SOP 209 - Ethylene Glycol Quantitation by Gas Chromatography/Mass Spectrometry

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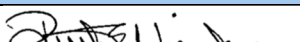
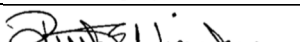
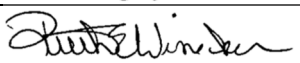
North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	**Revision:**	**Revision Date/Initials:**
	10.1.2 – Updated RT acceptance range 10.1.7 & 10.1.8 – Add calibrator acceptance criteria 11.1 – Added validation table 4 – Added standards prep instructions 6 – Updated Instrumentation and instrument parameters	MSF - 5/11/2015 MSF – 04/13/17 MSF – 5/25/2017 MSF – 06/05/2017

Approving Authority Name	Approving Authority Signature	Approval Date
Ruth E. Winecker, Ph.D.		04/08/2015
Ruth E. Winecker, Ph.D.		08/25/2016
Ruth E. Winecker, Ph.D.		12/07/2017

SOP 209 - Ethylene Glycol Quantitation by Gas Chromatography/Mass Spectrometry

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. From an aliquot of biological sample, ethylene glycol is derivatized with phenylboronic acid to form a boronate ester. Following a basic wash, the sample is made acidic with concentrated HCl and extracted with methylene chloride. The extract is injected onto a GC/MS for quantitation.

2. **Specimens**

    2.1. Approximately 0.1mL of blood, serum, vitreous, urine, or 0.2g tissue homogenate.

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

    3.1. Ethylene Glycol

    3.2. Ethylene Glycol-d6

    3.3. Acetone

    3.4. Deionized water

    3.5. Methylene Chloride

    3.6. Phenylboronic acid

    3.7. Potassium hydroxide

    3.8. Concentrated hydrochloric acid

    3.9. Sodium Sulfate, anhydrous

    3.10. 1% Phenylboronic acid solution

    3.11. 0.1% Phenylboronic acid solution

    3.12. 0.1N potassium hydroxide

4. **Standards, Controls, and Solutions**

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

4.2. **Ethylene Glycol-d6 Stock Solution (5000 mg/dL)**

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

4.2.2. Using a pasture pipette, transfer 0.5g Ethylene Glycol-d6, dropwise, into the flask.

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

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

4.3. **Ethylene Glycol-d6 Internal Standard (100 mg/dL)**

4.3.1. Into a 10mL volumetric flask, add 0.2mL of Ethylene Glycol-d6 Stock Solution (5000 mg/dL) with a micropipette.

4.3.2. 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.

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

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

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

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

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

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

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

4.5.2. 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.

4.6. **Ethylene Glycol Calibration Standard (Cal 1 - 10mg/dL)**

4.6.1. Into a 10mL volumetric flask, add 1 ml of Ethylene Glycol Calibration Standard (Cal 2-6 - 100mg/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. **Ethylene Glycol Stock Control Standard (10000mg/dL)**

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

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

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

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

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

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

4.8.2. 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.

4.9. **1% Phenylboronic acid solution**

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

4.10. **0.1% Phenylboronic acid solution**

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

4.11. **0.1N potassium hydroxide**

4.11.1. Weigh 2.8g potassium hydroxide, transfer to 500 mL volumetric flask and qs to volume with deionized H_2O. Store at room temperature for up to two years.

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 and Parameters**

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

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

6.3. Instrument method (GCMS13, GCMS15): "13_EG_QUANT", "15_EG_QUANT" – click here for instrument parameters.

6.4. Acquisition mode: SIM

6.5. Column: DB5-MS

6.6. MS transfer line temperature: 280º C

6.7. Oven ramp program:

6.7.1. Equilibration time: 30 sec.

6.7.2. Initial temp: 50º C

6.7.3. Initial temp hold: 1.00 min.

6.7.4. Ramp 1: 35º C/min.

6.7.5. Ramp 1 final temp: 310º C

6.7.6. Ramp 1 hold: 0.5 min.

6.7.7. Total Run Time: 8.93 min.

6.8. Inlet Parameters

6.8.1. Mode: SPLIT

6.8.2. Split ratio: 50:1

6.8.3. Split vent: 677mL/min

6.8.4. Temperature: 275º C

6.8.5. Injection Volume: 1µL

6.8.6. Purge time: Constant

6.8.7. Column Flow: 1.4mL/min (Constant Flow)

7. **Target Ions**

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

7.2. Major Ions for Ethylene Glycol-d6 phenyl borate ester: 93, 152

8. **Procedure**

8.1. Prepare 0.1% phenylboronic acid solution. (See Section 4. Standards, Controls, and Solutions)

8.2. Add 100µL Ethylene Glycol-d6 IS to the appropriate number of 16x125mm test tubes.

8.3. Prepare calibrators/controls according to Standard & Control Worksheet.

8.4. Add 0.1mL blank blood to all calibrators and controls.

8.5. Aliquot case samples in duplicate at 0.1mL and 0.05mL.

8.6. Add 0.5mL DiH_2O to each tube and vortex.

8.7. Add 3.0mL 0.1% phenylboronic acid solution to each tube and vortex for 30 seconds.

8.8. Centrifuge at 2000 x *g* for 10 minutes.

8.9. Transfer supernatants to clean 16x125mm test tubes. Discard bottom layer (blood pellet).

8.10. Evaporate supernatants to approximately 0.5mL in Turbo-Vap at 60ºC for 6-8 min. (The purpose is to remove acetone and leave aqueous portion).

8.11. Remove tubes from Turbo-Vap and add 1mL of 0.1N KOH to each tube and vortex for 10 seconds.

8.12. Add 2mL methylene chloride to each tube and vortex for 30 seconds.

8.13. Centrifuge at 2000 x *g* for 10 minutes.

8.14. Transfer upper (aqueous) layer to labeled 16x125mm tubes. Discard bottom organic layer into halogenated waste bottle.

8.14.1. Note: It is OK to transfer a small amount of methylene chloride in this step.

8.15. Acidify samples with concentrated HCl (1 drop per tube) and vortex.

8.16. Add 2mL methylene chloride to each tube and vortex for 30 seconds.

8.17. Centrifuge at 2000 x *g* for 10 minutes.

8.18. Aspirate upper aqueous layer to waste.

8.19. Dry bottom organic layer by adding ~100mg (2 small spatulas) of anhydrous sodium sulfate to each sample and vortex for 15 seconds.

8.20. Centrifuge at 2000 x *g* for 5 minutes.

8.21. Transfer ~60µL of remaining organic layer to GC autosampler vials for analysis.

9. **Calculations**

9.1. Calibration

9.1.1. A linear regression resulting from the 6 standards is used to quantitate the analytes in the case. The area of the analyte divided by the area of the internal standard is used in the resulting formula of the calibration curve.

9.2. Dilution Factor

9.2.1. D = Total volume/Sample volume

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. Max/Min

9.5.1. Percent Difference = $((R_h / R_l)-1) \times 100$

9.5.1.1. R_h = high result

9.5.1.2. R_l = low result

9.6. Average

9.6.1. Average = $(R_1 + R_2) / 2$

9.6.1.1. R_1 = first result

9.6.1.2. R_2 = second result

9.7. Qualifier Ion Ratios

9.7.1.1.1. Ratio 1 = QL_1/QN

9.7.2. QL_1 = response of the quantifying product ion

9.7.3. 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 ±2% 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 ±20% 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. Analytical curves must have a coefficient of determination (R^2) of 0.992 or greater.

10.1.8. Each calibrator, when calculated against the calibration curve, must not deviate outside ± 20% of the target value (± 25% at LOQ).

10.1.9. Results must fall within the linear range of the assay. Results above or below the linear range can only be reported as "less than" the low control or "greater than" the highest control.

10.1.10. Results are reported with two significant figures. All results will be truncated to the nearest significant figure.

11. Validation of Method

11.1.

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.**
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)**

12. **Reporting**

12.1. The percent difference of duplicate analysis for an analyte must be less than or equal to 25% (see Max/Min in Calculations section)

12.2. Reporting of duplicate analysis should be done according to the table below:

Reporting Duplicates

- **Dilution factors of 1 and 2 (or other)**

Dil / Scenario	1	2	REPORT
A	**In curve**	**In curve**	**Average**
B	**In curve**	**BQL**	**"In" value**
C	**AQL**	**In curve**	**"In" value**
D	**In curve**	**ND (should be in)**	**Repeat**
E	**AQL/BQL**	**AQL/BQL**	**Less than/Greater than**
F	**BQL**	**ND**	**ND**
G	**In curve**	**ND (should be BQL)**	**"In" value**

12.2.1.

12.2.1.1. In Curve = Measured concentration (pre-multiplier) falls within the calibration rang

12.2.1.2. AQL = Measured concentration (pre-multiplier) falls Above Quantitation Limit

12.2.1.3. BQL = Measured concentration (pre-multiplier) falls Below Quantitation Limit

12.2.1.4. ND = None Detected

12.3. Averaging reportable values

12.3.1. Results for duplicate analysis (both falling within calibration curve) shall be truncated prior to averaging.

12.3.2. Enter calculated concentration for each specimen into toxlog.

12.4. Significant figures

12.4.1. Concentrations are truncated and reported with two significant figures in mg/dL.

13. **Load Assignment Packet Preparation**

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

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

13.1.2. Load specimen sheet.

13.1.3. Data summary

13.1.4. Chain of Custody.

13.1.5. Standard Sheet.

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

13.1.7. Running Sequences.

13.1.8. Chromatograms and results for calibrators, controls and specimens

13.1.9. 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.

14. **References**

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