHROMATOGRAPHY – MASS SPECTROMETRY

23.1 POLICY

This test method may be used to qualitatively identify ethylene glycol in biological specimens. Reporting of results following the application of this method will be contingent upon a thorough review and acceptance of quality control data and the qualification of individual results under the criteria for acceptance.

Any adjustments or deviations from the procedures below must be approved by a member of TLD Management, and appropriately documented in the batch file.

23.2 PURPOSE

The purpose of this standard operating procedure (SOP) is to provide technical direction for the analysis of biological specimens for the presence of ethylene glycol. This procedure will serve as the laboratory document describing sample preparation, criteria for acceptance and reporting of the specified compound.

23.3 PRINCIPLE

The target compound, ethylene glycol, and internal standard (1, 2-butanediol) are isolated from biological specimens using the liquid/liquid extraction procedure described below. Following derivatization with n-butylboronic acid, the specimens, now termed extracts are injected into a GC where they are separated between a gaseous mobile and liquid stationary phase. Each compound exits the GC at a reproducible time which is termed its retention time.

The GC is coupled to a mass spectrometer (MS) detector equipped with an electron ionization source. As each compound is ionized in the source, selected-ion-monitoring is used to measure the mass-to-charge ratios of each compound and its related fragments.

23.4 SPECIMENS

23.4.1 The specimen volume is 2 mL.

23.4.2 Acceptable specimens include whole blood, serum, plasma or tissue homogenate.

NOTE: Urine is not a suitable sample due to the fact that less than 1% of ethylene glycol is excreted unchanged in the urine.

23.4.3 Dilutions of specimens may be analyzed at the Forensic Scientist's discretion; however, this should be done in addition to testing the standard specimen volume, unless sample quantity dictates otherwise.

23.4.4 Analysis of larger specimen volumes must be approved and documented.

23.5 REAGENTS, MATERIALS AND EQUIPMENT

22.5.1 REAGENTS

23.5.1.1 Acetone

23.5.1.2 1, 2-butanediol

23.5.1.3 Blank blood

NOTE: As this assay is performed infrequently, and ethylene glycol is not detected by any other methods used in the laboratory, blood may not be previously certified as negative for ethylene glycol. However, it is highly unlikely the sources of blank blood used in the laboratory would contain the target compound.

23.5.1.4 Butylboronic acid (1-butaneboronic acid)

23.5.1.5 0.1% n-butylboronic acid in acetone

Add 0.1 g n-butylboronic acid to a 100 mL volumetric flask. Fill to the appropriate volume with acetone. Use on date of preparation only. Volumes may be adjusted provided that the proportions remain constant.

23.5.1.6 Deionized water (DI H_2O)

23.5.1.7 Ethylene glycol

23.5.1.8 Hydrochloric acid (HCl), concentrated 12N

23.5.1.9 Methylene chloride (CH_2Cl_2)

23.5.1.10 Potassium hydroxide (KOH)

23.5.1.11 0.1M Potassium Hydroxide

Add approximately 400mL DI H_2O to a 500 mL volumetric flask. Add 2.8 g KOH to the flask and stir to dissolve. Dilute with DI H_2O to a final volume of 500 mL. Store the solution at room temperature in a plastic bottle for up to 1 year. Volumes may be adjusted provided that the proportions remain constant.

23.5.1.12 Sodium sulfate (Na_2SO_4), anhydrous

23.5.2 MATERIALS

23.5.2.1 Autosampler vials, inserts and caps

23.5.2.2 Disposable 16 x 150mm tubes with closures

23.5.2.3 Disposable screw-cap tubes or conical centrifuge tubes with closures

23.5.2.4 Disposable pipette tips

23.5.2.5 GC column (Agilent HP-5; 30 m x 0.250 mm i.d. x 0.25 μm film thickness, or equivalent)

23.5.2.6 Glass serological and Pasteur transfer pipettes

23.5.2.7 Laboratory glassware (graduated cylinders, flasks)

23.5.2.8 Volumetric glassware (flasks)

23.5.3 EQUIPMENT

23.5.3.1 Agilent GC (6890 or equivalent)

23.5.3.2 Agilent MS (5973 or equivalent)

23.5.3.3 Calibrated, adjustable piston pipettes

23.5.3.4 Centrifuge

23.5.3.5 Evaporator (Caliper LS, formerly Zymark, TurboVap)

23.5.3.6 Rotary mixer

23.5.3.7 Vacuum aspirator

23.5.3.8 Vortex mixer

23.6 STANDARDS, CALIBRATORS AND CONTROLS

23.6.1 STANDARDS

23.6.1.1 Stock standard (50 mg/mL)

a. Using a calibrated pipette, measure 11.23 mL ethylene glycol (density @ 20°C = 1.115 g/mL) into a 250 mL class-A volumetric flask.
b. Add DI H_2O to the flask to the designated volume.
c. The final concentration of the stock standard is 50 mg/mL. The stock standard is made fresh with each analysis.
d. The lot number of ethylene glycol used is recorded in the batch paperwork for traceability.

23.6.1.2 Stock internal standard (1, 2-butanediol, 10 mg/mL)

a. Using a calibrated balance, weigh 1.0 g 1, 2-butanediol and transfer to a 100 mL class-A volumetric flask.
b. Add DI H_2O to the flask to the designated volume.
c. The final concentration of the stock standard is 10 mg/mL. The stock standard is made fresh with each analysis.
d. The lot number of 1, 2-butanediol used is recorded in the batch paperwork for traceability.

23.6.2 CALIBRATORS

23.6.2.1 Calibrators are prepared in blank blood at the time of analysis using the stock standard. The preparation of the calibrators is detailed in 23.7 SAMPLE PREPARATION. If necessary, calibrators may be prepared in alternate matrices (see note in 23.5.1.3).

23.6.3 CONTROLS

23.6.3.1 Negative Control

a. At least one negative control is tested with every batch. The negative control is prepared using blank blood.

b. When testing different sample types, wherever possible, include a negative control prepared from that matrix.

23.6.3.2 Positive Controls

No positive controls are included with the batch. The calibrators serve as the necessary retention time and ion ratio references for identification of ethylene glycol.

23.7 SAMPLE PREPARATION

23.7.1 Label a clean 16 x 150mm tube for each of the five calibrators, negative control, and case samples.

23.7.2 Pipette 2 mL blank blood into the appropriately labeled tubes for the calibrators and negative control.

23.7.3 Prepare a 1:10 dilution of the stock standard. (5.0 mg/mL)

a. Using a calibrated pipette, combine 0.1 mL of the stock ethylene glycol standard with 0.9 mL of methanol or acetonitrile in a labeled tube.
b. Cap and vortex mix. This dilution shall be disposed of after calibrator preparation.

23.7.4 Using the stock standard and prepared dilution, spike the calibrators according to the following table:

Calibrator Description	Volume (µL) Added	Standard Concentration (mg/mL)
Calibrator 1 (0.25 mg/mL)	100	5.0 mg/mL
Calibrator 2 (0.625 mg/mL)	25	50 mg/mL
Calibrator 3 (1.25 mg/mL)	50	50 mg/mL
Calibrator 4 (2.5 mg/mL)	100	50 mg/mL
Calibrator 5 (5.0 mg/mL)	200	50 mg/mL

23.7.5 Sample 2 mL of each case sample into its respective tube.

23.7.6 Add 50 µL of 1, 2-butanediol stock internal standard solution (10 mg/mL) to each tube and vortex mix. The final concentration of the internal standard is 0.25 mg/mL.

23.7.7 Add 10 mL of 0.1% n-butylboronic acid in acetone to each tube.

23.7.8 Cap the tubes and place on a rotary mixer for 10 minutes.

23.7.9 Centrifuge for 5 minutes at 2000 rpm to achieve separation.

23.7.10 Pour the supernatant into clean, labeled, conical centrifuge or screw-cap tubes.

23.7.11 Evaporate the extracts under a slow stream of air at 35°C, to approximately 3 mL volume.

23.7.12 Add 3 mL of 0.1M KOH and 5 mL CH_2Cl_2 to each tube. Cap the tubes, and vortex mix.

23.7.13 Centrifuge the tubes for 5 minutes at 2000 rpm to achieve separation.

23.7.14 Transfer the top (aqueous) layer into clean, labeled conical centrifuge or screw-cap tubes.

23.7.15 Using a glass Pasteur pipette, add 5 drops concentrated HCl to each tube.

23.7.16 Add 0.5 mL CH_2Cl_2 to each tube.

23.7.17 Cap the tubes and place on a rotary mixer for 10 minutes.

23.7.18 Centrifuge the tubes for 5 minutes at 2000 rpm to achieve separation.

23.7.19 Aspirate the top (aqueous) layer into chemical waste.

23.7.20 Add approximately 0.5 g Na_2SO_4 to each tube and vortex mix.

23.7.21 Cap and centrifuge the tubes for 5 minutes at 2000 rpm.

23.7.22 Transfer approximately 100µL of the extracts to labeled autosampler vials with inserts and cap.

23.8 INSTRUMENTAL PARAMETERS

The instrumental parameters can be found in Appendix A. Prepare a sequence table by first setting the data path in ChemStation to the date of the test. After entering all vial locations, sample descriptions, comments and/or lot numbers in the sequence table, ensure the method listing in the table is EGLYSIM.M for each line.

23.9 DATA ANALYSIS

23.9.1 Analysis of the batch data is conducted using the instrumental data analysis software in ChemStation.

23.9.2 A calibration curve is generated by internal standard, multi-point, linear regression with 1/a (inverse) weighting. The calibration curve is updated using the calibrator results for the batch; no historical calibration curves are permitted.

23.9.3 Printed reports for each vial in the batch are generated for review.

23.9.4 Technical review of the batch is conducted according to the criteria listed below.

23.10 CRITERIA FOR BATCH ACCEPTANCE

If the analysis of the batch meets the criteria listed below, the results for the specimens are accepted.

23.10.1 Calibrators and calibration curves

23.10.1.1 Chromatographic peaks for ethylene glycol and 1, 2-butanediol internal standard shall appear symmetrical (i.e. no co-elution, split peaks, or shoulders).

23.10.1.2 Retention times shall be within ±2% and ion ratios shall be within 20% of those in calibrator 4. These are inclusive ranges.

23.10.1.3 Quantitative results for ethylene glycol in each calibrator shall be within ±20% of their target values with the exception of calibrator 1 which shall be within ±25% of their targets. These are inclusive ranges. Result comparisons will use values truncated after the first decimal place in units of mg/mL.

NOTE: As this is a qualitative test, calibrator levels that do not meet the above criteria may be omitted from the curve. The concentration of the lowest calibrator in the batch that meets criteria listed above then becomes the LOD for that batch.

23.10.1.4 The calibration curves for target compounds shall have correlation coefficients ≥0.98.

NOTE: While the amount of ethylene glycol in a specimen is not quantified from the calibration curve, the curve is used to determine the concentration of the calibrators, which may then factor in the determination of the LOD for the batch.

23.10.2 Controls

23.10.2.1 The negative control shall not identify any target compound. Identification is based on a) acceptable retention time matching, b) distinct peaks present for all selected ions, and c) acceptable ion ratios.

23.11 CRITERIA FOR CASE SAMPLE ACCEPTANCE

If the criteria for batch acceptance have been satisfied, the results of individual case samples are deemed suitable for reporting if the following criteria are met.

23.11.1 Any chromatographic peaks for ethylene glycol and the internal standard shall appear symmetrical.

23.11.2 The retention times are ±2% and the ion ratios are within ±20% of those in calibrator 4. These are inclusive ranges.

23.12 REPORTING

23.12.1 Ethylene glycol is reported as qualitative (positive) only from this assay.

23.12.2 For qualitative reporting, any ethylene glycol initially identified using this method, must be confirmed on a separate sampling, using this or another test method.

NOTE: Extracts from this assay may also be run in full-scan acquisition mode to acquire a mass spectral match for each case, for comparison to a full-scan mass spectrum of a calibrator, or to mass spectrum from an approved reference database.

23.13 METHOD PERFORMANCE

23.13.1 Limit of detection: 0.25 mg/mL (or lowest acceptable calibrator level, see note in 23.10.1.3)

23.14 TRACEABILITY

23.14.1 Lot traceability (qualitative) of ethylene glycol and internal standard is provided through the certificates of analysis from the supplier(s).

APPENDIX A
INSTRUMENTAL PARAMETERS

GAS CHROMATOGRAPH

Split/splitless Inlet	
Mode	Split
Inlet liner	4 mm splitless w/glass wool plug
Split Ratio	30:1
Temperature	250°C
Gas Type	Helium
Gas Saver	On
Gas Saver Flow	15.0 mL/min
Gas Saver Time	2.0 min

Autosampler	
Injection volume	2.0 µL
Solvent Wash A	10 (Methanol)
Solvent Wash B	10 (Methanol)
Solvent Pumps	2

Oven	
Isothermic	90°C
Run time	10.0 min
Column	
Carrier Gas Mode	Ramped Flow
Initial Gas Flow	1.0 ml/min
Gas Ramp Rate 1	8.0 mL/min
Gas Flow	5.0 mL/min
Hold	0.0 min
Gas Ramp Rate 2	8 mL/min
Gas Flow	1.2 mL/min
Hold time	10.0 min

MASS SPECTROMETER

Solvent Delay	0.50 min	MS Quad Temperature	150°C
EM Offset	Set in tune	MS Source Temperature	230°C
Resolution	Low	Dwell Time	100 msec
Signals	Ions	Ion Ratios	
Ethylene Glycol	86, 99, 113	86/99, 86/113	
1, 2-butanediol Internal Std	126,127	126/127	

LIST OF CHANGES

Revision Date	Description	Page Number
07/08/13	Method approved by Washington State Toxicologist. See DRA dated 7/08/13. Method released for use in evidentiary testing on 7/08/13.	All
10/01/15	Changed “screening” in title to “”analysis.” Changed wording throughout to reflect that the test method may be used for qualitative identification/confirmation of ethylene glycol. Removed reference to quantitative confirmation in 23.12.2 and updated traceability statement.	1, 3, 6-7

Removed from current customer scope. 8/2021