SOP 109 – Ethylene Glycol Screen
by Gas Chromatography/Mass Spectrometry

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Ethylene Glycol Screen by Gas Chromatography/Mass Spectrometry		**109**
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	4 – Added standards prep instructions 6 – Updated instrumentation 11.1 – Added Validation Table	MSF – 05/25/2017 MSF – 05/26/2017 & 06/05/2017 MSF – 12/07/2017

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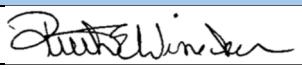
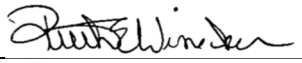
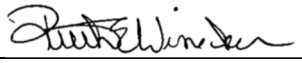
Ruth E. Winecker, Ph.D.		04/16/2015
Ruth E. Winecker, Ph.D		06/20/2016
Ruth E. Winecker, Ph.D		12/07/2017

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1. **Principle of Assay**

   1.1. Ethylene glycol is a common component of commercial anti-freeze preparations, automotive cooling systems, and hydraulic brake fluids. This, coupled with its sweet taste, results in its relatively common appearance in emergency departments and medical examiner laboratories.

   1.2. Ethylene glycol is completely miscible with water, and as such is extremely difficult to remove from blood and serum samples. This procedure describes a method which extracts the ethylene glycol present in blood or serum, such that it can be easily derivatized and identified by gas chromatography/mass spectrometry.

2. **Specimens**

   2.1. This procedure is applicable to serum, plasma, whole blood, vitreous humor, and urine specimens. A 100µL sample size is generally employed unless a dilution is required so that the calibration curve encompasses the expected range of the unknown specimens.

3. **Reagents and Materials (HPLC grade)**

   3.1. Ethylene Glycol

   3.2. 1,2-Butanediol

   3.3. Acetone

   3.4. Deionized water

   3.5. Methylene Chloride

   3.6. Phenylboronic acid

   3.7. 2,2-dimethoxypropane

4. Standards, Controls, and Solutions

4.1. Prepare Calibration, IS, and Control standards, as needed, according to SOP 10.

4.2. **1,2-Butanediol Internal Standard (100 mg/dL)**

4.2.1. Into a 10mL volumetric flask, add 0.05mL of 1,2-Butanediol with a micropipette.

4.2.2. Fill to the line with DiH20, insert stopper and invert three times to mix. Transfer to properly labeled 16x100mm screw topped test tubes and cap. Store in laboratory refrigerator (R1-2601). See SOP-010.

4.3. **Ethylene Glycol Stock Calibration Standard (10000mg/dL)**

4.3.1. Place a 10mL volumetric flask containing 1-2mL of DiH_20 onto a rough balance and tare.

4.3.2. Using a pasture pipette, transfer 1g Ethylene Glycol, dropwise, into the flask.

4.3.3. Fill to the line with DiH_20, insert stopper and invert three times to mix.

4.3.4. Transfer to properly labeled 16x100mm screw topped test tubes and cap. Store in laboratory refrigerator (R1-2601). See SOP-010.

4.4. **Ethylene Glycol Calibration Standard (Cal 2-6 - 100mg/dL)**

4.4.1. Into a 10mL volumetric flask, add 0.1 ml of Ethylene Glycol Stock Calibration Standard (10000mg/dL) with a class: A volumetric pipette.

4.4.2. Fill to the line with DiH_20, insert stopper and invert three times to mix. Transfer to properly labeled 16x100mm screw topped test tubes and cap. Store in laboratory refrigerator (R1-2601). See SOP-010.

4.5. **Ethylene Glycol Stock Control Standard (10000mg/dL)**

4.5.1. Place a 10mL volumetric flask containing 1-2mL of DiH_20 onto a rough balance and tare.

4.5.2. Using a pasture pipette, transfer 1g Ethylene Glycol, dropwise, into the flask.

4.5.3. Fill to the line with DiH_20, insert stopper and invert three times to mix.

4.5.4. Transfer to properly labeled 16x100mm screw topped test tubes and cap. Store in laboratory refrigerator (R1-2601). See SOP-010.

4.6. **Ethylene Glycol Control Standard (100mg/dL)**

4.6.1. Into a 10mL volumetric flask, add 0.1 ml of Ethylene Glycol Stock Control Standard (10000mg/dL) with a class: A volumetric pipette.

4.6.2. Fill to the line with DiH_20, insert stopper and invert three times to mix. Transfer to properly labeled 16x100mm screw topped test tubes and cap. Store in laboratory refrigerator (R1-2601). See SOP-010.

4.7. **1% Phenylboronic acid solution**

4.7.1. Weigh 1g Phenylboronic acid, transfer to 100 mL volumetric flask, and qs to volume with acetone. Store at 2-8ºC for up to 1 year.

4.8. **0.1% Phenylboronic acid solution**

4.8.1. Pipette 10 mL of 1% Phenylboronic acid solution into a 100 mL volumetric flask and QS to volume with acetone. Make fresh daily.

4.9. Phenylboronic Acid (5mg/mL) in 2,2-dimethoxypropane

4.9.1. To a 100 mL volumetric flask, add 0.500 grams of phenylboronic acid, and fill to the mark with 2,2-dimethoxypropane. PROTECT FROM MOISTURE.

5. Equipment and Special Supplies

5.1. Test tubes, 16x100 borosilicate glass
5.2. Micropipette – (10-100µL)
5.3. Pipette tips – (10-100µL)
5.4. Vortex mixer
5.5. Centrifuge (2000-3000 rpm)
5.6. GC autosampler vials
5.7. Nitrogen Evaporator
5.8. Pasteur Pipettes

6. Instrumentation

6.1. Agilent GC/MS – 6890/5973, Chemstation software, compatible computer and printer.

6.2. GCMS13 Instrument Method: "13ETHYLENEGLYCOL.M"
6.2.1. Click here for instrument parameters

6.3. GC/MS parameters (The instrument and conditions may be changed to permit improved performance).

6.3.1. Acquisition mode: SIM

6.3.2. Column: DB5-MS

6.3.3. MS transfer line temperature: 280º C

6.3.4. Oven ramp program:

6.3.4.1.	Equilibration time:	30 sec.
6.3.4.2.	Initial temp:	70º C
6.3.4.3.	Initial temp hold:	1.00 min.
6.3.4.4.	Ramp 1:	25º C/min.
6.3.4.5.	Ramp 1 final temp:	175º C
6.3.4.6.	Ramp 1 hold:	0.00 min.
6.3.4.7.	Ramp 2:	35º C/min.
6.3.4.8.	Ramp 2 final temp:	310º C
6.3.4.9.	Ramp 2 hold:	0.5 min.
6.3.4.10.	Total Run Time:	9.56 min.

6.3.5. Inlet Parameters

6.3.5.1.	Mode:	SPLIT
6.3.5.2.	Split ratio:	30:1
6.3.5.3.	Split vent:	51.0mL/min
6.3.5.4.	Temperature:	240º C
6.3.5.5.	Injection Volume:	2µL
6.3.5.6.	Purge time:	Constant
6.3.5.7.	Column Flow:	1.7mL/min (Constant Flow)

7. **Target Ions**

7.1. Major Ions for Ethylene glycol phenyl borate ester: **91**, 148, 118

7.2. Major Ions for 1,2-Butanediol phenyl borate ester: **147**, 91

8. **Procedure**

8.1. Label disposable 16x100 mm test tubes for each standard, blank, control and specimen.

8.2. Add 50µL of the 1,2-butanediol internal standard solution to all tubes.

8.3. Add the ethylene glycol standard solution and ethylene glycol quality control to appropriately labeled tubes.

8.4. Add 100 µL of negative blood bank blood to all standards, controls and blanks.

8.5. Add 100 µL of unknown specimen to appropriately labeled tubes.

8.6. Add 300 µL of acetone to each tube to deproteinate blood, vortex, and let sit for 3-5 minutes.

8.7. Centrifuge for 10 minutes at 2000-3000 x *g*.

8.8. Transfer 100 µL of supernatant to a clean, appropriately labeled 5 mL conical test tube.

8.9. Add 100 µL of 5µg/mL phenylboronic acid in 2,2-dimethoxypropane, and vortex.

8.10. Centrifuge for 5 minutes at 2000-3000 RPM.

8.11. Transfer 50 µL into a clean, labeled autosampler tube, and inject on the GC/MS. (Note: if two layers are formed in step 10, transfer only the top layer.)

9. **Calculations**

9.1. Calibration

9.1.1. A one point calibration curve is used.

9.2. Dilution Factor

9.2.1. D = Sample volume (curve) / Sample volume (unknown)

9.3. Multiplier for homogenates/dilutions and non-standard volumes

9.3.1. $M = (V_{curve} / V_{samp}) \times D$

9.3.1.1. M = Multiplier
9.3.1.2. D = dilution factor
9.3.1.3. V_{curve} = matrix volume of calibration curve
9.3.1.4. V_{samp} = matrix volume of specimen

9.4. Concentration

9.4.1. C = (A / V) * M

9.4.1.1. C = Concentration (ng/mL) of the analyte in the unknown case.

9.4.1.2. A = Amount of drug in sample
9.4.1.3. V = Volume of sample
9.4.1.4. M = Multiplier

9.5. Qualifier Ion Ratios

9.5.1. Ratio 1 = QL_1/QN

9.5.1.1. QL_1 = response of the quantifying product ion
9.5.1.2. QN = response of the qualifying product ion

10. **Quality Control**

10.1. For an analysis to be acceptable the following criteria must be met:

10.1.1. Chromatography must be acceptable with a symmetrical (Gaussian) shape. Each analyte of interest must have near baseline resolution from any other peaks in the chromatogram.

10.1.2. The retention time of each analyte should be within ±5% of the expected retention time based on the calibrators and the relative retention time to the internal standard.

10.1.3. The quality control samples shall have an analytical value as stated on the standard and control worksheet, and shall not deviate ±30% from the expected value.

10.1.4. The blank shall represent a specimen of "none detected" and should not contain analyte signal with appropriate ion ratios above 10% of the low standard.

10.1.5. The internal standard areas of the samples shall not deviate more than 50-200% from the average internal standard areas of the calibrators.

10.1.6. Calculated ion ratios shall not deviate more than 20% to that of the average ion ratios of the calibrators.

10.1.7. Results are reported qualitatively.

11. **Validation of Method**

Parameter	Result
Bias (all matrix - blood, urine, hd liver)	L: -10.86% H: -9.37%
Precision (all matrix - blood, urine, hd liver)	L: 2.21% H: 0.83%
Calibration model	Linear: Equal weighting
Carryover	No carryover observed following high calibrator.
Interference Studies	Not evaluated -
Ion Ratios	Ion ratios were noted to be concentration dependent. Ion ratio range for unknowns shall be determined using the closest calibrator concentration ion ratio(s) as the target.
Ionization/Suppression: (Not needed if IS coelutes within 0.05 min.)	N/A - IS coelutes with target analyte (0.1 min).
LOD (Determined experimentally)	1.35 mg/dL
LOQ (Set to lowest calibrator with acceptable Bias/Precision).	10 mg/dL
Dilution Integrity	Average % difference of diluted specimens is 3.51% - lowest: 0.44% Highest 6.63% (n=8)
Processed Sample Stability - (re-analyze after 8 days)	Not Determined
Recovery	Not Determined

11.1.

12. **Load Assignment Packet Preparation**

12.1. After completing all data generation and reviewing for corrections, the analyst will assimilate the data in the following order:

12.1.1. Load assignment sheets, followed by any additional notes to file pertaining to load.

12.1.2. Load specimen sheet.

12.1.3. Data summary

12.1.4. Chain of Custody.

12.1.5. Standard Sheet.

12.1.6. GC/MS Method and Calibration Report(s).

12.1.7. Running Sequences.

12.1.8. Chromatograms and results for calibrators, controls and specimens

12.1.9. The Load Checklist should be initialed and dated to acknowledge completion of load. The finished data package will be placed in the data review box.

13. **References**

13.1. Baselt, Randall C. *Disposition of Toxic Drugs and Chemicals in Man*. Foster City, CA: Chemical Toxicology Institute, 2000. 406-08. Print.