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	☒ Screening ☒ Confirmation ☒ 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 ≥ 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*	EI 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-d_4 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	2000 (high)	15	75
3	600		30	60
4	1000		50	40
5	1400		70	20
6	1800		90	0

5.6.2.6 Negative Control Blood (NCB)

Purchased from Cliniqa 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.

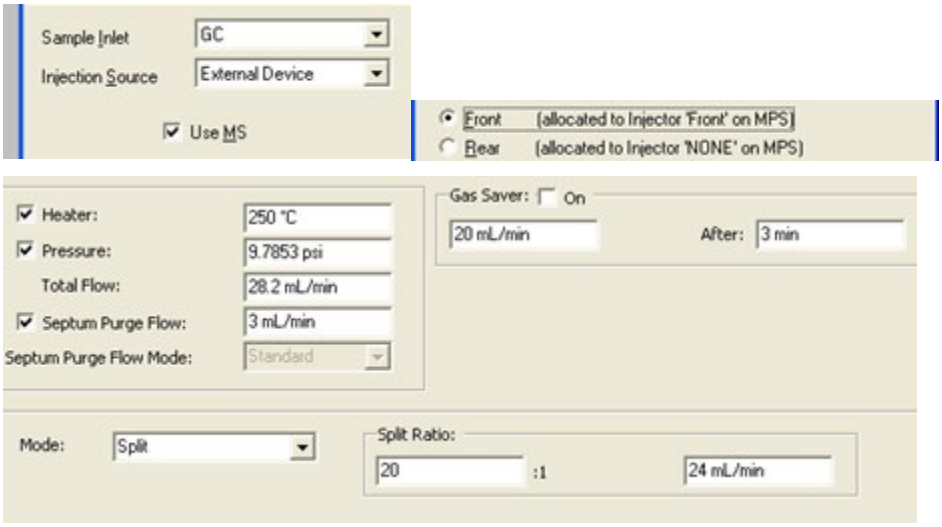
6 PROCEDURE

Step		Activity	Note	Reference/Lot
6.1	☐	Label Eppendorf tubes for each sample, control or calibrator. Number of tubes: Qualitative: 4 + samples Quantitative: 11 + samples (duplicate)		
6.2	☐	Add 0.1 mL of negative control blood (NCB) to a tube.	NCB	
6.3	☐	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 $_3$	
6.4	☐	Prepare calibrators for quantitative analysis. Add to 0.1mL of NCB. Calibrator Scheme	Cal W/S Low Cal W/S High Methanol $_3$	
6.5	☐	Add 0.1 mL of case specimen to a tube (in duplicate for quantitative analysis).		
6.6	☐	Add 90 µL of makeup methanol to all case samples and negative control(s).	Methanol	
6.7	☐	Add 25 µL of d4-EG Internal Standard Solution (400 µg/mL) to each case sample, calibrator and control.	EG-d4 ISS	
6.8	☐	Add 400 µL of Phenylboronic Acid Derivatizing Reagent.	Derivatizing Reagent	
6.9	☐	Cap each tube and vortex-mix for 10 seconds.		
6.10	☐	Centrifuge at 10,000 rpm for 3 minutes.		
6.11	☐	Remove 20 µL of the acetone layer to a labeled 10 mL headspace vial and immediately cap.		
6.12	☐	Analyze by HS-GC/MS(EI) after verifying instrument performance with S3.		

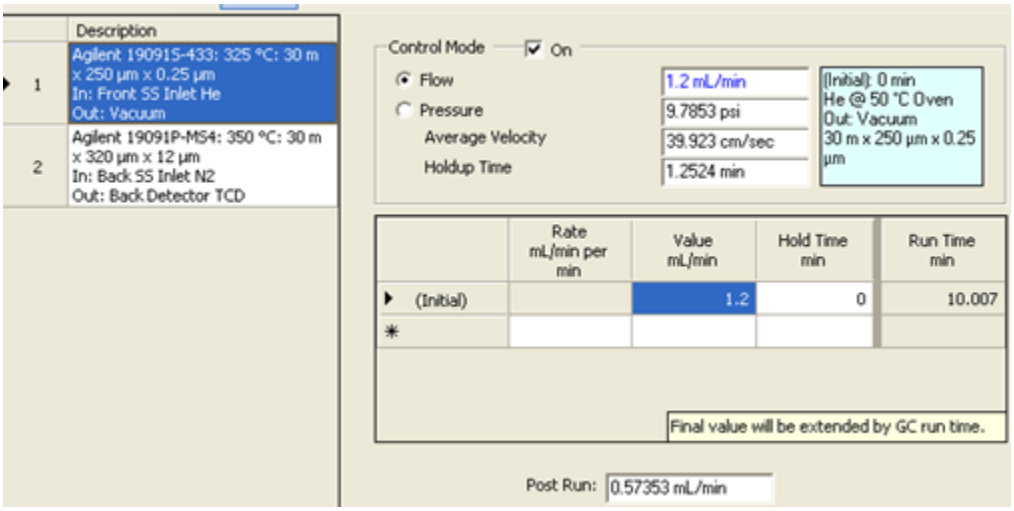
7 ANALYTICAL PARAMETERS

7.1 Gas Chromatography

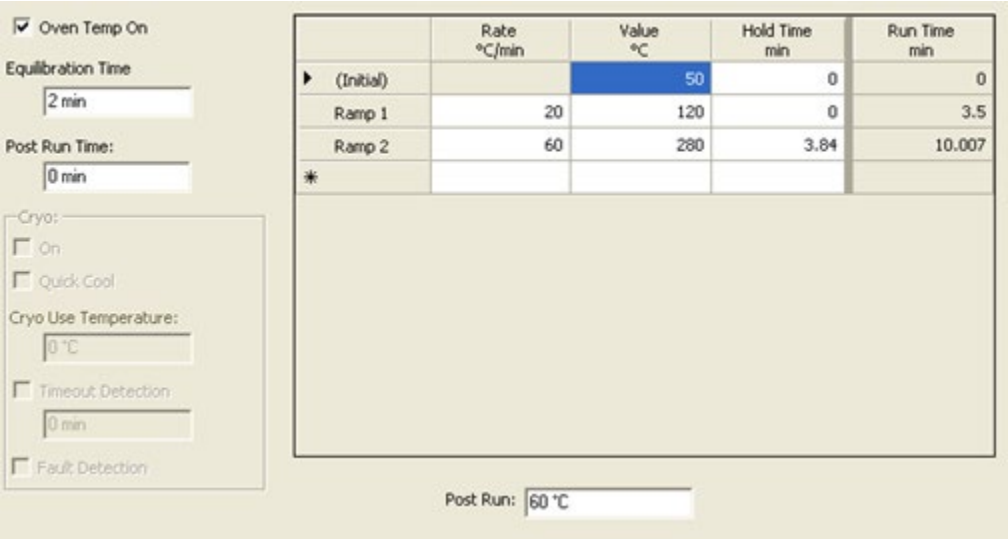
7.1.1 Inlet and Injector



7.1.2 Column

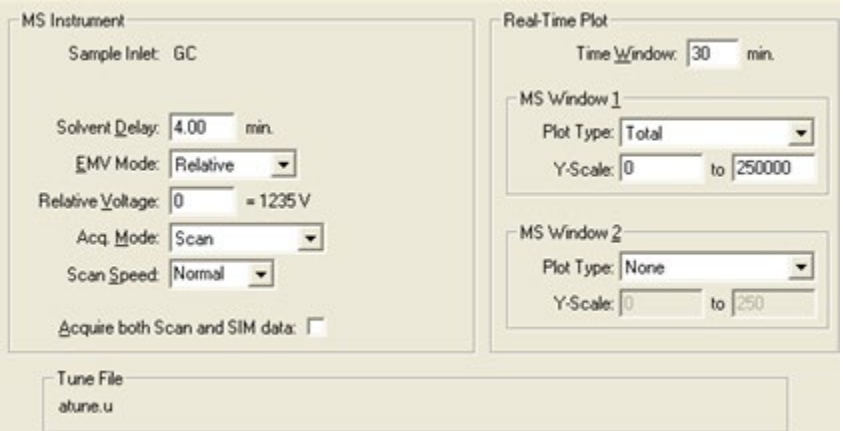


7.1.3 Oven



7.2 Mass Spectrometry Parameters

7.2.1 SIM/Scan



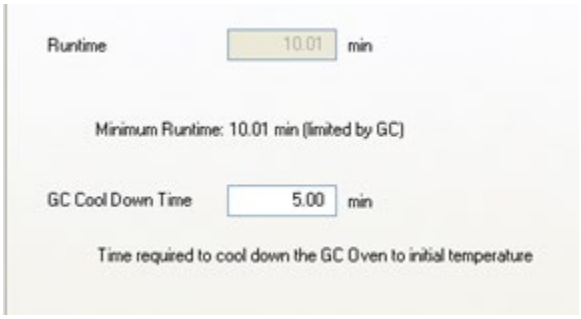
7.2.2 Scanning Mass Range

7.2.3 Mass Spectrometer Temperatures

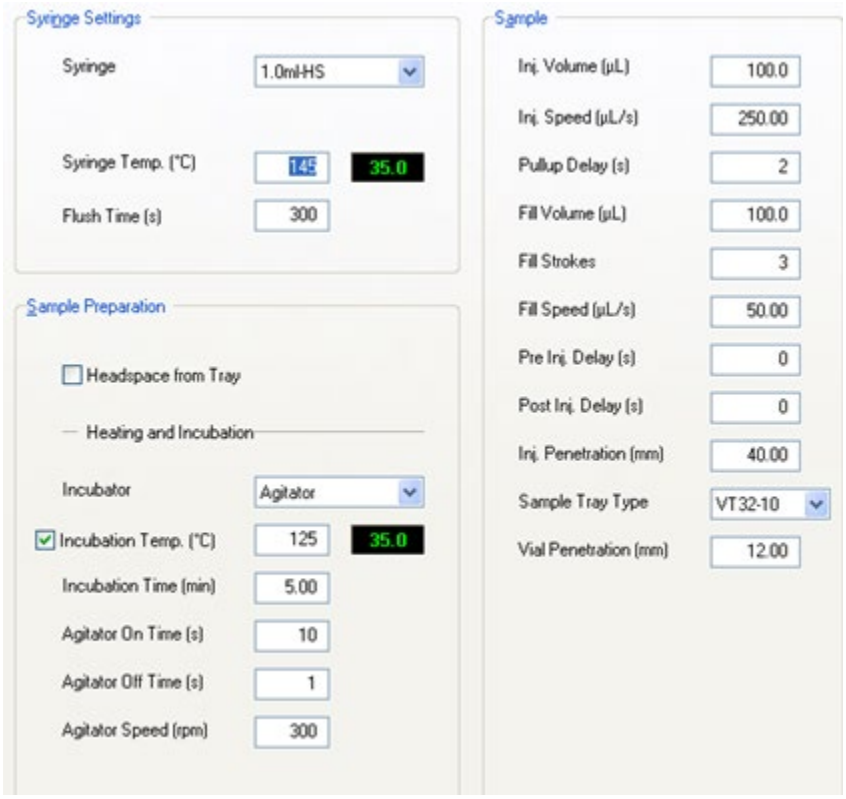
	Setpoint	Actual
MS Source	230	230
MS Quad	150	150

7.3 Gerstel Autosampler Parameters

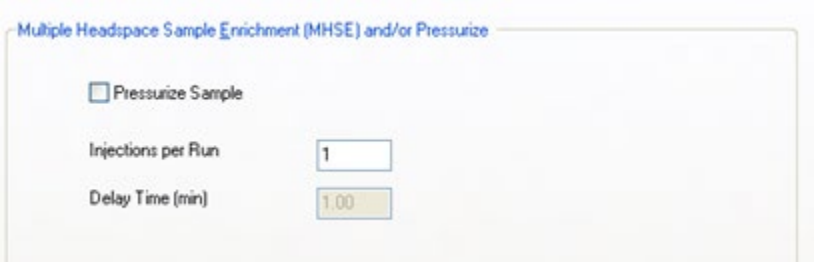
7.3.1 Timing



7.3.2 Headspace Injection Settings



7.3.3 Options


Multiple Headspace Sample Enrichment (MHSE) and/or Pressurize
Pressurize Sample
Injections per Run
1
Delay Time (min)
1.00

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 ±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 µg/mL. Results can also be reported in mg/dL. In order to convert from µg/mL to mg/dL, divide by ten. For example, 500 µg/mL = 50.0 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 µg/mL
Limit of Detection	40 µg/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 stearic 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.