

Analysis of Ethylene Glycol from Blood

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1 INTRODUCTION

Ethylene glycol (EG) is a toxic glycol used in coolants and antifreezes. It causes central nervous system depression like that caused by ethanol. It is metabolized in the body to oxalic acid, which is damaging to the kidneys.

2 SCOPE

Analyses	<input checked="" type="checkbox"/> Screening <input checked="" type="checkbox"/> Confirmation <input checked="" type="checkbox"/> Quantitation
Matrices	Blood
Analytes	Ethylene Glycol (EG)
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.

3 PRINCIPLE

After addition of an internal standard, blood specimens are mixed with phenylboronic acid in acetone. A small amount of the acetone layer is added to a headspace vial and sealed. The vial is thermostatted to cause volatilization of the acetone and ethylene glycol derivative. The headspace is analyzed by HS-GC/MS(EI) (headspace gas chromatography mass spectrometry in electron ionization mode).

4 SPECIMEN CRITERIA

Blood (0.1 mL per analysis)

5 EQUIPMENT

5.1 Equipment

Item	Description
Vortex mixer	n/a
Centrifuge	10,000 rpm capable
Volumetric pipets with appropriate tips	0.09-1 mL range
Laboratory Balance	minimum \geq 100 g capacity and 0.1 mg or better resolution

5.2 Consumables

Item	Description
Volumetric pipets with appropriate tips	0.09-1 mL range
Eppendorf centrifuge tubes*	1-2 mL volume
Headspace vials with magnetic caps	10 mL volume

*use of an equivalent product is allowable

5.3 Instruments

Item	Description
GC/MS with Headspace Autosampler*	El ionization, Gerstel autosampler
GC Column*	Agilent DB-5 30 m x 0.25 mm x 0.25 µm film thickness or equivalent

*use of an equivalent product is allowable

5.4 Software

Component	Software	Version
Operating System	Microsoft Windows	XP Professional SP2
GC/MS	Xcalibur	2.0.7 SP1
	Enhanced Chemstation	E.02.00.493

5.5 Chemicals/Reagents

5.5.1 Purchased

Item	Supplier*	Description	Part Number*
Phenylboronic acid	Sigma-Aldrich	purum, ≥97.0% (HPLC)	78181-1G
Acetone	Fisher	Optima	A929-1
Methanol	Fisher	Optima	A454-1

*use of an equivalent product is allowable

5.5.2 Prepared

Depending upon the batch size, the absolute amounts may be adjusted so long as the ratios of components are maintained.

5.5.2.1 *Phenylboronic Acid Derivatizing Reagent (5 mg/mL)*

Step	Action	Amount	Component/Information
1	Acquire	1	Volumetric flask, 25 mL
2	Add	125 mg	Phenylboronic acid
3	QS	25 mL	Acetone
4	Mix		
5	Transfer		Amber glass
6	Storage		Refrigerated
7	Stability		≥ 1 month
8	Prepares	25 mL	(62 samples)

5.6 Standards/Controls

5.6.1 Purchased

5.6.1.1 Primary Standards

Analyte	Supplier*	Description	Part Number*
EG (Cal)	Sigma-Aldrich	Pharmaceutical standard, neat	PHR1046-1G
EG (Control)	LGC Standards	≥ 99%, neat	DRE-C13327000
EG-d ₄	Sigma-Aldrich (Isotec)	≥ 98%, neat	347442-1G

*Use of an equivalent product is allowable. Store refrigerated. Stability determined by manufacturer.

5.6.2 Prepared

5.6.2.1 EG Stock Standards (10 mg/mL)*

Step	Action	Amount	Component/Information
1	Acquire	1	volumetric flask, glass, 10 mL. Tare on laboratory balance.
2	Add	100 mg	EG
3	QS	10 mL	methanol
4	Mix		
5	Transfer		Amber glass
6	Storage		about 5°C
7	Stability		≥ 1 year

*For quantitative analysis, separate stock standards are made for calibrator and controls

5.6.2.2 High Working Standards (2000 µg/mL in methanol)*

Step	Action	Amount	Component/Information
1	Acquire	1	volumetric flask, glass, 5 mL
2	Add	1.0 mL	EG Stock Standard (10 mg/mL)
3	QS	5 mL	methanol
4	Mix		
5	Transfer		Amber glass
6	Storage		about 5°C
7	Stability		≥ 1 year

*For quantitative analysis, separate working standards are made for calibrator and controls

5.6.2.3 Low Working Standards (200 µg/mL in methanol)*

Step	Action	Amount	Component/Information
1	Acquire	1	volumetric flask, glass, 10 mL
2	Add	0.2 mL	EG Stock Standard (10 mg/mL)
3	QS	10 mL	methanol
4	Mix		

5	Transfer		Amber glass
6	Storage		about 5°C
7	Stability		≥ 1 year

*For quantitative analysis, separate working standards are made for calibrator and controls

5.6.2.4 EG-d₄ Internal Standard Solution (ISS) (400 µg/mL)

Step	Action	Amount	Component/Information
1	Acquire	1	volumetric flask, glass, 25 mL. Tare on laboratory balance.
2	Add	10 mg	EG-d ₄
3	QS	25 mL	methanol
4	Mix		
5	Transfer		Amber glass
6	Storage		about 5°C
7	Stability		≥ 1 year

5.6.2.5 Calibrator and Control Scheme

Reference Section 6.3 and 6.4 for aliquot scheme.

5.6.2.5.1 Control

Control Level	EG Conc (µg/mL)	EG Control Working Stock Conc (µg/mL)	Volume EG Control Working Stock (µL)	Volume Makeup Methanol (µL)
1 + S ³	120	200 (low)	60	30
2	1500	2000 (high)	75	15

5.6.2.5.2 Calibrator

Cal. Level	EG Conc (µg/mL)	EG Cal. Working Stock Conc. (µg/mL)	Volume EG Calibrator Working Stock (µL)	Volume Makeup Methanol (µL)	
1	40	200 (low)	20	70	
2	300		15	75	
3	600		30	60	
4	1000		2000 (high)	50	40
5	1400			70	20
6	1800			90	0







5.6.2.6 Negative Control Blood (NCB)

Purchased from Cliniqua or another approved vendor. Storage and stability are determined by the manufacturer.

5.6.2.7 *System Suitability Sample (S3)(120 µg/mL)*

An additional Level 1 Positive Control is used to verify system performance.

6 PROCEDURE

Step		Activity	Note	Reference/Lot
6.1	<input type="checkbox"/>	Label Eppendorf tubes for each sample, control or calibrator. Number of tubes: Qualitative: 4 + samples Quantitative: 11 + samples (duplicate)		
6.2	<input type="checkbox"/>	Add 0.1 mL of negative control blood (NCB) to a tube.	NCB 	
6.3	<input type="checkbox"/>	Prepare low control(s), high control(s) and S3 (positive controls in duplicate for quantitative analysis): Control Scheme is added to 0.1 mL of NCB	Control W/S Low Control W/S High Methanol 	
6.4	<input type="checkbox"/>	Prepare calibrators for quantitative analysis. Add to 0.1mL of NCB. Calibrator Scheme	Cal W/S Low Cal W/S High Methanol 	
6.5	<input type="checkbox"/>	Add 0.1 mL of case specimen to a tube (in duplicate for quantitative analysis).		
6.6	<input type="checkbox"/>	Add 90 µL of makeup methanol to all case samples and negative control(s).	Methanol 	
6.7	<input type="checkbox"/>	Add 25 µL of d4-EG Internal Standard Solution (400 µg/mL) to each case sample, calibrator and control.	EG-d4 ISS 	
6.8	<input type="checkbox"/>	Add 400 µL of Phenylboronic Acid Derivatizing Reagent.	Derivatizing Reagent 	
6.9	<input type="checkbox"/>	Cap each tube and vortex-mix for 10 seconds.		
6.10	<input type="checkbox"/>	Centrifuge at 10,000 rpm for 3 minutes.		
6.11	<input type="checkbox"/>	Remove 20 µL of the acetone layer to a labeled 10 mL headspace vial and immediately cap.		
6.12	<input type="checkbox"/>	Analyze by HS-GC/MS(EI) after verifying instrument performance with S3.		

7 ANALYTICAL PARAMETERS

7.1 Gas Chromatography

7.1.1 Inlet and Injector

Sample Inlet: GC
 Injection Source: External Device
 Use MS

Front (allocated to Injector 'Front' on MPS)
 Rear (allocated to Injector 'NONE' on MPS)

Heater: 250 °C
 Pressure: 9.7853 psi
 Total Flow: 28.2 mL/min
 Septum Purge Flow: 3 mL/min
 Septum Purge Flow Mode: Standard

Gas Saver: On
 20 mL/min After: 3 min

Mode: Split
 Split Ratio: 20 : 1
 24 mL/min

7.1.2 Column

	Description
1	Agilent 190915-433: 325 °C: 30 m x 250 µm x 0.25 µm In: Front SS Inlet He Out: Vacuum
2	Agilent 19091P-M54: 350 °C: 30 m x 320 µm x 12 µm In: Back SS Inlet N2 Out: Back Detector TCD

Control Mode On

Flow
 Pressure

Average Velocity: 39.923 cm/sec
 Holdup Time: 1.2524 min

1.2 mL/min
 9.7853 psi
 (Initial): 0 min
 He @ 50 °C Oven
 Out: Vacuum
 30 m x 250 µm x 0.25 µm

	Rate mL/min per min	Value mL/min	Hold Time min	Run Time min
▶ (Initial)		1.2	0	10.007
*				

Final value will be extended by GC run time.

Post Run: 0.57353 mL/min

7.1.3 Oven

Oven Temp On

Equilibration Time:

Post Run Time:

Cryo: On
 Quick Cool

Cryo Use Temperature:

Timeout Detection

Fault Detection

	Rate °C/min	Value °C	Hold Time min	Run Time min
▶ (Initial)		50	0	0
Ramp 1	20	120	0	3.5
Ramp 2	60	280	3.84	10.007
*				

Post Run:

7.2 Mass Spectrometry Parameters

7.2.1 SIM/Scan

MS Instrument

Sample Inlet: GC

Solvent Delay:

EMV Mode:

Relative Voltage: = 1235 V

Acq. Mode:

Scan Speed:

Acquire both Scan and SIM data:

Tune File:

Real-Time Plot

Time Window:

MS Window 1

Plot Type:

Y-Scale: to

MS Window 2

Plot Type:

Y-Scale: to

7.2.2 Scanning Mass Range

Scanning Mass Range | Threshold and Sampling Rates | Plotting

	Start Time (minutes)	Start at Mass... (amu)	End at Mass... (amu)
Scan Group 1 <input checked="" type="checkbox"/>	<input type="text" value="4.00"/>	<input type="text" value="35.00"/>	<input type="text" value="200.00"/>
Scan Group 2 <input type="checkbox"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
Scan Group 3 <input type="checkbox"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

Summary of Settings

Group	Start Time	Low Mass	High Mass	Threshold	Samples	S
1	4.00	35.00	200.00	100	2	7

7.2.3 Mass Spectrometer Temperatures

	Setpoint	Actual
MS Source	230	230
MS Quad	150	150

7.3 Gerstel Autosampler Parameters

7.3.1 Timing

Runtime min

Minimum Runtime: 10.01 min (limited by GC)

GC Cool Down Time min

Time required to cool down the GC Oven to initial temperature

7.3.2 Headspace Injection Settings

Syringe Settings	Sample
Syringe <input type="text" value="1.0ml-HS"/>	Inj. Volume (µL) <input type="text" value="100.0"/>
Syringe Temp. (°C) <input type="text" value="145"/> <input type="text" value="35.0"/>	Inj. Speed (µL/s) <input type="text" value="250.00"/>
Flush Time (s) <input type="text" value="300"/>	Pullup Delay (s) <input type="text" value="2"/>
	Fill Volume (µL) <input type="text" value="100.0"/>
	Fill Strokes <input type="text" value="3"/>
	Fill Speed (µL/s) <input type="text" value="50.00"/>
	Pre Inj. Delay (s) <input type="text" value="0"/>
	Post Inj. Delay (s) <input type="text" value="0"/>
	Inj. Penetration (mm) <input type="text" value="40.00"/>
	Sample Tray Type <input type="text" value="VT32-10"/>
	Vial Penetration (mm) <input type="text" value="12.00"/>

Sample Preparation
<input type="checkbox"/> Headspace from Tray
— Heating and Incubation —
Incubator <input type="text" value="Agitator"/>
<input checked="" type="checkbox"/> Incubation Temp. (°C) <input type="text" value="125"/> <input type="text" value="35.0"/>
Incubation Time (min) <input type="text" value="5.00"/>
Agitator On Time (s) <input type="text" value="10"/>
Agitator Off Time (s) <input type="text" value="1"/>
Agitator Speed (rpm) <input type="text" value="300"/>

7.3.3 Options

Multiple Headspace Sample Enrichment (MHSE) and/or Pressurize

Pressurize Sample

Injections per Run

Delay Time (min)

8 DATA ANALYSIS

8.1 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this procedure. In general, compound identification will be based on comparison of the chromatography and mass spectrometry to a Calibrator, Sample or Positive Control. In most cases, all of the below should be met in order to identify ethylene glycol within a biological specimen.

8.1.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak in a known sample analyzed on the same system in the same analytical runs. Additionally, the following two criteria should be met.

8.1.1.1 *Retention Time*

The retention time of the peak should be within $\pm 2\%$ of the retention time (relative or absolute) obtained from injection of an extracted Positive Control.

8.1.1.2 *Signal-to-Noise*

To justify the existence of a peak, its baseline signal to noise ratio should exceed 3. Further, the baseline signal for the peak from the sample of interest should be at least 10-fold greater than that for any observed peak at a similar retention time in a Negative Control or solvent blank injected just prior to that sample.

8.1.2 Mass Spectrometry

The following ions are characteristic of the phenylboronic acid derivative of ethylene glycol: 148, 118, and 91. The mass spectrum of the derivatized ethylene glycol should match that of an extracted positive control or calibrator. See the Guidelines for Comparison of Mass Spectra standard operating procedure (TOX-104) for further guidance.

8.1.3 Batch Acceptance

8.1.3.1 *Control Criteria*

Target analyte(s) shall not be detected in the Negative Control. Positive Control(s) shall have all target analytes identified.

8.1.3.2 *Internal Standard*

The internal standard shall be recovered for all samples.

8.2 Calculations

Refer to TOX-101 and CHEM-100 for guidance.

Ethylene glycol is quantitated by calculating the area of derivatized EG to the area of its internal standard (148:152) and plotting these ratios against concentration. For additional guidance in

performing quantitations, see TOX-101. Results in this method are calculated in the units $\mu\text{g/mL}$. Results can also be reported in mg/dL . In order to convert from $\mu\text{g/mL}$ to mg/dL , divide by ten. For example, $500 \mu\text{g/mL} = 50.0 \text{mg/dL}$.

8.2.1 Curve Model

Linear regression is used to find the best fit line through the data using $1/x$ weighting.

9 REPORTING

9.1 Measurement Uncertainty

Refer to TOX-101 and CHEM-100 for guidance.

10 CORRECTIVE MEASURES

Refer to TOX-101 for potential responses to QC failure(s).

11 PERFORMANCE CHARACTERISTICS

Accuracy	Range of -2.73% to +0.50% at three measured concentrations
Calibration Range	40 – 1800 $\mu\text{g/mL}$
Limit of Detection	40 $\mu\text{g/mL}$
Precision	Range of 8.73% – 11.24% at three measured concentrations

11.1 Carryover

There was negligible carryover for the target analyte signal after analysis of the highest level calibrator.

12 LIMITATIONS

Processed Sample Stability	Not thoroughly evaluated; samples should be analyzed on the day of preparation.
Interferences	None known. Laboratory experiments have demonstrated that diethylene glycol, triethylene glycol, propylene glycol, glycerol and 1,4-butanediol do not interfere with the method.
Degradation of Sample(s)	Postmortem production of glycols may occur under some conditions. Interpretation of analyses on putrefied or otherwise degraded samples should be done with caution.
Detection of Other Glycols	Due to steric hindrance encountered during the derivatization step, this procedure is not effective for the detection of other glycols.

13 SAFETY

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

14 REVISION HISTORY

Revision	Issued	Changes
03	02/11/2022	Document reformat. Minor updates to language.