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United States Environmental Protection Agency

The Verification of a Method for Detecting and Quantifying Diethylene Glycol, Triethylene Glycol, Tetraethylene Glycol, 2-Butoxyethanol and 2-Methoxyethanol in Ground and Surface Waters

RESEARCH AND DEVELOPMENT

The Verification of a Method for Detecting and Quantifying Diethylene Glycol, Triethylene Glycol, Tetraethylene Glycol, 2-Butoxyethanol and 2-Methoxyethanol in Ground and Surface Waters

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Notice

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The EPA Quality System and the Hydraulic Fracturing Research Study

EPA requires that all data collected for the characterization of environmental processes and conditions are of the appropriate type and quality for their intended use. This is accomplished through an Agency-wide quality system for environmental data. Components of the EPA quality system can be found at http://www.epa.gov/quality. EPA policy is based on the national consensus standard ANSI/ASQ E4-2004 *Quality Systems for Environmental Data and Technology Programs: Requirements with Guidance for Use*. This standard recommends a tiered approach that includes the development and use of Quality Management Plans (QMPs). The organizational units in EPA that generate and/or use environmental data are required to have Agency-approved QMPs. Programmatic QMPs are also written when program managers and their QA staff decide a program is of sufficient complexity to benefit from a QMP, as was done for the study of the potential impacts of hydraulic fracturing (HF) on drinking water resources. The HF QMP describes the program's organizational structure, defines and assigns quality assurance (QA) and quality control (QC) responsibilities, and describes the processes and procedures used to plan, implement, and assess the effectiveness of the quality system. The HF QMP is then supported by project-specific QA project plans (QAPPs). The QAPPs provide the technical details and associated QA/QC procedures for the research projects that address questions posed by EPA about the HF water cycle and as described in the *Plan to Study the Potential Impacts of Hydraulic Fracturing on Drinking Water Resources* (EPA/600/R-11/122/November 2011; www.epa.gov/hfstudy). The results of the research projects will provide the foundation for EPA's study report.

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Executive Summary

This verification study was a special project designed to determine the efficacy of a draft standard operating procedure (SOP) developed by US EPA Region 3 for the determination of selected glycols and glycol ethers in drinking waters that may have been impacted by active unconventional oil and gas operations utilizing hydraulic fracturing (HF) extraction. HF has become increasingly prevalent as a method of extracting energy resources from "unconventional" reservoirs, such as coalbeds, shales, and tight sands. Concerns have been raised about the potential for hydraulic fracturing fluid chemical additives to enter ground water aquifers that, in turn, may be used as drinking water sources.

One group of hydraulic fracturing fluid chemical additives that concerns have been raised about includes the additives: 2-methoxyethanol (2-ME), 2-butoxyethanol (2-BE), diethylene glycol (Di-EG), triethylene glycol (Tri-EG), and tetraethylene glycol (Tetra-EG). The primary objective of this study was to verify the performance of the draft standard operating procedure developed by US EPA Region 3 in multiple laboratories. This study verified a simple and rapid high performance-liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) method for the quantitation of these five chemical additives in aqueous samples. The draft method was quick, required little to no sample preparation, and utilized the sensitivity that HPLC/MS/MS provides.

The verification of the draft SOP included volunteer federal, state, municipal, and commercial analytical laboratories. Each laboratory tested the efficacy of the draft SOP using the HPLC/MS/MS instrumentation present in their laboratories. Four different water matrices were used to spike batches of samples at various concentrations. Three source matrix waters were collected from bulk water samples acquired from three drinking water source wells (prior to treatment) around the country in areas where active shale oil and gas operations are occurring or where they may occur in the future. The source matrix waters were collected at Avella, Pennsylvania, Raleigh, North Carolina, and Ada, Oklahoma. Laboratory deionized water, from Las Vegas, Nevada, was used as a fourth matrix. Batches of 36 blind samples, prepared by EPA, were distributed to the volunteer laboratories for analysis following the draft SOP.

To ensure that data of known and documentable quality are generated by the participating analytical laboratories, data quality indicators (DQIs) were defined to examine key parameters and to determine if the key parameters met their acceptance criterion. The key parameters included: verification of the calibration curves, determination of any laboratory blank contamination issues, examination of the precision and accuracy of the laboratory control and matrix spike samples, substantiation of sample precision from duplicate samples, second source check standard verification, confirmation of reporting limits and appropriate method detection limits, and continuing calibration verification after each batch of samples.

To determine if the draft glycol SOP could be followed and meet the performance criteria, blind samples submitted to the analytical laboratories and several key factors were examined. The key factors included: accuracy (defined as the difference between the known and measured concentration) within and among the laboratories, precision within the analytical laboratory, and investigating whether matrix effects from the four waters used were present.

The data generated by the analytical laboratories following the draft SOP were statistically analyzed to determine if differences existed among the laboratories as related to key factors used to determine the analytical performance of the draft SOP. Accuracy, determined by comparing the measured result to the known spiked concentration, met the performance criteria but a few statistical outliers were identified. The precision within the analytical laboratories met the performance criteria indicating that reproducible results were being generated. Matrix effects between the four different water

matrices were not identified in any of the analytical laboratories for any of the compounds of interest, indicating that the method could produce the same results in the four water matrices tested.

The reporting limits and calibration ranges of the draft SOP were similar among different instruments used at the analytical laboratories with reporting limits typically ranging between 5 and 10 µg/L. However, a few discrepancies were noted. Some laboratories had better sensitivity on their instruments, wider calibration ranges; and had different optimum calibration fits (i.e., a linear calibration fit was specified in the draft SOP but a quadratic fit of the calibration point data yielded better results). Differences in instrument sensitivity were found where 2-BE could not be successfully detected at one laboratory and two laboratories could not successfully detect 2-ME.

Overall, the draft glycol SOP presented a method that was accurate and precise by meeting the established performance criterion nearly all the time. No matrix effects on the chemical recoveries were exhibited for the four waters tested when the compounds of interest were detectable. The multi-laboratory verification of the draft SOP resulted in the generation of several recommendations in order to construct an improved analytical method including: allowing best calibration fit parameters, incorporating surrogate spikes, conducting a sample preservation and holding time study, conducting a filtering unit study, allowing for greater adjustment of chromatographic conditions, initiating second source verification, and applying calibration check verification concentration consistency.

List of Acronyms

2-BE	2-Butoxyethanol
2-ME	2-Methoxyethanol
CCV	Continuing Calibration Verification
CV	Coefficient of Variation
Di-EG	Diethylene Glycol
DQI	Data Quality Indicator
FS	Field Sample
GC	Gas Chromatography
HF	Hydraulic Fracturing
HPLC	High Performance Liquid Chromatography
HPLC/MS/MS	High Performance Liquid Chromatography/Mass Spectrometry/Mass Spectrometry
LC/MS/MS	Liquid Chromatography/Mass Spectrometry/Mass Spectrometry
LCS/LCSD	Laboratory Control Sample/ Laboratory Control Sample Duplicate
MDL	Method Detection Limit
MS	Mass Spectrometry
MS/MS	Mass Spectrometry/Mass Spectrometry
MS/MSD	Matrix Spike/Matrix Spike Duplicate
N/A	Not Applicable
NERL	National Exposure Research Laboratory
ORD	Office of Research and Development
QA	Quality Assurance
QA/QC	Quality Assurance/Quality Control
QC	Quality Control
QAPP	Quality Assurance Project Plan
QCCS	Quality Control Check Sample
QMP	Quality Management Plan
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
SOP	Standard Operating Procedure
Tetra-EG	Tetraethylene Glycol
Tri-EG	Triethylene Glycol
US EPA	United States Environmental Protection Agency

1.0 Introduction

Glycols and glycol ethers are solvents and chemical intermediates commonly used during the production of many resins, plasticizers, adhesives, surfactants, and cosmetics. Due to their useful properties, many glycols and glycol ethers, including 2-methoxyethanol (2-ME), 2-butoxyethanol (2-BE), diethylene glycol (Di-EG), triethylene glycol (Tri-EG), and tetraethylene glycol (Tetra-EG), have been classified as high-production volume chemicals by the United States Environmental Protection Agency (US EPA)[1]. Additionally, these compounds have frequently been used during oil and gas production. For example, ethylene glycol, Di-EG, Tri-EG, and Tetra-EG are commonly used during the dehydration processes of natural gas[2], and glycol ethers are used as foaming agents and in breaker fluids during hydraulic fracturing[3].

1.1 Background

Hydraulic fracturing (HF) has become increasingly prevalent as a method of extracting energy resources from "unconventional" reservoirs, such as coalbeds, shales, and tight sands. Concerns have been raised about the potential for hydraulic fracturing fluid chemical additives to enter ground water aquifers that, in turn, may be used as drinking water sources. Of concern for this project are diethylene glycol (CASRN #111-46-6), triethylene glycol (CASRN #112-27-6), tetraethylene glycol (CASRN #112-60-7), 2-butoxyethanol (CASRN #111-76-2), and 2-methoxyethanol (CASRN #109-86-4). In response to this concern, the US EPA Region 3 Environmental Science Center in Fort Meade, Maryland developed a quick, draft method for the determination and quantification of these compounds. This draft method, prepared in the form of a standard operating procedure (SOP; Appendix A), needed to be verified to determine its efficacy in determining these compounds in laboratory and various drinking water matrices.

1.2 Project Description and Objectives

The *Multi-laboratory Verification of Diethylene Glycol, Triethylene Glycol, Tetraethylene Glycol, 2- Butoxyethanol and 2-Methoxyethanol in Ground and Surface Waters by Liquid Chromatography/Tandem Mass Spectrometry* study was designed to determine the efficacy of a draft method developed by US EPA Region 3 for the determination of glycols and glycol ethers in drinking waters collected from drinking water wells. The objectives of this study were to verify a simple and rapid liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the quantitation of 2-ME, 2-BE, Di-EG, Tri-EG, and Tetra-EG in aqueous samples by examining three key performance areas: (1) can the analytical laboratory follow the SOP, (2) can the analytical laboratory meet the SOP requirements as related to quality assurance/quality control measures, and (3) how well did the method perform in terms of bias/accuracy, precision, and the absence of matrix effects. This report describes the interlaboratory verification results for the described method.

For the verification of the method, eight analytical laboratories were invited to participate in the analyses of a series of multiple blind samples (spiked and unspiked) in multiple matrices (e.g., laboratory waters and drinking well waters).

The following laboratories were invited to participate:

1. US EPA National Exposure Research Laboratory, Environmental Sciences Division, Las Vegas, NV,

2. US EPA National Exposure Research Laboratory, Microbiological & Chemical Exposure Assessment Research Division, Cincinnati, OH,

3. US EPA Region 3 Environmental Science Center, Fort Meade, MD,

4. US EPA Region 5 Chicago Regional Laboratory, Chicago, IL,

5. Eurofins Lancaster Testing Laboratories, Lancaster, PA,

6. TestAmerica, Inc, Arvada, CO,

7. Philadelphia Water Department, Philadelphia, PA, and

8. Metropolitan Water District of Southern California, La Verne, CA.

Two laboratories had instrument issues or business time constraints and were unable to participate. All participating laboratories did so *a gratis*.

To ensure that these study objectives were met, all participating laboratories strictly adhered to the requirements that:

- Each laboratory verified and optimized the liquid chromatography/mass spectrometry/mass spectrometry conditions in sections 10 and 11 of the draft SOP (Appendix A) on their instrumentation and determined the reporting limits on their LC/MS/MS systems.
- Each laboratory followed all analytical and quality control procedures in the approved quality assurance project plan (QAPP)[4].
- Each laboratory documented any deviations from the SOP or QAPP.
- All data produced were capable of being verified by an independent person reviewing the analytical data package.
- Each laboratory had a verifiable QA program, equal to or exceeding EPA requirements, in placeand operating throughout the study to ensure that the data produced are of appropriate and documented quality.

2.0 Method Verification Procedure

2.1 Laboratory Initial Demonstration of Capability and Unknown Samples

For the verification study, each participating laboratory was sent a copy of the draft SOP as Appendix A of the QAPP. The conditions in the SOP were to be used as a starting point in order to optimize each LC/MS/MS instrument within the limits identified in the SOP. The goal of optimization was to: familiarize the analyst with the analytes and method, determine the range of the calibration curves, and determine the method detection and reporting limits on their instrument. At least seven replicates at a low level were used in order to determine a method detection limit (MDL) for each analyte in each laboratory (40 CFR Part 136 Appendix B).

Once optimized, the reporting limit was calculated to be at least 3 times the MDL and may be different between the laboratories because of varying sensitivities of the LC/MS/MS systems used. A reporting limit target of 5 µg/L was established for this study. Each laboratory determined their reporting limits (Table 1). If the actual reporting limit, as calculated using the MDL, was determined to be less than the reporting limit target at the laboratory, the target 5 µg/L reporting limit was used. If the determined reporting limit was greater than the target reporting limit of 5 µg/L, the new value was reported and used for this study. All concentrations reported were required to be at or above the reporting limit for statistical analysis.

Table 1. Laboratory Reporting Limits.

Analyte	Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 5	Laboratory 6	Laboratory 8
2-ME	Non-Detect*	5 µg/L	Non-Detect	25 µg/L	20 µg/L	5 µg/L
2-BE	Non-Detect	5 µg/L	10 µg/L	10 µg/L	8 µg/L	5 µg/L
Di-EG	5 µg/L	5 µg/L	5 µg/L	5 µg/L	8 µg/L	5 µg/L
Tri-EG	5 µg/L	5 µg/L	5 µg/L	5 µg/L	8 µg/L	5 µg/L
Tetra-EG	5 µg/L	5 µg/L	5 µg/L	5 µg/L	8 µg/L	5 µg/L

** Non-Detect indicates that the analytical laboratory could not detect this compound.*

For the verification study, four water matrix sets of nine samples each were prepared for a total of 36 blind samples. Samples were prepared by an independent scientist (i.e., one not involved with the draft glycol method verification study). The various blind samples were prepared from bulk water samples acquired from multiple drinking water source wells around the country in areas where active shale oil and gas operations are occurring or may occur in the future. Several gallons of each bulk water matrix were collected in clean, capped amber glass containers and labeled with the source and date of sampling. The matrix waters were collected from the drinking water system prior to any treatment at the source. Bulk samples were stored at 4 °C ± 2 °C. The matrix waters were collected at Avella, Pennsylvania, Raleigh, North Carolina, and Ada, Oklahoma. Laboratory deionized water, from Las Vegas, Nevada, was used as a fourth matrix. The sample identifiers, FS-1 through FS-4, were utilized throughout this report to reference the different sample matrices (Table 2).

Table 2. Source Waters.

Sample Identifier*	Source
FS-1	Avella, PA Drinking Well Water
FS-2	Raleigh, NC Well Water
FS-3	Ada, OK Ground Water
FS-4	EPA-Las Vegas, NV Deionized Laboratory Water

** FS = Field Sample.*

2.2 Blind Sample Description and Spike Concentrations

The blind spiked samples were produced in bulk such that all equivalent samples came from the same volumetric flask, and were bottled and shipped to the participating analytical laboratories on the same day to ensure that each participating laboratory received the same samples. Each analytical laboratory received 9 samples per matrix. Seven samples were spiked and 2 samples (bottles 8 and 9) were blank (non-spiked source water). Of the seven spiked bottles (bottles 1-7), five bottles had low-mid concentration of each target analyte, one bottle had a high concentration of each target analyte, and one bottle was not spiked with one of the analytes (Table 3). Actual concentrations of the low-mid and high concentration samples were varied among the analytes in an effort to avoid pattern recognition among the laboratories (i.e., all concentrations for all analytes were the same in all samples). Additionally, the one sample that was not spiked with one of the target analytes was used to ensure that the laboratory was confident in their analyses and reported a non-detect even though the remaining four analytes were present in the sample.

Table 3. Concentrations of Analytes in Unknown Sample Bottles.

Bottles	Di-EG (µg/L)	Tri-EG (µg/L)	Tetra-EG (µg/L)	2-BE (µg/L)	2-ME (µg/L)
1	10	80	100	60	40
2	100	80	100	60	40
3	10	80	200	Blank	40
4	10	80	100	180	Blank
5	Blank	200	100	60	40
6	10	80	Blank	60	100
7	10	Blank	100	60	40
8	Blank	Blank	Blank	Blank	Blank
9	Blank	Blank	Blank	Blank	Blank

2.3 Statistical Analyses

The total number measurement values generated was 840 (6 laboratories × 5 analyte × 4 sample × 7 bottle) per each type of calibration curve (i.e., linear or quadratic curves) used. Values less than or equal to the reporting limit (RL) were excluded prior to statistical analyses. The concentrations that are below reporting limit do not reflect precise or accurate measurements. However, the removal of the data points and the resulting unbalanced design can be overcome by applying the general linear model (GLM). Additionally, not all laboratories were able to provide measurements on all analytes because of the detecting capability on their analytical instruments. This limitation is described within each section for the analyte. A split plot design ANOVA[5] was assembled for this data set as:

$$Measurement = \mu + Lab + SW + Lab * SW + Spike + Spike * Lab + Spike * SW + \varepsilon \quad \text{Eqn 1}$$

where: μ is the overall mean of the observations; *Lab*, *SW*, and *Spike* are class variables; *Lab* for analytical laboratory, *SW* for source waters sample, and *Spike* is unknown concentration of the compound. The error for S*W* is *Lab*SW* and others were tested against the residual errors (mean square error or ε).

The above model was used to statistically analyze all analytes except for diethylene glycol, which was determined using the linear calibration curve for the low concentration samples. For diethylene glycol, Laboratory 6 did not report any data for the low level spiked samples; hence, spike level is no longer a class variable. With only one spike level, a randomized block design for ANOVA was applied as presented in Eqn 2:

$$Measurement = \mu + Lab + SW + \varepsilon \quad \text{Eqn 2}$$

Reliability of the study can be measured by the coefficient of variability (CV), which is the overall experimental error standardized by the overall mean (grand mean) of all measurements (Eqn 3):

$$CV(\%) = \frac{\sqrt{MSE}}{grandmean} * 100$$

where $\sqrt{MSE}$ is the square root of mean square error. The value of CV can be used as an index for model reliability. Model reliability increases as the value of CV decreases. If CV > 30%, then caution has to be taken when describing model reliability and model output[6]. All of our models exhibited low values of CV, where they ranged from 1.7 to 12.7.

Diagnostic checking on residuals was carried on for each model with the outliers removed. Model residuals were tested for normality where probability of Shapiro-Wilk test was ≥ 0.05 for all models. Means, standard error, and the 95% confidence limit for each class level, or their combinations, are presented in figures to explain statistical differences.

The statistical analyses were performed using a general linear model (Proc GLM) in SAS® with the least-square means (LSMEANS) option to account for the missing values in the unbalanced design. The probability of t-statistics was used for a multiple comparison of means between class variables and their combinations. Mean and standard error values in figures with ±20% and ±30% thresholds were determined using Proc Means in SAS®. The significance level was 0.05 for all statistical analyses.

3.0 Quality Assurance

The QAPP entitled, "Quality Assurance Project Plan for the Multi-Laboratory Verification of Diethylene Glycol, Triethylene Glycol, Tetraethylene Glycol, 2-Butoxyethanol and 2-Methoxyethanol in Ground and Surface Waters by Liquid Chromatography/Tandem Mass Spectrometry", was approved on March 5, 2013. The Data Quality Indicators (DQIs) and their acceptance criteria for the measurement of the data generated by the laboratories consisted of seven key parameters that were requested during the verification study (Table 4). Some of the DQI key parameters did not meet the acceptance criteria in the QAPP and these deviations are documented and explained in this section. The data generated from the blind samples with quality assurance/quality control (QA/QC) resulted in variances that appeared to be random with the exception of negative biases in the initial calibration curves (see section 3.1). Where the variances occurred, the majority of the associated QA/QC acceptance parameters were met so the data were used to assess the method performance.

Table 4. Data Quality Indicators and their Acceptance Criteria.

QC Check	Precision*	Accuracy
5-Point Initial Calibration	N/A	Correlation Coefficient $r^2 \geq 0.99$
Instrument Blank	N/A	< Reporting Limit
Laboratory Control Sample/ Laboratory Control Sample Duplicate (LCS/LCSD)	RPD ≤ 30%	±30% of Known Value
Laboratory Matrix Spike/Laboratory Matrix Spike Duplicate (MS/MSD)	RPD ≤ 30%	Recovery Between 70 and 130% of Spike Concentration
Laboratory Replicate	RPD ≤ 30%	N/A
Quality Control Check Standard (QCCS)	N/A	±20% of Known Value
Continuing Calibration Verification Sample (CCV)	N/A	±30% of Known Value

* RPD = relative percent difference; N/A = not applicable.

3.1 Initial Calibration

Linear initial calibration curves were required to be determined for each chemical. Although not specified in the draft SOP, quadratic calibration curves were also requested since: (a) the allowance to use both forms of initial calibration is being incorporated into the revised SOP, and (b) fitting the calibration points to a quadratic calibration curve is a simple, non-time consuming process for the analytical laboratories. In most cases, using the quadratic calibration curve reduced/removed most of the low calibration point(s) biases. The initial calibrations met the acceptance criteria of having a r^2 value ≥ 0.99 for both linear and quadratic fits in most cases.

Laboratory 6 did not meet the correlation coefficient parameter for 2-BE ($r^2 = 0.979$). A negative bias for 2-BE at the low concentration level (-144% at the 5 µg/L concentration) in the calibration curve using the linear calibration fit was observed. The remainder of the calibration curve, whether linear or quadratic, displayed a range in positive and negative biases (-45 to 77%) among the calibration points. This broad range in biases is believed to be the cause for the failure of Laboratory 6 to meet the $r^2 \geq 0.99$ criterion for 2-BE.

Similar to the negative bias seen in Laboratory 6 for 2-BE, Laboratory 6 also had negative biases (-75 to -267%) at the lower concentration levels (5 and 10 µg/L) in the linear calibration curves for Tetra-EG, Tri-EG and Di-EG. These biases were not as pronounced in the quadratic calibration curve for these compounds. Laboratory 6 had a negative bias (-74 to -104%) in both the linear and quadratic calibration fits for 2-ME at the lower concentration levels (5 and 10 µg/L) forcing the low level calibration standard to be dropped and the reporting limit to be raised to 20 µg/L (Table 1).

The linear calibration curves in Laboratory 1 met the correlation coefficient requirement; however, Tri-EG and Tetra-EG had negative biases (-67 and -51%, respectively) at the 5 µg/L concentration standard. The calibration curves for Di-EG, Tri-EG and Tetra-EG were established from 5-100 µg/L requiring dilution of the samples that were over the calibration curve. All reported Laboratory 1 results were used in this verification study and were in statistical agreement with the participant laboratory's data.

Laboratory 5 linear calibration curves all met the correlation coefficient requirement but 4 out of 5 of the target analytes had negative biases near the reporting limit. The Di-EG calibration curve did not display a negative bias. The 5 and 10 µg/L calibration standards were not incorporated in the linear calibration curve due to very strong negative bias for 2-ME resulting in a raised reporting limit to 25 µg/L (Table 1). The Tetra-EG higher concentration standards of the calibration curve were not used due to negative bias resulting in an abbreviated calibration curve from 5-100 µg/L. The abbreviated calibration curve; however, still contained the -62% negative bias near the reporting limit (5 µg/L). Some results were reported above the calibration curve and they were considered to be semi-quantitative. The results were used in this verification study as they were in statistical agreement with the other participating laboratories data.

Linear calibration curves for Laboratories 2, 3, and 8 met the correlation coefficient requirement; however, some negative biases were seen near the reporting limit. In Laboratory 2, Tri-EG and Tetra-EG had negative biases (-65 and -51%, respectively) at the reporting limit (5 µg/L). In Laboratory 3, Di-EG, Tri-EG and Tetra-EG had negative biases (-45, -60 and -106%, respectively) at reporting limit (5 µg/L) while in Laboratory 8, a -34% bias at 5 µg/L was identified for Tetra-EG.

In two instances, the initial calibration was not done by the laboratories because the analytical laboratories were not able to ionize the analytes. No calibration curves were submitted for 2-ME and 2-BE by either Laboratory 1 nor for 2-ME by Laboratory 3.

3.2 Instrument Blank

The instrument blank results were acceptable in all cases except for Laboratory 6 which initially had blank contamination issues with 2-BE. Laboratory 6 used an isocratic gradient which is a deviation from the draft SOP. The generated Laboratory 6 data were accepted and included in the statistical data evaluation.

3.3 Laboratory Control Sample/Laboratory Control Sample Duplicate (LCS/LCSD)

Overall, the LCS data were acceptable for all the laboratories except for Laboratory 6. Laboratory 6 had a few random exceedances of the acceptance criteria where the LCS determined value was biased high for 2-ME and biased low for Di-EG and the LCSD was biased low for 2-ME.

3.4 Laboratory Fortified Matrix/Laboratory Fortified Matrix Duplicate (MS/MSD)

The MS/MSD data were generally acceptable. The laboratories were required to decide which samples to use for MS/MSD samples. Laboratory 2 had a positive bias for Di-EG with acceptable RPDs between the MS and MSD. Laboratory 1 had randomly positive and negative biases for 11 out of 14 MS/MSD samples for Di-EG, Tri-EG, and Tetra-EG with acceptable RPDs between the replicates. Laboratory 6 had: low recoveries for Di-EG with an acceptable RPD between the duplicate samples; one biased high recovery with an exceeded RPD for 2-BE; all samples biased high with acceptable RPDs for 2-ME and Tetra-EG; and one MS with low recovery that exceeded the RPD acceptance limit for Tri-EG.

3.5 Laboratory Replicate (Duplicate)

Laboratory duplicates were not analyzed by Laboratories 5 and 6 so the MS/MSD RPDs were used to determine duplicate reproducibility in these cases with the identified discrepancies being explained in Section 3.4. All other duplicate data met the ≤30% RPD acceptance criterion.

3.6 Quality Control Check Standard (QCCS)

The laboratories chose a QCCS standard to analyze with each of their batches. The QCCS was either purchased as a prepared diluted standard/mix or purchased as a neat material. (This is not a comparison study of analytical standards so no sources of standards are mentioned.) Laboratory 6 did not analyze a QCCS sample. Laboratory 2 had a slight positive bias for Di-EG while Laboratory 3 had slight positive bias for Tetra-EG and Tri-EG. Laboratory 5 had slight positive bias for Di-EG, Tri-EG, and Tetra-EG.

3.7 Continuing Calibration Verification (CCV)

The CCV check samples were generally within acceptance criterion with exceptions at two of the participating analytical laboratories. At Laboratory 5, the 2-ME CCV checks exceeded the acceptance limit for 2 out of 4 check samples with a 45% recovery of the 25µg/L CCV sample and a 132% recovery of the 50 µg/L CCV sample. Laboratory 6 CCV check samples were negatively biased for Di-EG in 4 out of 7 check samples; positively biased for 2-BE and 2-ME in 4 out of 7 check samples; and positively biased for Tetra-EG in 3 out of 7 check samples.

3.8 Holding Times

There are no preservation or holding time studies of these analytes in reagent water or the matrices of concern. A fourteen day holding time was used for this study. Laboratory 1 initiated the analysis of the samples on day 14 and had a few samples that were analyzed after that due to an instrument failure. Laboratories 3 and 6 performed their analysis on day 14 with a few samples being run early on day 15 at Lab 3. Laboratories 2, 5, and 8 performed their analyses within 6-8 days, 2-3 days, and 2 days, respectively. With no formal holding times being established, all the data were used based on this criterion.

3.9 Audits

Each participating laboratory was asked to perform a readiness review, surveillance audit, and audit of data quality during the study. Reports were submitted from Laboratories 2, 3, 5, 6, and 8. Laboratory 1 did not provide the requested audit reports. In each case, no major findings were identified. Discrepancies, when noted, have been identified in this section of the report. In all cases, discrepancies were deemed to not have a major effect on the resultant data due to the passage of a majority of the quality assurance/quality control measures; therefore, all data was used during the statistical analysis (see section 2.4) of the results.

In addition to these three audits, Laboratories 3 and 8 performed additional surveillance audits/quality assurance inspections and technical systems audits. During one of the surveillance audits/quality assurance inspections, 2 blind sample bottles were mislabeled. Results from these samples were removed from statistical consideration. A comprehensive quality perspective (i.e., overview) was performed at Laboratory 8 over several months of this study with no findings being identified.

This report was reviewed by the NERL Director of Quality Assurance according to the requirements of the QAPP, "Quality Assurance Project Plan for the Multi-Laboratory Verification of Diethylene Glycol, Triethylene Glycol, Tetraethylene Glycol, 2-Butoxyethanol and 2-Methoxyethanol in Ground and Surface Waters by Liquid Chromatography/Tandem Mass Spectrometry" (approved on March 5, 2013) and deemed acceptable on January 31, 2014.

4.0 Results and Discussion

To determine the performance characteristics of the draft glycol method, several key factors needed to be examined. The key factors included: (a) examining the accuracy (defined as the difference between the known and measured concentration) within and among the laboratories, (b) determining the precision within the analytical laboratory, and (c) investigating whether matrix effects from the four waters used were present.

When examining the accuracy within and among the laboratories, the ±20% and ±30% of the known concentration lines will be plotted in the appropriate figures. These two percentages are commonly cited, acceptance criteria and are the two acceptance criteria used for accuracy determinations in this study (Table 4). The means of the data was examined first to see if it passed the ±20% criterion which would indicate a high degree of accuracy. If the means of the data do not meet the ±20% criterion, then they should meet the ±30% criterion to be fully acceptable.

The laboratory numbers do not coincide with the laboratory list in Section 1.2; this was done to provide anonymity to the participating analytical laboratories.

The following sections and figures discuss the results generated from the linear calibration curves. If differences in the statistical interpretation of the results (i.e., acceptance vs failure to meet the acceptance criterion) occurred when the data were generated from the quadratic calibration curves, these differences, and how they affected our interpretation of the data, are clearly delineated in the text.

4.1 Tetraethylene Glycol

All six participating laboratories provided data. All submitted blind blank samples (see Table 3) showed no target analytes at or above the reporting limits of 5 or 8 µg/L depending on the sensitivity of the instrument at the participating analytical laboratory. The blind samples were spiked at 100 or 200 µg/L.

The accuracy among the analytical laboratories was within 20% of the known concentrations, except for Laboratory 6 for the low level spiked samples which were biased high (Figures 1 and 2). The results for any given laboratory included all samples, regardless of matrix, at the given concentration level.

The precision within the laboratory can be determined by examining the results of the individual bottles shipped to the analytical laboratory. In each case, the bottle number (represented as the last digit on the x-axis identifiers) represents the mean of the four different water matrices for that bottle number (Figure 3). By checking for significant differences among the 5 bottles spiked at the 100 µg/L concentration, the precision within an analytical laboratory can be determined. In general, precision met the performance criteria among all the samples spiked at the same concentrations with a few exceptions. For example, Laboratories 1 and 2 showed statistical differences between bottles 1 and 6 (Figure 4). These exceptions; however, do not affect our interpretation of the study results as all of the bottles determined concentrations were within the study's accuracy acceptance criterion.

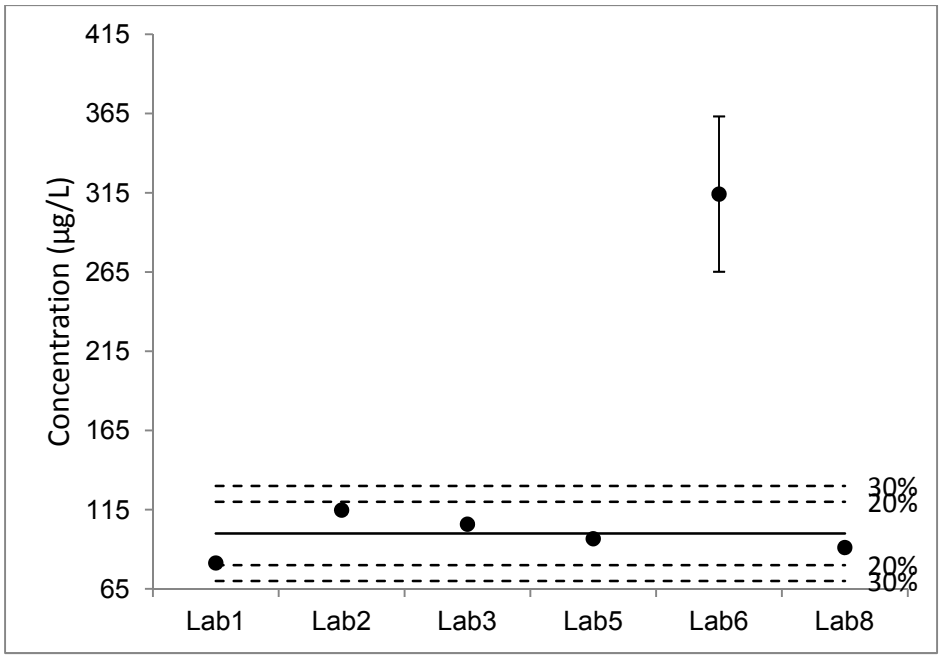

Figure 1. **Low Concentration (100 µg/L Spike) Average Recovery for Tetraethylene Glycol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)**

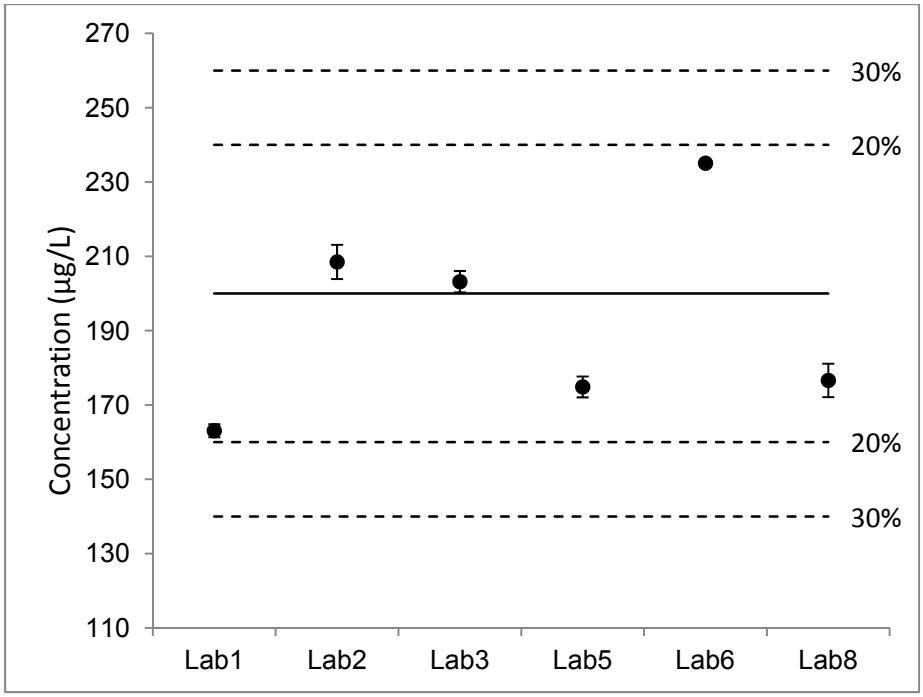

Figure 2. **High Concentration (200 µg/L Spike) Average Recovery for Tetraethylene Glycol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)**

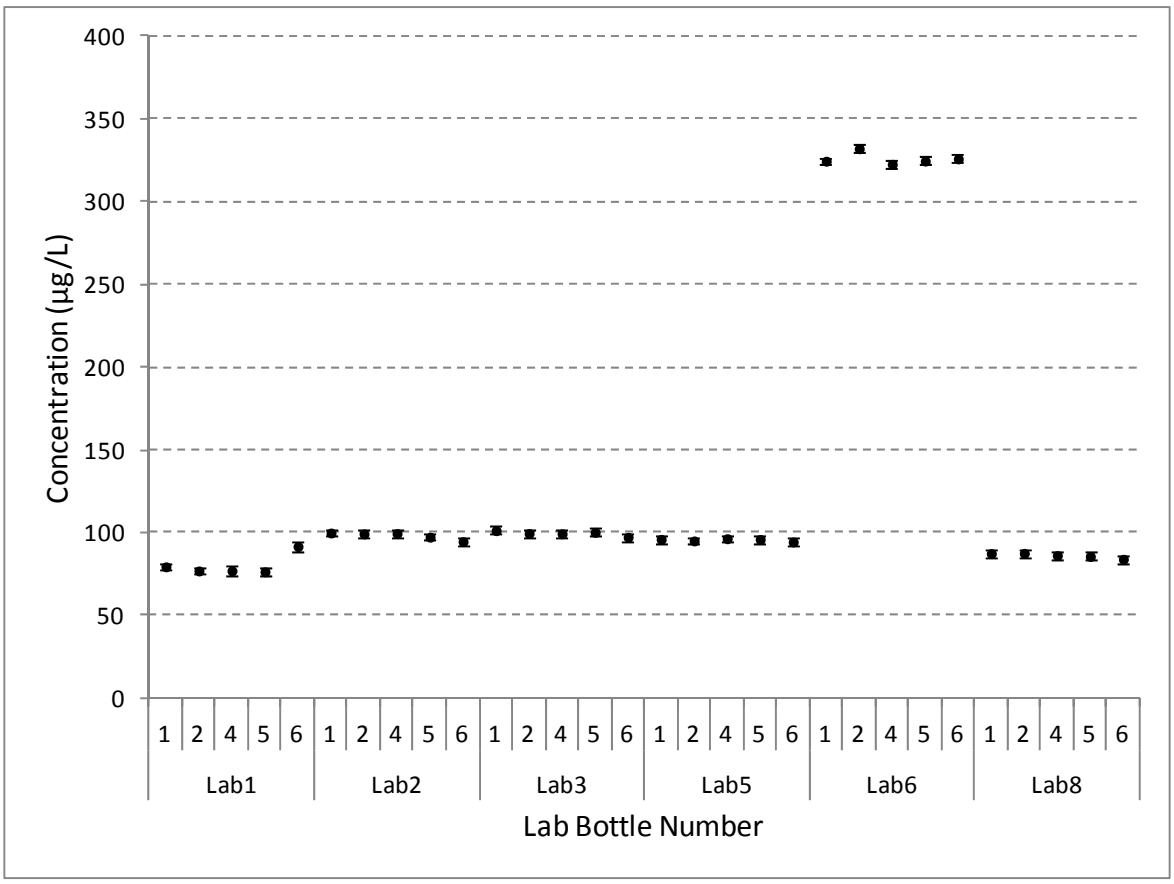

Figure 3. Precision among Low Concentration (100 µg/L) Sample Recoveries for Tetraethylene Glycol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is 95% Confidence Limits.)

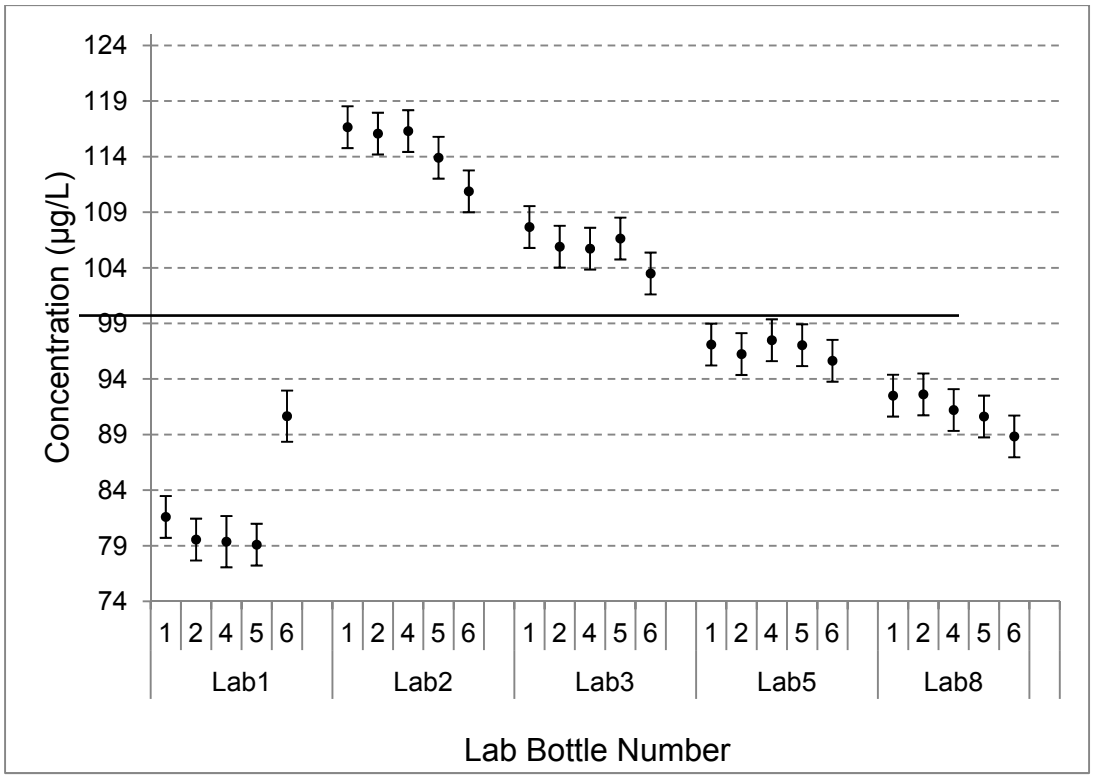

Figure 4. Precision among Low Concentration (100 µg/L) Sample Recoveries for Tetraethylene Glycol among the Analytical Laboratories excluding Laboratory 6 Data. (Closed Circle is Mean Concentration and Whisker is 95% Confidence Limits. The Points with No Bottle Numbers are for High Concentration Samples.)

The influence of the different matrices used to make the blind samples, upon first examination, appear to have a strong influence on the analytical results at the low concentration level (Figure 5). Matrices FS-1 and FS-3 have means outside the ±30% acceptance criterion. In contrast, matrices FS-2 and FS-4 fall very close to the known concentrations and well within the ±20% accuracy acceptance criterion. These anomalous findings are the results of the influence of the high biased concentrations found in the low-level sample concentrations from Laboratory 6 (Figure 2). Once the results from Laboratory 6 are removed from the statistical analysis, the data indicate that there are no matrix affects among the analytical laboratories and all mean concentrations are statistically similar ($p > 0.5$) and fall within the ±20% accuracy acceptance criterion (Figure 6). Similarly, no matrix effects were observed among the analytical laboratories at the high concentration levels (Figure 7) with all mean concentrations falling within the ±20% accuracy acceptance criterion.

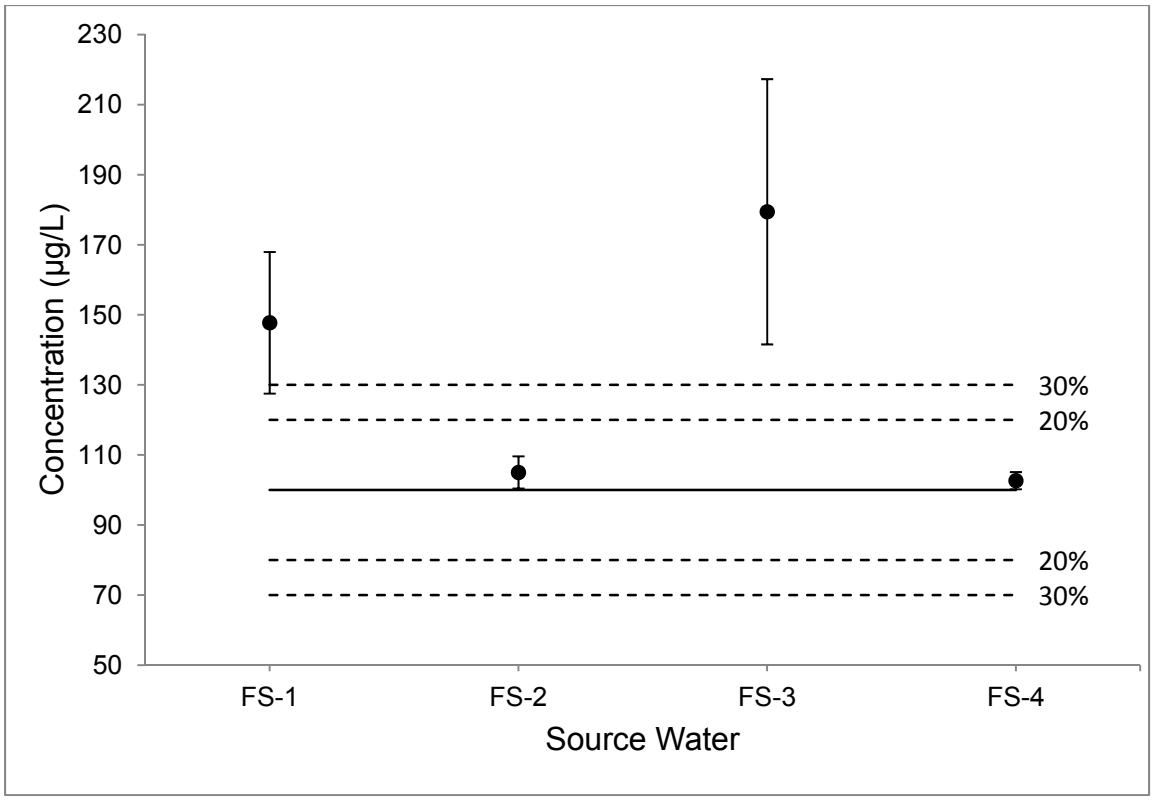

Figure 5. **Low Concentration (100 µg/L) Average Recovery for Tetraethylene Glycol by Matrix. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)**

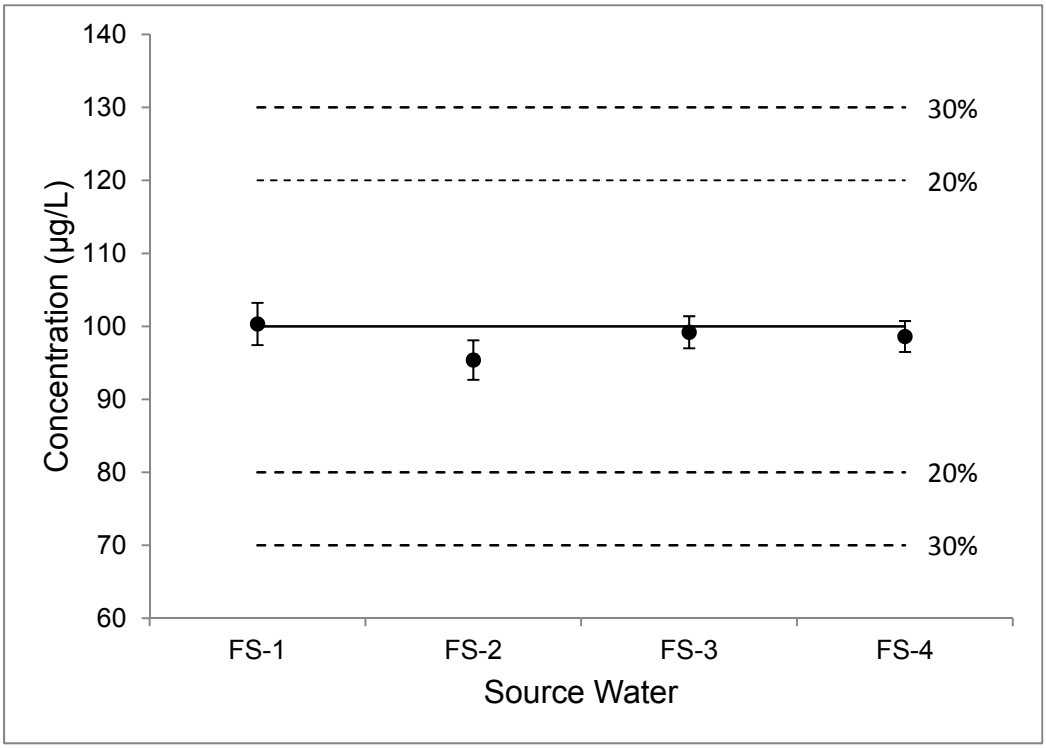

Figure 6. **Low Concentration (100 µg/L) Average Recovery for Tetraethylene Glycol by Matrix excluding Laboratory 6 Data. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)**

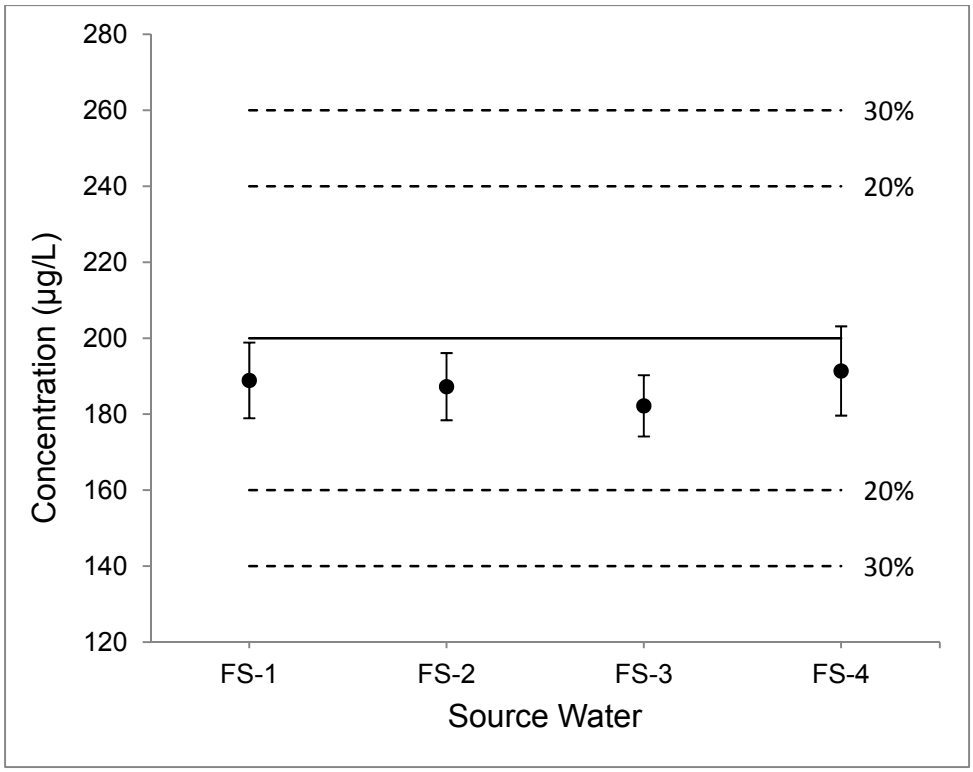

Figure 7. High Concentration (200 µg/L) Average Recovery for Tetraethylene Glycol by Matrix. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

4.2 Triethylene Glycol

All six participating laboratories provided data for triethylene glycol. All submitted blind blank samples (see Table 3) showed no target analytes at or above the reporting limits of 5 or 8 µg/L, depending on the sensitivity of the instrument at the participating analytical laboratory. The blind samples were spiked at 80 or 200 µg/L.

The accuracy among the analytical laboratories was within 20% of the known concentrations, except for Laboratory 6 for the high level spiked samples which fell within the ±30% acceptance criterion (Figures 8 and 9). The results for any given laboratory included all samples, regardless of matrix, at the given concentration level. A slight difference was seen in the results when the concentrations were determined using either linear or quadratic calibration curves. When the concentrations of triethylene glycol are calculated using the quadratic calibration curve, Laboratory 6 falls outside the ±20% acceptance limit for the low level spiked sample but within the ±30% acceptance criterion while for the high level spiked sample, Laboratory 6 falls outside the ±30% acceptance criterion.

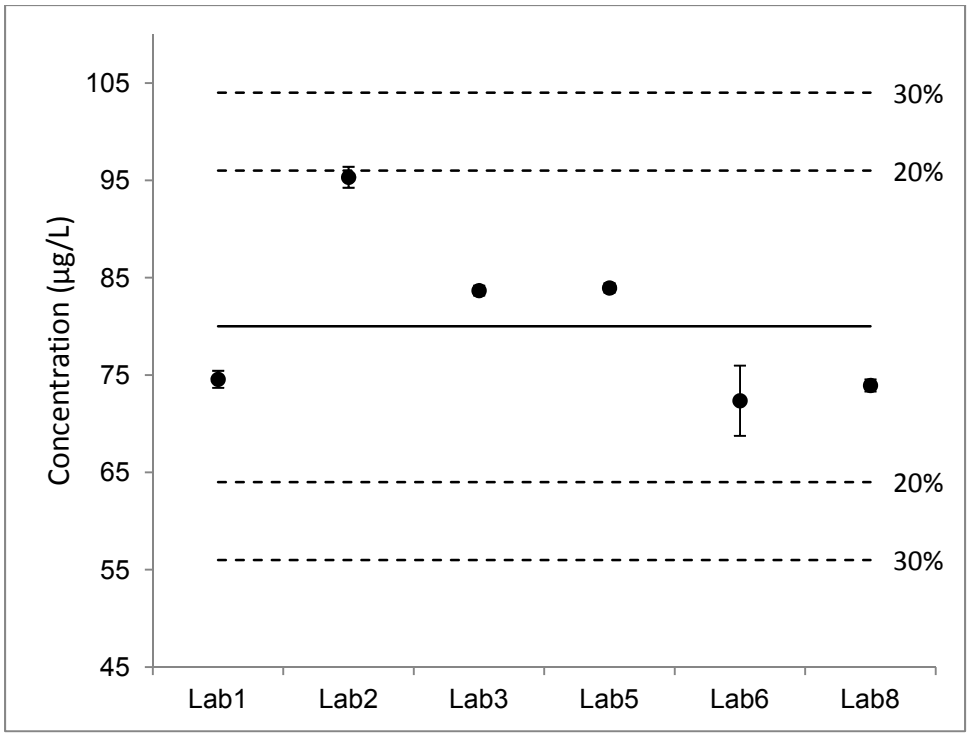

Figure 8. **Low Concentration (80 µg/L Spike) Average Recovery for Triethylene Glycol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)**

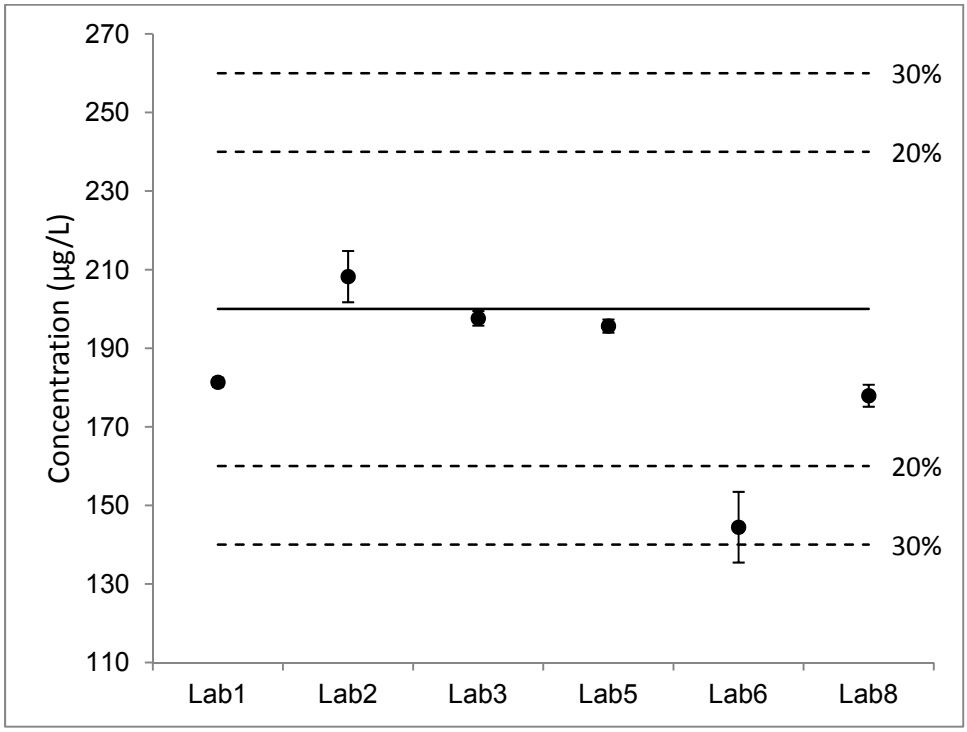

Figure 9. **High Concentration (200 µg/L Spike) Average Recovery for Triethylene Glycol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)**

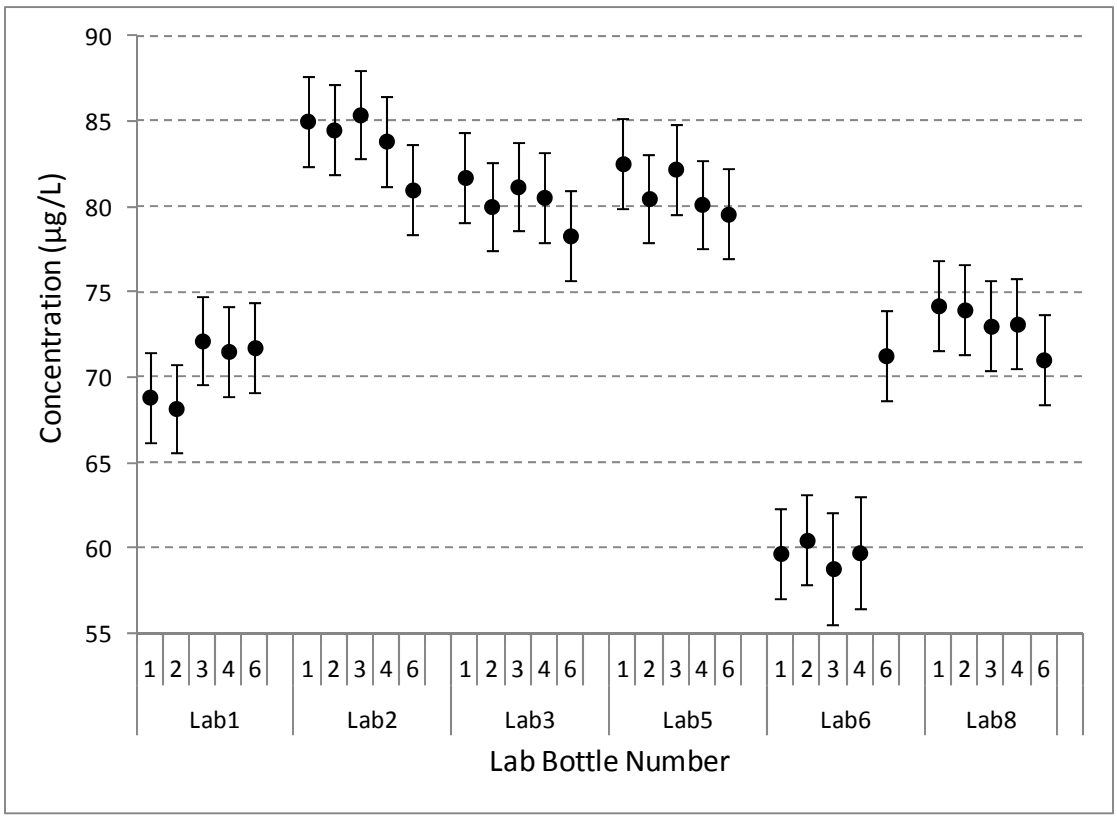

Figure 10. Precision among Low Concentration (80 µg/L) Sample Recoveries for Triethylene Glycol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is 95% Confidence Limits. The Points with No Bottle Numbers are for High Concentration Samples.)

The precision within the laboratory can be determined by examining the results of the individual bottles shipped to the analytical laboratory. In each case, the bottle number (represented as the last digit on the x-axis identifiers) represents the mean of the four different water matrices for that bottle number (Figure 10). By checking significant differences among the 5 bottles spiked at the 80 µg/L concentration, the precision within an analytical laboratory was determined. In general, precision met the performance criteria among all the bottles spiked at the same concentrations. The results from Laboratory 6 showed that bottle 6 was statistically different ($p<0.003$) than the other four bottles received at the laboratory.

The influence of the different matrices used to make the blind samples showed a slight statistical difference for the FS-2 matrix, which was slightly lower than the FS-4 matrix samples (Figure 11). This anomaly in mean values may be the result of very tight precision within the participating analytical laboratories for each matrix and slight accuracy differences resulting in the appearance of meaningful differences when none really exist. No matrix effects were seen for the high concentration blind samples among the analytical laboratories (Figure 12) with all mean concentrations being statistically similar ($p > 0.52$) and falling within the ±20% accuracy acceptance criterion.

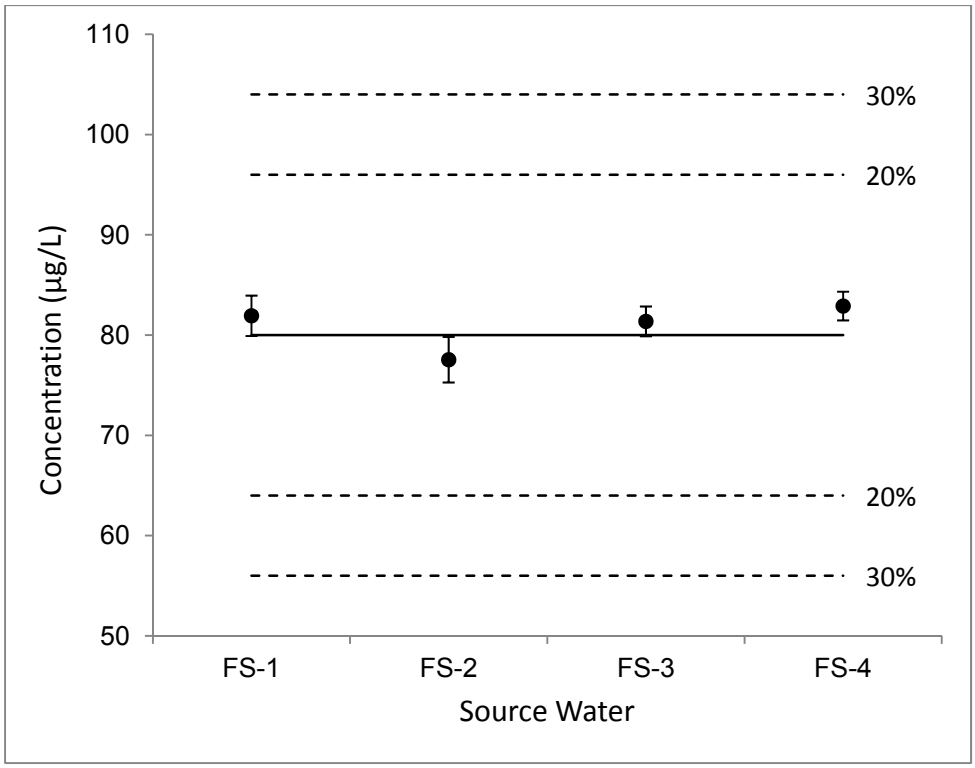

Figure 11. Low Concentration (80 µg/L) Average Recovery for Triethylene Glycol by Matrix. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

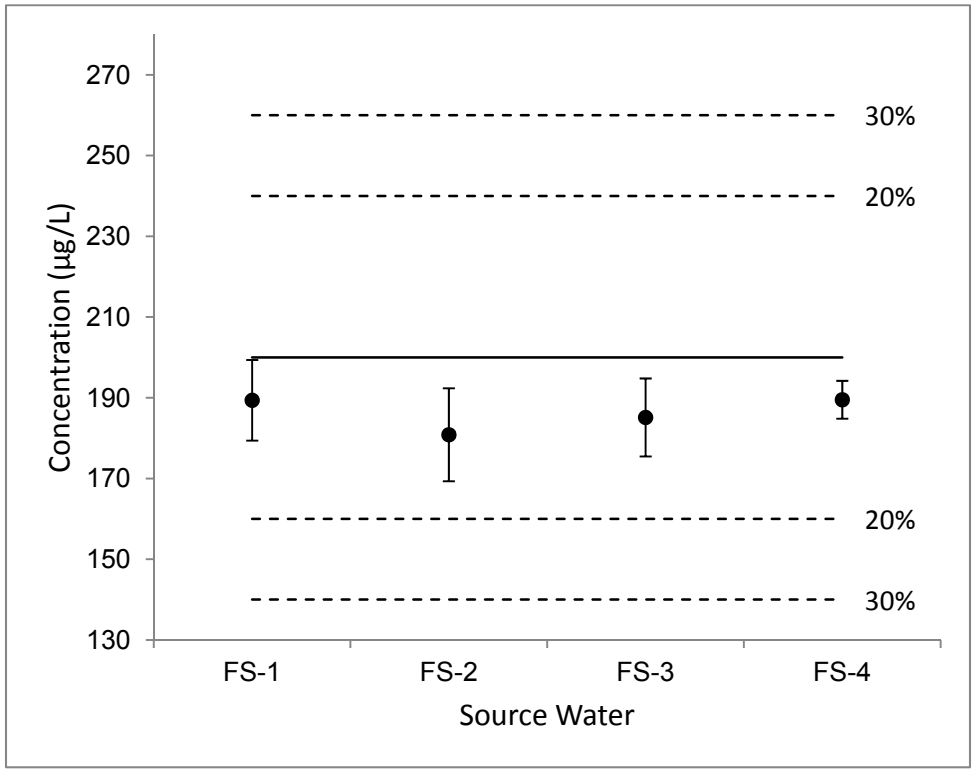

Figure 12. High Concentration (200 µg/L) Average Recovery for Triethylene Glycol by Matrix. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

4.3 Diethylene Glycol

All six participating laboratories provided data for diethylene glycol. All submitted blind blank samples (see Table 3) showed no target analytes at or above the reporting limits of 5 or 8 µg/L, depending on the sensitivity of the instrument at the participating laboratory. The blind samples were spiked at 10 or 100 µg/L.

A non-normal data distribution was found when using both spiked sample concentrations together; therefore, the two populations (i.e., low and high spiked datasets) were treated separately statistically. The primary cause for the existence of the two populations is believed to be a result of the low level spiked sample concentration being so close to the reporting limits.

The accuracy among the participating analytical laboratories was within 20% of the known concentrations for the low level spiked samples for Laboratories 1 and 5 and within ±30% for Laboratory 3 (Figure 13). Laboratory 6 did not report any data for the low level spiked samples as the spike levels were too close to their laboratory reporting limit. Laboratory 2 results were high and Laboratory 8 results were low for the low level spiked samples with both laboratories exceeding the ±30% acceptance criterion. For the high level spiked samples, all analytical laboratories fell within the ±20% acceptance criterion (Figure14) except Laboratory 2 which had results above the 20% acceptance criterion.

The precision within the laboratory for diethylene glycol was determined by examining the results of the individual bottles shipped to the analytical laboratory. In each case, the bottle number (represented as the last digit on the x-axis identifiers) represents the mean of the four different water matrices for that bottle number (Figure 15). By checking significant differences among the 5 bottles spiked at the 10 µg/L concentration, the precision within an analytical laboratory was determined. In general, precision meeting the performance criteria was seen in Laboratories 5 and 8 while variability among all the bottles spiked existed within each of the other participating laboratories. This variability within the laboratories may be due to the closeness of the spike concentration (10 µg/L) to the reporting limits (5 µg/L).

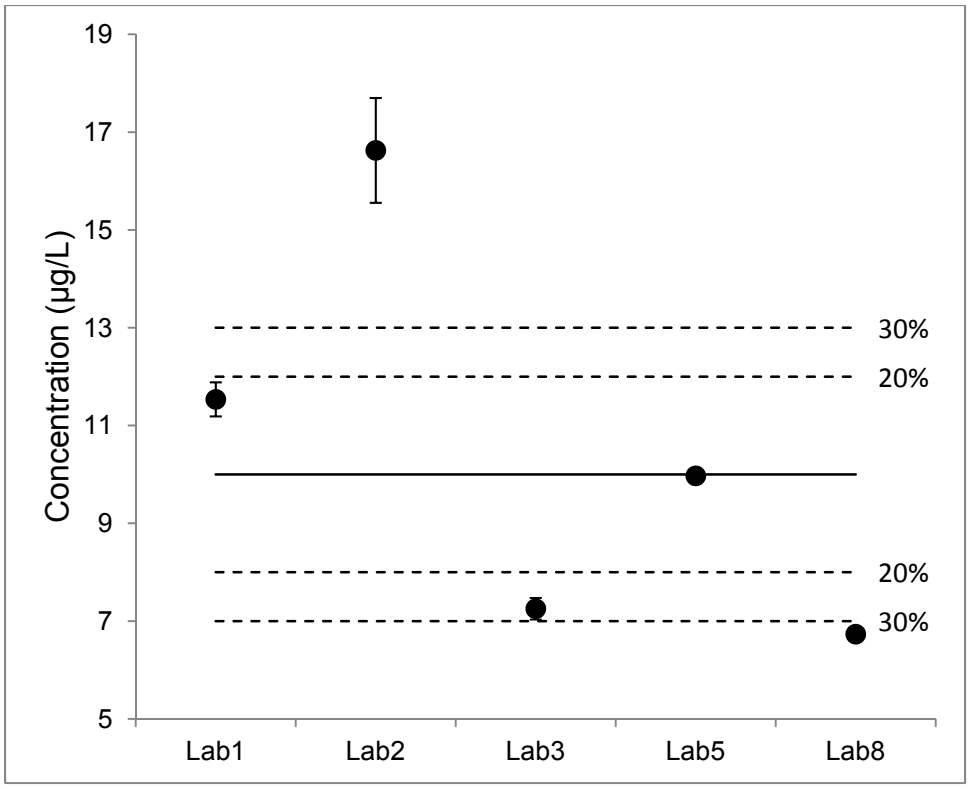

Figure 13. **Low Concentration (10 µg/L Spike) Average Recovery for Diethylene Glycol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)**

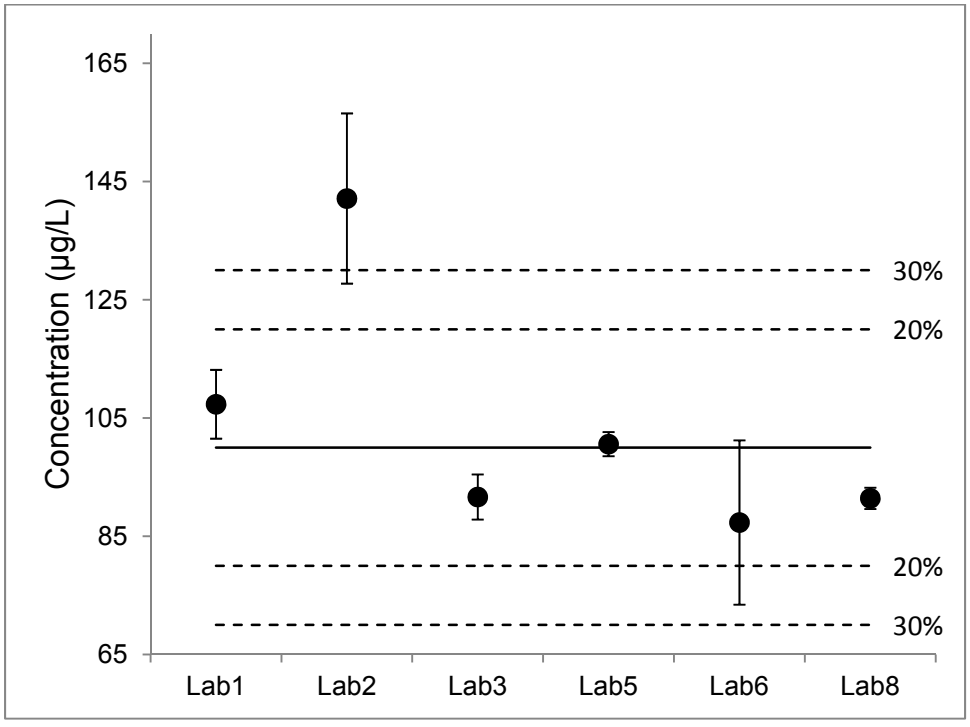

Figure 14. **High Concentration (100 µg/L Spike) Average Recovery for Diethylene Glycol among the Analytical Laboratories. (Closed circle is Mean Concentration and Whisker is One Standard Error.)**

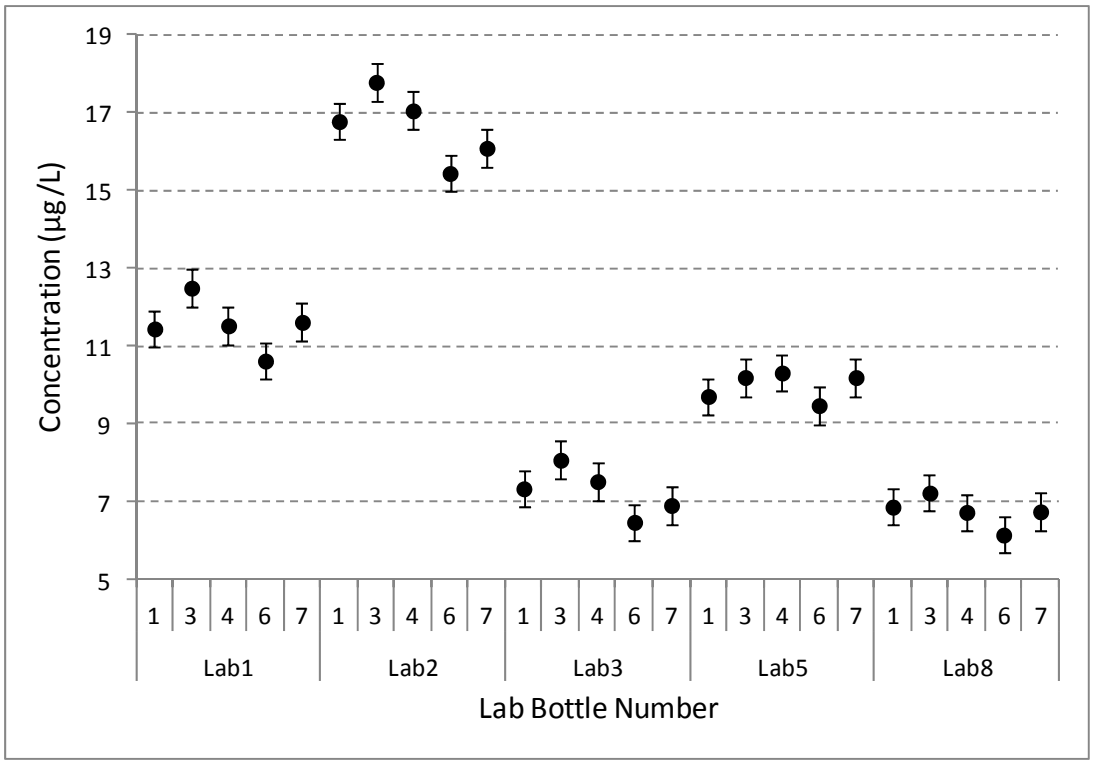

Figure 15. Precision among Low Concentration (10 µg/L) Sample Recoveries for Diethylene Glycol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is 95% Confidence Limits.)

No matrix effects were seen at both high and low diethylene glycol concentration among the analytical laboratories (Figures 16 and 17) with no significant difference among all the mean diethyl glycol concentrations ($p > 0.6$ for Figure 20 and $p > 0.25$ for Figure 21) and falling within the ±20% accuracy acceptance criterion.

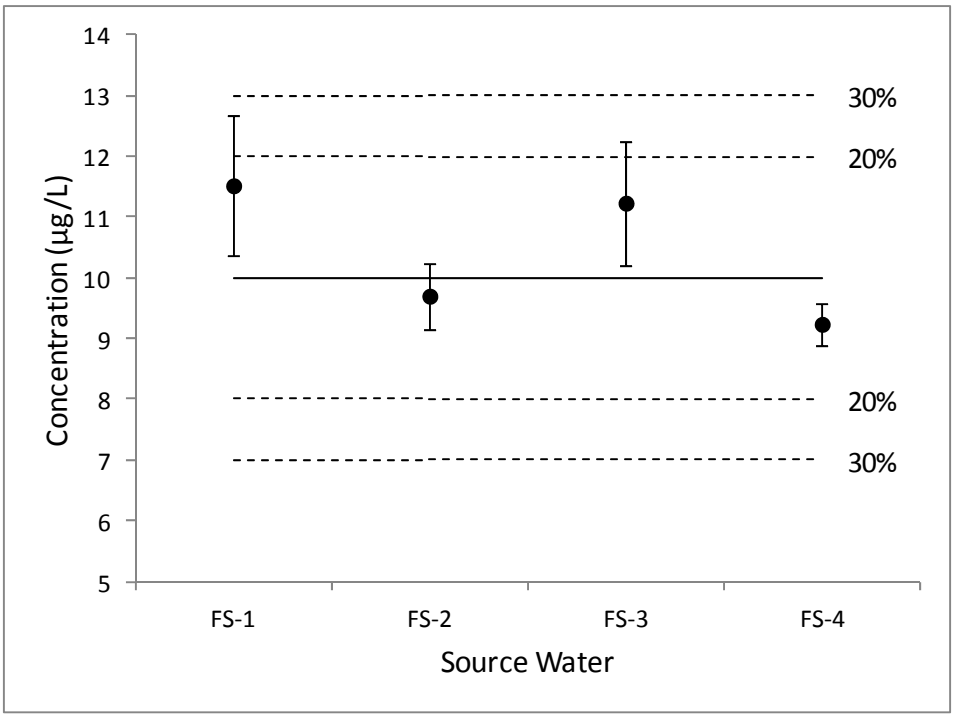

Figure 16. Low Concentration (10 µg/L) Average Recovery for Diethylene Glycol by Matrix. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

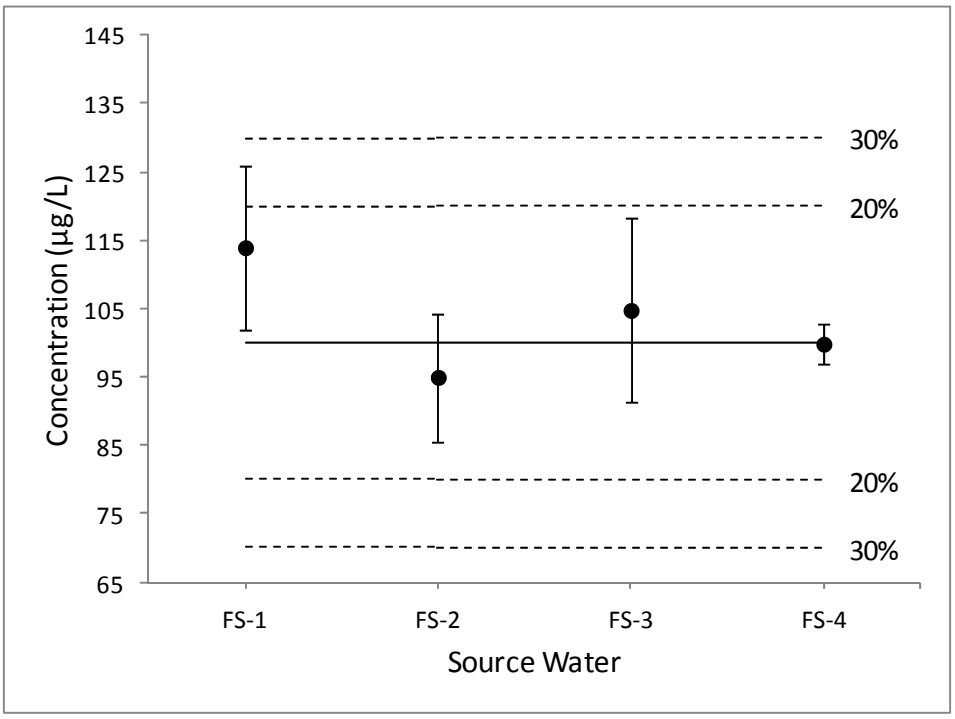

Figure 17. High Concentration (100 µg/L) Average Recovery for Diethylene Glycol by Matrix. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

4.4 2-Butoxyethanol

Five participating laboratories provided data for 2-butoxyethanol. Laboratory 1 could not detect 2-butoxyethanol on their instrument. All submitted blind blank samples (see Table 3) showed no target analytes at or above the reporting limits of 5, 8, or 10 µg/L, depending on the sensitivity of the instrument at the participating analytical laboratory. The blind samples were spiked at 60 or 180 µg/L.

The accuracy among the analytical laboratories was within 20% of the known concentrations for both the low level and high level spiked samples in all analytical laboratories (Figures 18 and 19).

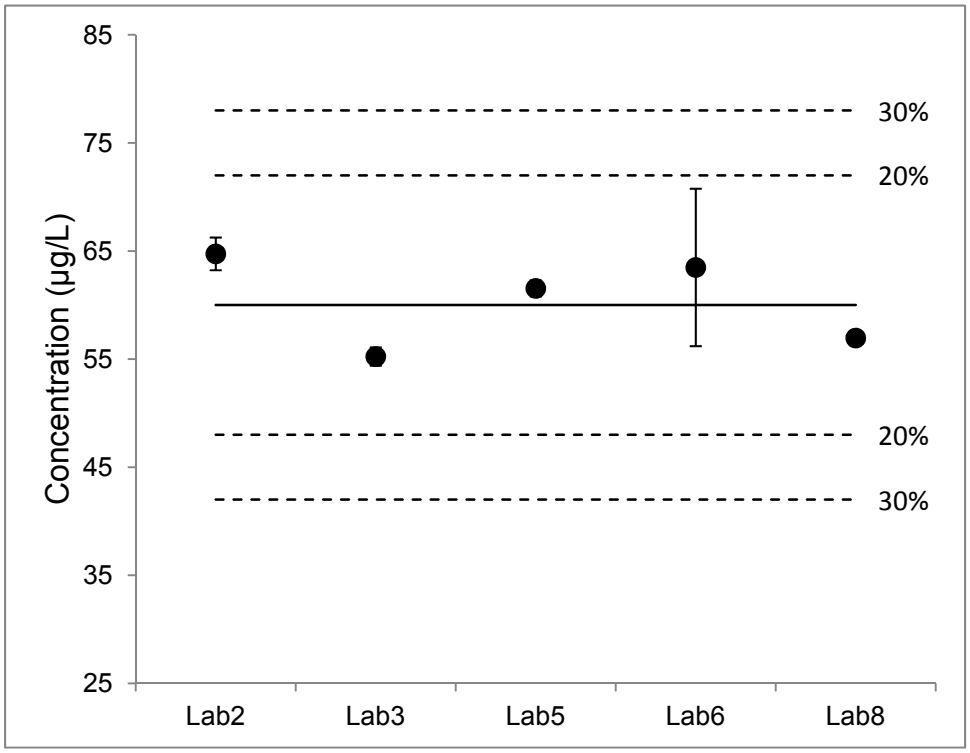

Figure 18. Low Concentration (60 µg/L Spike) Average Recovery for 2-Butoxyethanol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

The precision within the laboratory can be determined by examining the results of the individual bottles shipped to the analytical laboratory. In each case, the bottle number (represented as the last digit on the x-axis identifiers) represents the mean of the four different water matrices for that bottle number (Figure 20). By checking significant differences among the 5 bottles spiked at the 60 µg/L concentration, the precision within each analytical laboratory was determined. No statistically significant differences were found among the bottles within the individual participating laboratory ($p>0.43$).

No matrix effects ($p>0.10$) were seen for either concentration blind samples among the participating laboratories (Figures 21 and 22) with all mean concentrations being statistically similar and falling within the ±20% accuracy acceptance criterion.

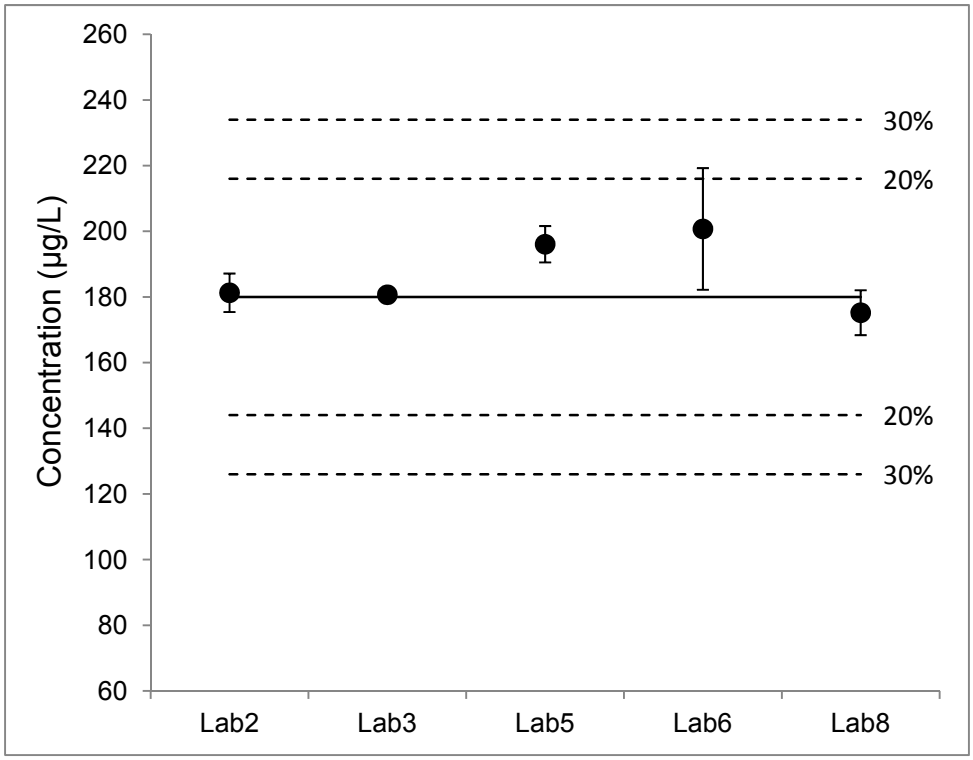

Figure 19. High Concentration (180 µg/L Spike) Average Recovery for 2-Butoxyethanol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

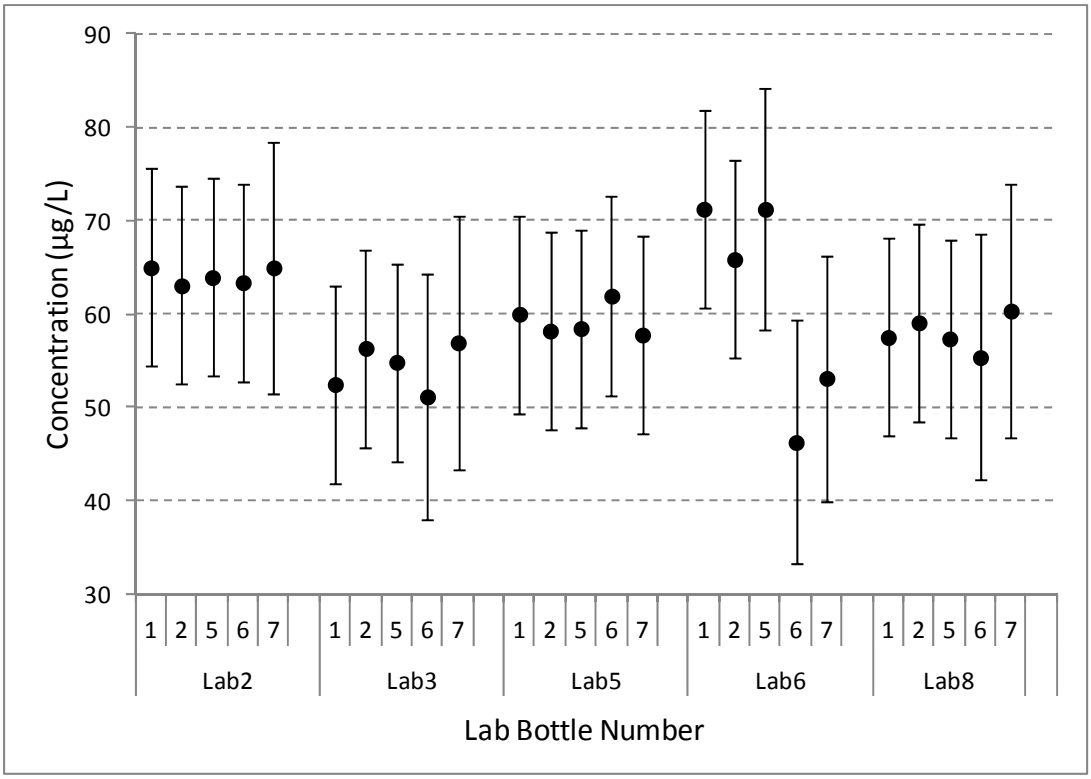

Figure 20. Precision among Low Concentration (60 µg/L) Sample Recoveries for 2-Butoxyethanol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is 95% Confidence Limits. The Points with No Bottle Numbers are for High Concentration Samples.)

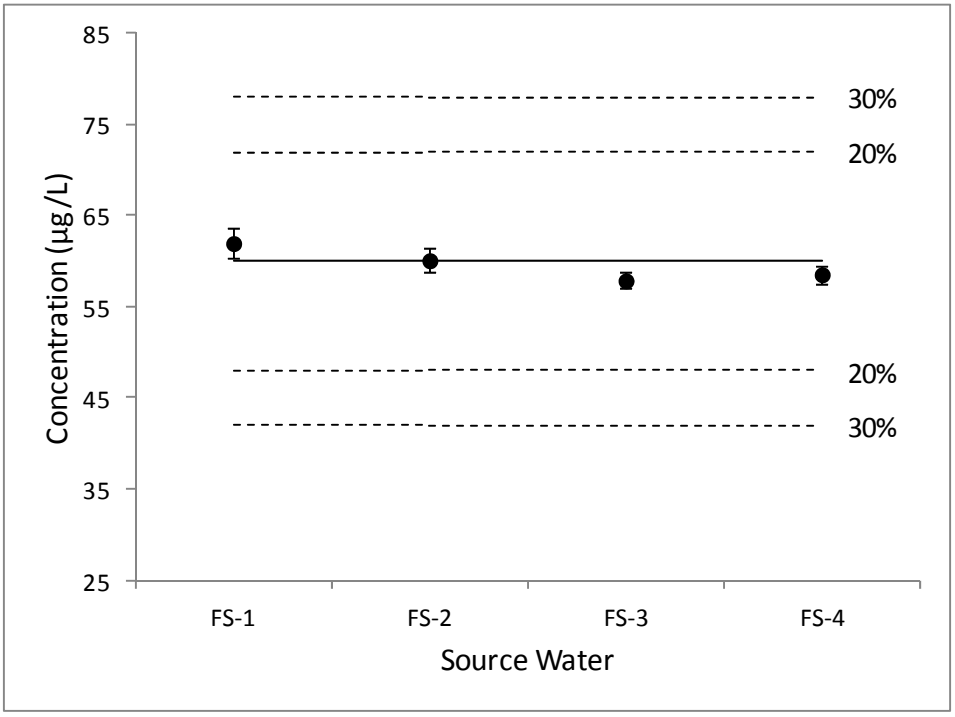

Figure 21. Low Concentration (60 µg/L) Average Recovery for 2-Butoxyethanol by Matrix. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

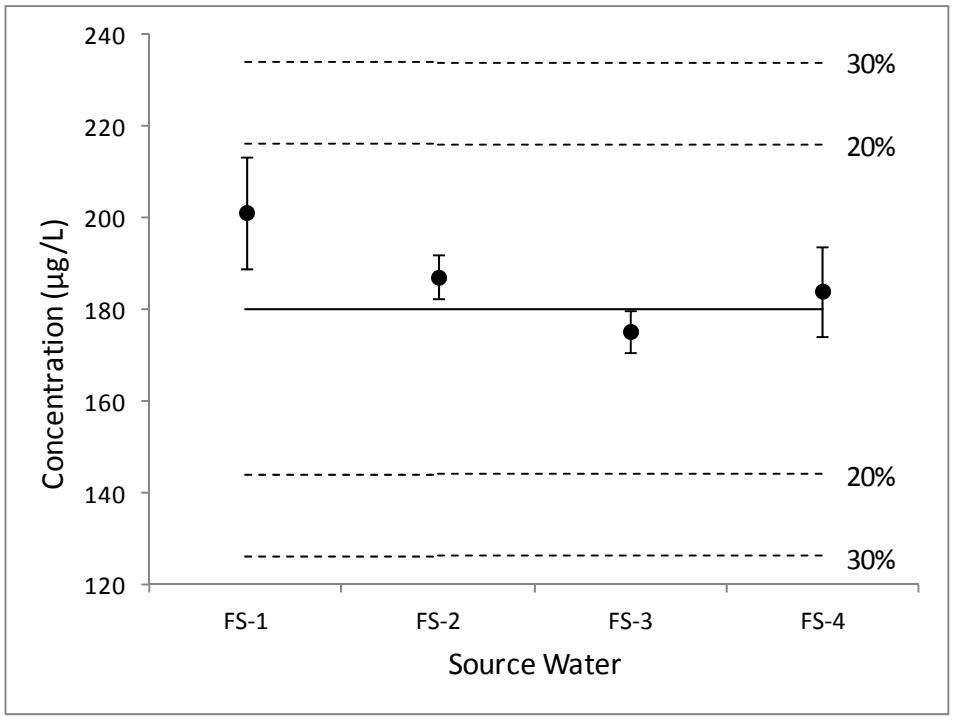

Figure 22. High Concentration (180 µg/L) Average Recovery for 2-Butoxyethanol by Matrix. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

4.5 2-Methoxyethanol

Four laboratories provided data for 2-methoxyethanol. Laboratories 1 and 3 could not detect 2-methoxyethanol on their analytical instruments. All submitted blind blank samples (see Table 3) showed no target analytes at or above the reporting limits of 5, 20, or 25 µg/L, depending on the sensitivity of the instrument at the analytical laboratory. The blind samples were spiked at 40 or 100 µg/L.

At the higher concentration, Laboratory 6 was significantly different from the others laboratories ($p<0.05$); whereas, Laboratory 5 was significantly different only from Laboratory 2 ($p<0.001$). At the lower concentration, there were no significant differences between labs ($p> 0.136$). The accuracy among the analytical laboratories was within 20% of the known concentrations for both the low level and high level spiked samples in all analytical laboratories with the exception of the 40 µg/L samples analyzed at Laboratory 6 (Figures 23 and 24). Laboratory 6 results were positively biased and were just slightly higher than the ±30% acceptance limit.

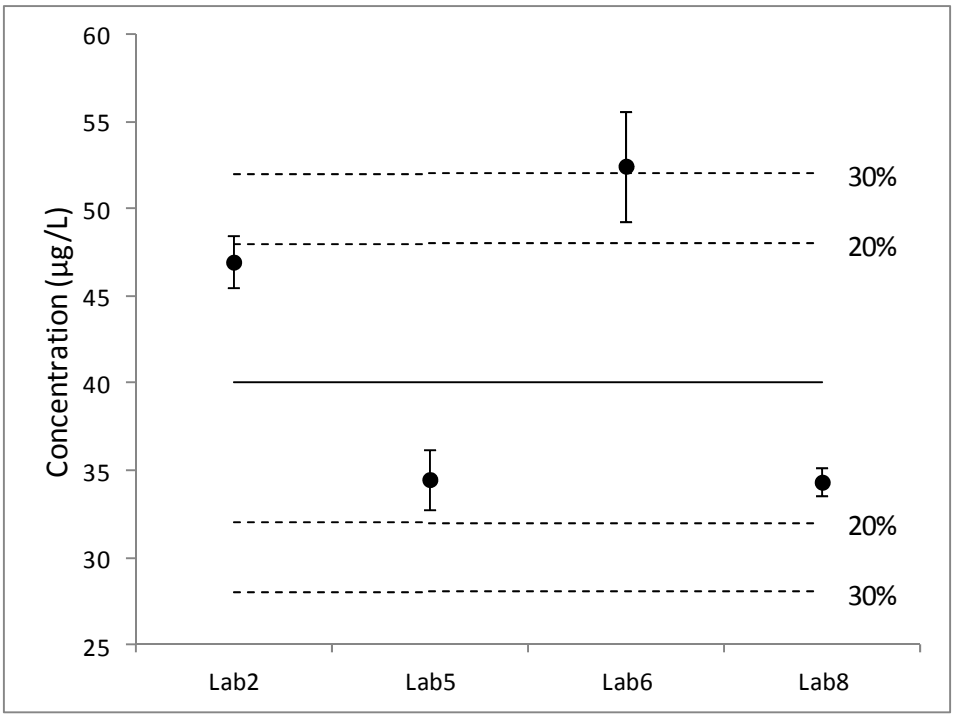

Figure 23. Low Concentration (40 µg/L Spike) Average Recovery for 2-Methoxyethanol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

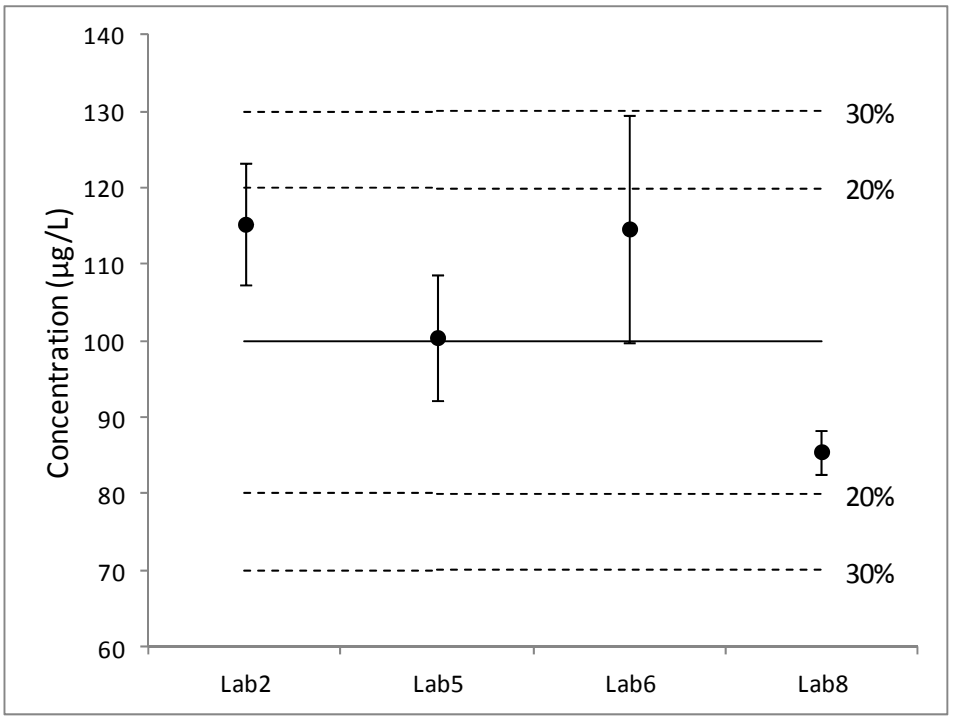

Figure 24. High Concentration (100 µg/L Spike) Average Recovery for 2-Methoxyethanol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

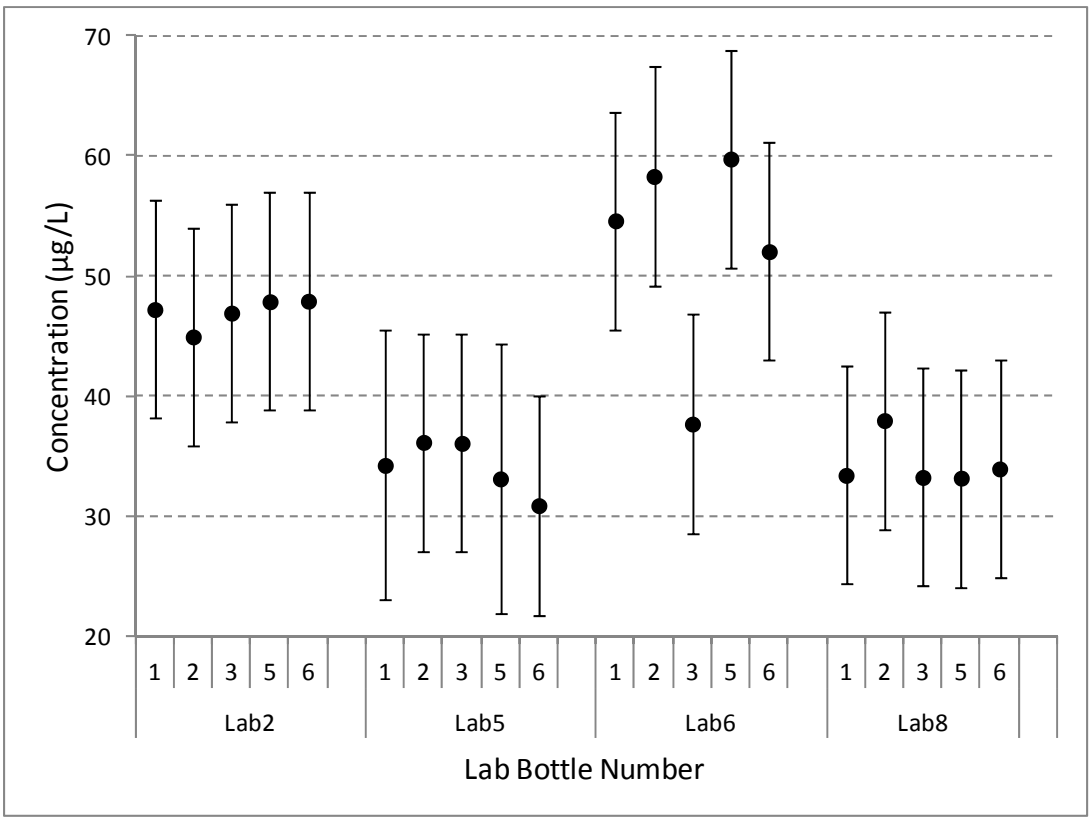

Figure 25. Precision among Low Concentration (40 µg/L) Sample Recoveries for 2-Methoxyethanol among the Analytical Laboratories. (Closed Circle is Mean Concentration and Whisker is 95% Confidence Limits. The Points with No Bottle Numbers are for High Concentration Samples.)

The precision within the laboratory was determined by examining the results of the individual bottles shipped to the analytical laboratory. In each case, the bottle number (represented as the last digit on the x-axis identifiers) represents the mean of the four different water matrices for that bottle number (Figure 25). By checking significant differences among the 5 bottles spiked at the 40 µg/L concentration, the precision within an analytical laboratory was determined. No statistically significant differences ($p>0.14$) were found among the bottles within the individual participating laboratory except for Laboratory 6 which showed statistical differences, but not significant differences, between bottle 3 and bottles 2 ($p>0.22$) and 5 ($p>0.14$; Figure 25).

No matrix effects were seen for either concentration blind samples ($p >0.083$). Overall mean concentrations for water matrices are within the ±20% accuracy acceptance criterion (Figures 26 and 27).

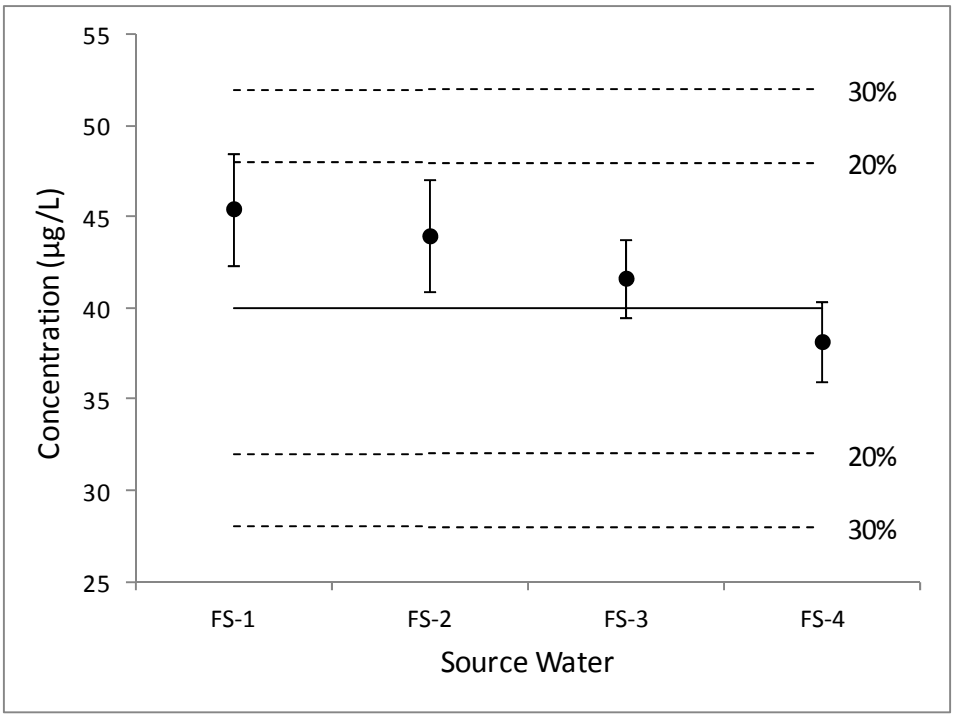

Figure 26. Low Concentration (40 µg/L) Average Recovery for 2-Methoxyethanol by Matrix. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

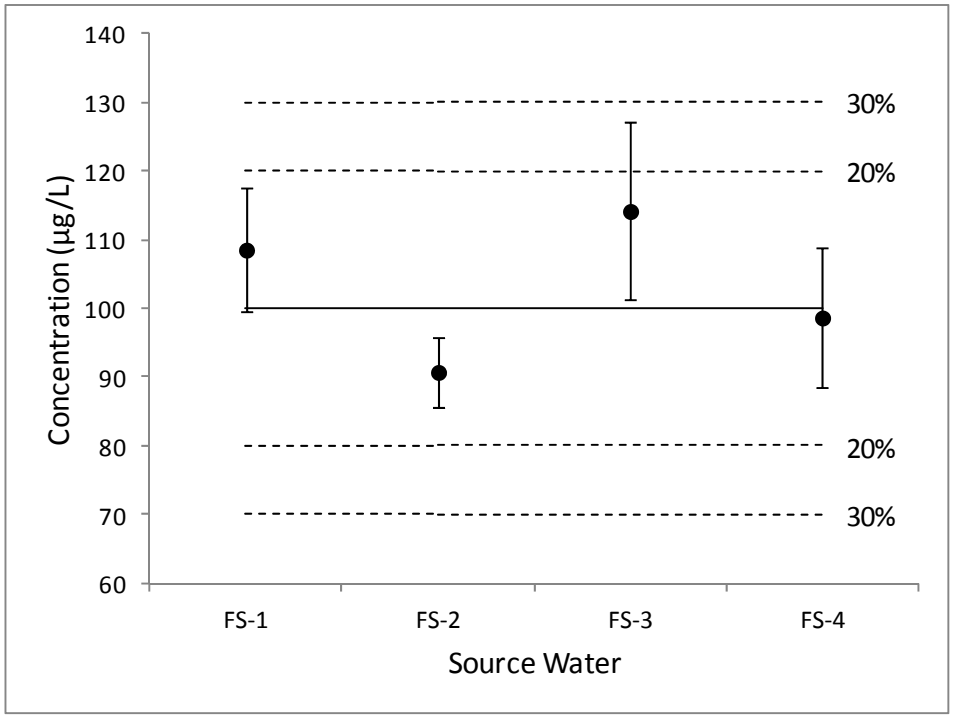

Figure 27. High Concentration (100 µg/L) Average Recovery for 2-Methoxyethanol by Matrix. (Closed Circle is Mean Concentration and Whisker is One Standard Error.)

5.0 Summary and Conclusions

Several key factors were used to determine the performance characteristics of the draft glycol method including: detectability of the compounds, accuracy, precision, and the presence/absence of matrix effects. A batch of 36 blind samples spiked with the five compounds of interest in four different water matrices was submitted to six analytical laboratories. Each laboratory was provided with a copy of the draft glycol SOP and asked to optimize their LC/MS/MS system to perform the analyses of the blind samples. Once optimized, the blind samples were analyzed following the QA/QC requirement identified in the project QAPP. Each laboratory provided the resultant data as determined using both linear and quadratic calibration curves. The data were statistically analyzed, after checking the data for normalcy, by running least square means analyses to determine if statistical differences existed among the laboratories and if the data fit within the acceptance criterion for the key factors of interest.

Diethylene glycol, triethylene glycol, and tetraethylene glycol were detectable at all participating analytical laboratories. 2-butoxyethanol could not be successfully detected at Laboratory 1 while Laboratories 1 and 3 could not successfully detect 2-methoxyethanol.

While statistical differences between laboratories were present, the accuracy of the laboratory analyses were typically within the acceptance criteria of either ±20 or 30% of known value. A few notable exceptions were identified including:

a) Laboratory 6 was not within ±30% for tetraethylene glycol with a positive bias at the low concentration,
b) Laboratory 6 was not with ±20% for triethylene glycol with a negative bias at the high concentration,
c) Laboratory 2 was not within ±30% for diethylene glycol with a positive bias at the low concentration,
d) Laboratories 3 and 8 were not within ±20% and ±30%, respectively, for diethylene glycol with a negative bias at the low concentration, and
e) Laboratory 6 was not within ±30% for 2-methoxyethanol with a positive bias at the low concentration.

Precision within the analytical laboratories met the performance criteria indicating that reproducible results were being produced at the analytical laboratories. All replicate bottles were statistically the same with the exceptions of diethylene glycol across the analytical laboratories and 2-methoxyethanol in Laboratory 6. The variability within the laboratories during the analysis of diethylene glycol was most likely due to the closeness of the spike concentration (10 µg/L) to the reporting limits (5 or 8 µg/L). Laboratory 6 did not report any concentrations for diethylene glycol as a result of the spiked concentration of the low level sample being too close to their reporting limit.

Matrix effects were not identified for the tested water matrices at any of the analytical laboratories indicating that the method could produce the same results in the four water matrices tested.

Overall, the draft glycol SOP presented a method that was accurate and precise, when the compounds of interest were detectable, by meeting the established performance criterion nearly all the time. Further, the draft method exhibited no matrix effects in the four waters tested for any of the compounds of interest. The detection of 2-ME and 2-BE was problematic and instrument/laboratory dependent. With the few method variations and QA/QC deviations that were identified throughout the study among the analytical laboratories, a strong QA/QC program to monitor the resultant data is essential. The QA/QC program should incorporate blind samples of known concentrations to ensure quality of the resultant data and that the results are within the specified accuracy acceptance limits.

6.0 Recommendations

The interlaboratory verification of the draft glycol SOP resulted in some recommendations in order to further improve the analytical method. These recommendations are presented below:

a) The draft glycol SOP states that at least a 5-point initial linear calibration curve should be used. This restriction was found to limit the effectiveness of the method. Many calibration curves generated during the study were best fit using quadratic formulae yet the linear curves were also acceptable. The deviations from the calibration curve were lessened, especially at the low concentration standards when incorporating a quadratic fit. A minimum 6-point quadratic fit should be allowed with stipulations on the allowable deviations of the calibration points from the curve, such as ±25% deviation from the curve with a correlation coefficient, $r^2 \geq 0.99$.

b) Surrogate spikes should be incorporated in order to have recovery data with every sample. There are now commercially available isotopically labeled 2-BE and Di-EG that do not require custom synthesis. Recovery limits should be set in reagent water and matrix waters for the surrogate spikes with correlations back to the native analytes.

c) There are no preservation or holding time studies of these analytes in reagent water or matrices of concern. A fourteen day holding time was used for this study. Studies should be conducted to determine if holding at 4 ±2º C is adequate to preserve sample integrity. The multi-laboratory study did, however, demonstrate that the matrices studied did not affect the integrity of the spiked sample from the time of collection to the time of analysis in the participating laboratories.

d) The preparation of samples in the laboratory does not require a filtering procedure but leaves it as an option to use a 0.45 µm Teflon® filter unit. There are no data presented demonstrating the performance of a filter unit and if target analyte contamination is an issue. Various glycols may be used in the manufacturing or cleaning of the filter units and may cause bias in the results. A filter unit study should be conducted to determine their performance and effects on contaminant concentrations.

e) The liquid chromatography conditions in the draft glycol SOP rise to a maximum of 15% acetonitrile. This may result in a build-up of organic contaminants on the column that will lessen the performance over time. A higher organic content gradient for the acetonitrile should be used in order to elute non-target analytes from the column with each injection cycle.

f) Mass calibration/tuning appear to be required annually in the draft glycol SOP. This should be checked routinely and re-calibration should be required before analysis if mass shift is noticed that will affect the sample results.

g) Second source standards are an issue with this method. Standard concentrations need to be verified between the different vendors.

h) The final CCV should have a concentration near the mid-point of the calibration curve. During this verification study, varying CCV concentrations covering the calibration curve range were used and depending on the concentration selected, different recovery biases may be encountered.

7.0 References

(1) US EPA Office of Pollution Prevention and Toxics: High Production Volume (HPV) Challenge. http://www.epa.gov/hpv/pubs/general/opptsrch.htm, Accessed October 25, 2012.

(2) Sorensen, J. A.; Gallagher, J. R.; Hawthorne, S. B.; Aulich, T. R., JV Task 3 - Gas Industry Groundwater Research Program: Final Report. DOE NETL, 2000.

(3) US EPA Office of Water: Evaluation of Impacts to Underground Sources of Drinking Water by Hydraulic Fracturing of Coalbed Methane Reservoirs (816-R-04-003). Washington, DC, 2004.

(4) Quality Assurance Project Plan for the Multi-Laboratory Verification of Diethylene Glycol, Triethylene Glycol, 2-Butoxyethanol and 2-Methoxyethanol in Ground and Surface Waters by Liquid Chromatography/Tandem Mass Spectrometry. http://www2.epa.gov/sites/production/files/documents/glycol-qapp-rev0-0.pdf

(5) Peterson R.G. 1985. Design and Analysis of Experiments. Marcel Dekker, Inc. New York. 1985.

(6) Federer W. T. (1955) Experimental design. Oxford & IBH publishing CO. PVT. LTD. New Delhi.

Appendix A

Glycol Analysis of Aqueous Samples by Direct Injection HPLC/MS/MS

Glycol Analysis of Aqueous Samples by Direct Injection HPLC/MS/MS

Effective Date: March 2012

EPA Region 3
Office of Analytical Services and Quality Assurance
701 Mapes Road
Fort Meade, Maryland 20755

Approved by:

Jill Bilyeu
Quality Assurance Officer

Date

Prepared by: Jennifer L. Gundersen, Ph.D.
Chemist, Laboratory Branch

Reviewed by:
Peer-Reviewer, Laboratory Branch

Updates Table

Peer reviewer's initials indicate that changes meet the NELAC and regulatory requirements described in Section 9.4 in SOP R3-QA060

Responsible Person	Date	Description of Change	Peer Reviewer	Date
		New SOP		

1 Scope and Application

1.1 This Standard Operating Procedure (SOP) documents and provides a descriptive method to perform glycol analysis by HPLC/MS/MS on liquid matrices.

1.2 This SOP is based on EPA SW-846 Method 8321B, 8000C and ASTM D7731-11^{E1} and applies to the measurement of glycols listed in Table 1.

Table 1: Analyte List

Analyte	CAS #	MDL (aqueous, ug/l)	NQL (aqueous, ug/l)
Diethylene glycol	111-46-6	In prep	25
Triethylene glycol	112-27-6	In prep	25
Tetraethylene glycol	112-60-7	In prep	25
2-Butoxyethanol	111-76-2	In prep	5
2-Methoxyethanol	109-86-4	In prep	10

2 Summary of the Method

2.1 The method employs high performance liquid chromatography (HPLC) coupled with positive electrospray ionization (ESI+) tandem mass spectrometry (MS/MS) for the determination of a suite of glycols in aqueous matrices.

2.2 A sample aliquot is directly injected into the HPLC/MS/MS system without extraction or derivitization. Concentration of each identified analyte is performed through linear, external standard calibration.

2.3 Target compounds are identified by retention time and one or more MRM (Multiple Reaction Monitoring) transition.

3 Definitions

3.1 Refer to the ESC Quality Manual for applicable definitions

3.1.1 MRM: Multiple Reaction Monitoring is the application of selected reaction monitoring to multiple product ions from one or more precursor ions.

4 Interferences

4.1 Suspended solids in the sample can clog frits in the sample management system and on the column. If site history suggests, samples may be filtered prior to introduction to the

HPLC/MS/MS system.

4.2 Matrix interferences may be caused by contaminants in the sample.

4.3 All reusable glassware must be cleaned according to procedures for cleaning glassware used in organic compound analyses per R3QA-054 Glassware Preparation for Organic Analyses.

5 Safety

5.1 Before beginning any procedures, refer to the Chemical Hygiene Plan (CHP) in the OASQA Quality Assurance Manual for general safety precautions and guidelines.

5.2 All sample prep work should be conducted in a fume hood.

5.3 The toxicity or carcinogenicity of each reagent used in this method may not have been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable.

5.4 Material Safety Data Sheets (MSDS) must be maintained in the facility for all reagents used in the laboratory. This information must be made available to all personnel prior to the performance of this SOP and upon staff request. The MSDS (hard copies) are currently located in the library as well as electronically on CD-ROM and online.

5.5 All applicable safety and compliance guidelines set forth by the EPA and by federal, state, and local regulations must be followed during the performance of this SOP. In addition, all procedures outlined in the OASQA Chemical Hygiene Plan must be adhered to. Stop all work in the event of a known or potential compromise to the health and safety of any person and immediately notify the Safety Officer, and other appropriate personnel as outlined in the CHP.

5.6 All laboratory waste must be handled in accordance with guidelines established in the CHP and the appropriate waste disposal procedures identified in Section 15.0 (Waste Management).

5.7 Analysts must be cognizant of all instrumental hazards (i.e. dangers from electrical shock, heat or explosion etc.).

5.8 All chemicals used in the performance of this SOP, as well as the samples, should be handled with caution. Adequate protective gear should be worn. At a minimum, this includes ANSI approved safety glasses and a lab coat to protect from chemical splashes, and powderless gloves made from acid resistant materials such as nitrile, latex, neoprene, butyl or PVC.

5.9 Spill procedures: Follow the procedures outlined in the ESC Occupant Emergency Plan (OEP), Hazardous Material Spills section. For minor spills (which can be handled by the analyst) wear safety glasses, lab coat, and gloves to clean up the material. For significant spills, immediately contact the SHEM Manager.

6 Equipment and Supplies

6.1 HPLC/MS/MS system: Analytical instrument and accessories suitable for automated injection of samples onto analytical HPLC columns and fragmentation and detection by a tandem mass spectrometer.

6.2 System used at R3-ESC: Waters (Milford, MA) TQD HPLC/MS/MS system: equipped with a 1 to 50 µL or 1 to 100 µL loop injector and electrospray (ESI) tandem mass spectrometer (MS/MS) capable of multiple reaction monitoring (MRM) and negative and positive ion mode.

6.3 HPLC column: Waters (Milford, MA) Atlantis dC18 3µm , 2.1 x 150mm. Other columns may be used if they provide sufficient retention and separation of the target analytes.

6.4 Data System: Computer system with software capable of accepting and processing raw detector data from the HPLC/MS/MS. The system must have the following capabilities:

Integrate peaks from raw data.
Provide peak height and peak area information.
Calculate and store calibration information.
Identify peaks of interest by retention time.
Quantitate peaks of interest using calibration obtained.
Produce chromatograms.
Allow overlay and comparison of chromatograms.
Produce reports with quantitation information.
Provide a vehicle for storing data.
Define manually integrated data on report.
The current system for operation and processing is Waters Empower2 (current revision)

6.5 Disposable 0.45um syringe tip filters, Teflon, if needed to remove suspended solids.

6.6 Disposable luer tip syringes, sized as appropriate, if needed to remove suspended solids.

6.7 Volumetric flasks - Class A glass: sized as appropriate

6.8 Micro syringes or Class A graduated (to deliver) pipets, sized as appropriate

6.9 Autosampler vials- Glass, 2 mL crimp top or screw top with Teflon-lined septum

6.10 Graduated cylinders, sized as appropriate

6.11 Disposable Pasteur pipets

7 Reagents and Standards

7.1 Reagents

7.1.1 Acetonitrile - HPLC grade or equivalent. Optima grade is preferred.

7.1.2 Organic-free, deionized water: ASTM Type III water provided and monitored in-house according to R3-QA065 (current revision) and further polished at a point of use Millipore unit to a resistivity of 18 MΩ-cm and a total organic carbon of less than 50 ppb.

7.1.3 Nitrogen gas, provided by liquid nitrogen dewars

7.1.4 Argon gas, provided by liquid argon dewars

7.1.5 Formic Acid, reagent grade.

7.1.6 Sodium Cesium Iodide, NaCsI. For instrument tuning. Provided annually by manufacturer with system preventive maintenance (PM) kit.

7.1.7 Mobile phase: Reservoir A1: H2O with 0.1% formic acid, Reservoir B1: Acetonitrile with 0.1% formic acid.

7.2 Standards

7.2.1 All standards are to be labeled with the Element standard number and the preparer's initials. This is a unique identifier and all standard information is referenced in Element. Other information may include: expiration date, concentration, and manufacturer.

7.2.2 Standards must be stored in glass containers at 4 +/-2°C.

7.2.3 Stock standard solution 100 mg/L (ppm) glycol mix – This solution can be purchased commercially as a certified standard. Stock standards should be stored at 4-6°C or according to manufacturer's suggestions until manufacturer's expiration. Expiration dates should be clearly specified on the label.

7.2.4 Intermediate standard solution (1.0 and 10 mg/L glycol mix) – Prepared by dilution of stock standard solution to 10 or 100 mL with reagent water. Intermediate standards may be stored at 4±2 °C for a period of up to 6 months. Expiration dates should be clearly specified on the label.

7.2.5 Calibration standards – Prepare dilutions of the intermediate standard solution to prepare five calibration standards Due to the varied responses of the analytes, recommended standard concentrations for establishing a calibration curve are: 5, 10, 25, 50, 100, 200, and 400µg/L (ppb). This range may be extended provided that the linear response can be adequately verified through satisfaction of all calibration criteria and quality control requirements. The low standard must be equivalent to or below the lowest result to be reported. All reported results must be within the calibration range.

8 Sample Collection, Preservation and Storage

8.1 This SOP does not describe sample collection procedures; however, the following guidelines are followed once samples are received at the laboratory.

8.2 Samples must be stored in tightly sealed glass at 4 +/- 2°C in a designated sample refrigerator. Recommended sample container is 40mL vial with Teflon septa without the use of acid preservation.

8.3 Analyze samples within 14 days of collection.

8.4 Samples extracted outside of holding time should be noted in the case narrative and qualified according to the lab QM.

9 Quality Control

9.1 Batch QC. The following are relevant QC criteria for this method taken from the OASQA Laboratory Quality Manual (current revision).

NELAC Requirement	Minimum Frequency	Acceptance Criteria	Corrective Action
Method Blank – BLK (clean matrix **processed**)	One per sample preparation batch[1]	Fails if the concentration of a targeted analyte in the blank is at or above the reporting limit, AND is greater than 1/10 of the amount measured in any sample. Criteria do not apply to sample results reported as less than values and mandated methods that require correction for blanks.	If outside acceptance criteria reprep affected samples or qualify sample results.
Laboratory Control Sample (LCS) – **BS** (clean matrix spiked with analytes of interest)	One per sample preparation batch [1]	±20% of expected value for aqueous samples. As per 8000C. LCS/BS is equivalent to CCV because there is no extraction. Sec 11.7.6	If outside acceptance criteria, first re-analyze the failed QC to verify difficulty. If still failing, perform corrective actions and reprep. affected

NELAC Requirement	Minimum Frequency	Acceptance Criteria	Corrective Action
			samples or qualify results.
Matrix Spike – MS (spiked or fortified sample)	One per 20 samples per matrix Selection of sample [3]	±30% of expected value for aqueous samples. This is a conservative /demanding limit based on acceptance criteria for spikes into clean matrix (LCS-BS) per 8000C Section 9.5.4..	If outside acceptance criteria, qualify the sample associated with failing QC results.
Matrix Spike Duplicate –MSD (analysis of second fortified aliquot, processed)	One per 20 samples per matrix and site Selection of sample [3]	Relative percent difference: 25, as per Method 8000C. ±30% of expected value for aqueous samples. As per Method 8000C, Sec 9.5.4. RPD≤25	If outside acceptance criteria, qualify the sample associated with failing QC results. Re-analyze the sample (holding time and sample volume permitting). If MS/MSD recoveries are high, first examine raw ion data for possible interference. If the problem is confirmed by re-analysis, include explanation in analytical report. If the MS/MSD recover problems are not confirmed and recoveries from the second analysis are within the QC limits, then report the second analysis and reject the first.
Initial Calibration –	At least five calibration standards with one at the Level of Quantitation (not to include the blank)	$r^2 \geq 0.99$ as per Method 8000C Sec 9.3.2. Minimum of 5 concentrations Method 8000C Sec 7.4.1.1	If the initial instrument calibration results are outside established acceptance criteria, corrective actions must be performed. Results associated with an unacceptable initial instrument calibration must be qualified. Results of samples not bracketed by initial instrument calibration standards (within calibration range) must be reported as having less certainty.
Second Source Quality Control Standard (QCS) – **SCV** (material is from a second source; **source independent** of calibration standards, not processed)	One per initial calibration	±20% of expected value as per Method 8000C. Sec 9.3.6.	If outside acceptance criteria, first re-analyze or reprep. the failed QC to verify difficulty. If still out, correct problem then recalibrate or qualify results.
Continuing Instrument	One at beginning, end and	±20% of expected value as per	If outside acceptance criteria,

NELAC Requirement	Minimum Frequency	Acceptance Criteria	Corrective Action
Calibration Verification – CCV	every 20 samples (analytical batch).	Method 8000C. Sec 1.1.7.6	first re-analyze or reprep the failed QC to verify difficulty. If reanalysis passes the first time, then continue run. If reanalysis fails but routine corrective actions correct the problem, then there must be two consecutive passing QCs before continuing the run. If it still fails, then recalibrate and reanalyze all samples since the last acceptable CCV or stop analysis (additional analyses shall not occur) and if any samples in the batch cannot be re-analyzed report data specifying the direction of the bias if clearly indicated.
Selectivity – Retention Time	All chromatography methods	All analytes in initial calibration standards, LCS/BS, SCV and CCV within windows established per method or in-house limits. The Empower software processing method currently sets the retention time window at ±5% of the analyte's retention time in the mid-point calibration curve.	If outside acceptance criteria, first re-analyze or reprep the failed QC to verify difficulty. If still out, correct problem then recalibrate or qualify results.
Surrogate – SUR	Organic only - All samples, standards, QC (Surrogate compounds as per SOP and mandated methods). Not currently used, may be added at a future date.	Not currently used, may be added at a later date.	If outside acceptance criteria, qualify results associated with failing QC.
Tuning	Mass spectrometry methods - before each analytical batch [1] ASTM D7731-11 states that tuning should be done according to manufacturer's directions. Because hardware tuning is done with NaCsI, tuning is recommended to be done yearly with the PM so that salts do not build up on the quadrupole.	According to manufacturer's directions.	Perform instrument maintenance and rerun tuning standard. Data associated with an unacceptable tune shall not be reported.

[1] ***Batch:*** *environmental samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A* ***preparation batch*** *is composed of one to 20 environmental samples of the same NELAC-defined matrix, meeting the above mentioned criteria and with a maximum time between the start of processing of the first and last sample in the batch to be 24 hours. An* ***analytical batch*** *is composed of prepared environmental samples (extracts, digestates or concentrates) which are analyzed together as a group. An analytical*

batch can include prepared samples originating from various environmental matrices and can exceed 20 samples. (NELAC Quality Systems Committee)

[2] *The components to be spiked shall be as specified by the mandated test method. Any permit specified analytes, as specified by regulation or client requested shall also be included. If there are no specified components, the laboratory shall spike per the following:*
For those components that interfere with an accurate assessment such as spiking simultaneously with technical chlordane, toxaphene and PCBs, the spike should be chosen that represents the chemistries and elution patterns of the components to be reported.
For those test methods that have extremely long lists of analytes, a representative number may be chosen using the following criteria for choosing the number of analytes to be spiked. However, the laboratory shall insure that all targeted components are included over a two year period.
For methods that include 1-10 targets, spike all components.
For methods that include 11-20 targets, spike at least 10 or 80%, whichever is greater.
For methods that include 21 or more targets, spike at least 16 components.
(NELAC, Section D.1.1.3.1c)

[3] *The selected sample shall be rotated among client samples so various matrix problems may be noted and/or addressed.*

10 Calibration and Standardization

10.1 Refer to the Batch QC table for calibration criteria.

10.2 While many mass spectrometry methods require daily tuning to assure proper mass identification prior to each sample batch, ASTM Method D7731-11 states that tuning/mass calibration should be according to manufacturer's directions. According to the TQD Operator Manual, unless problems are noted, this system is only required to be tuned for proper mass identification annually with the system PM. Tuning is done with a NaCsI solution and repeated introduction of NaCsI can cause buildup of salt in the system and result in reduced sensitivity and will necessitate frequent cleaning.

10.3 Tuning to determine the correct system settings (cone voltage, desolvation temperature, source temperature etc) for a particular analyte is done as needed and according to manufacturer's directions. Representative settings for the analytes in this method are listed in Section 11.

10.4 Records of the annual system PM are maintained in the instrument maintenance log.

10.5 Suggested concentrations for the initial calibration levels are 5.0 to 400.0 ppb. If a wider calibration range is needed, more standard levels should be added provided the calibration curve remains linear. Suggested 5-point calibration levels is 5, 10, 25, 50, 100.

10.6 Linear calibration may be used if the $r^2 \geq 0.99$ and all continuing calibrations and calibration verifications pass. If linear calibration fails, calibration must be re-run.

10.7 The average of the retention times of the mid-level concentrations is to be used in the

processing method as the analyte retention time.

10.8 Certificates of analysis are stored in G201.

11 Procedure

11.1 Sample Preparation

11.1.1 Transfer sample to an autosampler vial using a glass Pasteur pipet. If necessary, filter the sample through a 0.45µm syringe tip filter and dispense into autosampler vial.

11.1.2 Prepare matrix spike samples in a 10.0 mL volumetric flask. Fill to about 50% with sample; add an appropriate volume of spike solution to achieve the needed concentration. The volume of spike added should not be more than 100-200ul (1-2% of the total sample volume) or it could affect the concentration in the source sample. Fill the volumetric flask to the mark with sample and mix by inverting several times. If necessary, filter the sample through a 0.45µm syringe tip filter and dispense into autosampler vial.

11.2 HPLC/MS analysis

11.2.1 Calibrate the HPLC/MS/MS with NaCsI, according to manufacturer's directions, during annual preventive maintenance. More frequent calibration with NaCsI can leave residue on the quadrupoles and should only be done following significant instrument repair.

11.2.2 Appropriate MRMs were determined during method development (see 11.2.6 below) but can be reevaluted as needed, by tuning with authentic, individual standards to determine the most abundant MRMs. Tuning may be done via the Waters Intellistart™ automated tuning program or manually through the tune page.

11.2.3 Mobile phases.

11.2.3.1 For 2-methoxyethanol, isocratic elution at 0.3ml/min at 98% A1 and 2% B1 is used.

11.2.3.2 For the other analytes a gradient is used.

Time (min)	Flow rate ml/min	% A1	% B1	Curve
Initial	0.4	98	2	Linear
3.0	0.4	98	2	Linear
10.5	0.4	85	15	Linear
12.5	0.4	85	15	Linear
13	0.4	98	2	Linear
13-19	0.4	98	2	Equilibration before next injection

11.2.4 The typical injection volume is 30 µL.

11.2.5 The gradient may be modified to achieve separation of target analytes in one run.

11.2.6 The following MRMs are monitored but may be adjusted depending on instrument response. The MRM marked * has a higher response and is used as the primary MRM for calibration and quantitation. The second MRM may be monitored and for supplementary confirmation but due to the lower response, cannot be used to confirm concentrations at the lower portions of the calibration curve. ASTM D7731-11 uses only one MRM per analyte.

Diethylene Glycol, Time: 0-5min, span: 0.2 Da, retention time (RT): 1.8min

Precursor (Da)	Product (Da)	Dwell (sec)	Cone voltage (V)	Collision energy V)
106.94	44.9*	0.2	18	48
106.94	88.4	0.2	18	22

Triethylene Glycol, Time:0-5min, span 0.2 Da, RT: 2.9min

Precursor (Da)	Product (Da)	Dwell (sec)	Cone voltage (V)	Collision energy V)
150.97	45.10*	0.2	24	26
150.97	89.00	0.2	24	24

Tetraethylene Glycol: Time 5-13min, span 0.2 Da, RT: 5.6 min

Precursor (Da)	Product (Da)	Dwell (sec)	Cone voltage (V)	Collision energy V)
195.05	45.10*	0.2	22	22
195.05	89.00	0.2	22	20

2-Butoxyethanol: Time, 5-13min, span 0.2 Da, RT: 10.6min

Precursor (Da)	Product (Da)	Dwell (sec)	Cone voltage (V)	Collision energy V)
118.93	57.10	0.2	16	20
118.93	63.00*	0.2	16	14

2-Methoxyethanol: Time 0-4min, span 0.2 Da, RT: 2.6min

Precursor (Da)	Product (Da)	Dwell (sec)	Cone voltage (V)	Collision energy V)
76.91	59.10*	0.2	12	8

11.2.7 MS/MS settings may be adjusted to meet quantitation limit requirements but are generally as follows:

	2-methoxyethanol	All other analytes
Desolvation temperature	350°C	400°C
Source temperature	150°C	150°C
Collision gas flow (Argon)	0.1ml/min	0.1ml/min
Cone gas	25 L/hr	25 L/hr
Desolvation gas	600 L/hr	800 L/hr
Ion Mode	Electrospray positive (ESI+)	Electrospray postive (ESI+)

Column temperature	30°C	30°C
Sample chamber	4°C	4°C
Inter-channel delay	0.005s	0.005s
Inter-scan delay	0.005s	0.005s

12 Data Analysis and Calculations

12.1 Refer to the current version of the Laboratory QM for Quality Control related equations and the policy on reporting significant figures.

12.2 Refer to R3QA-067 (current revision) for policies on manual integration.

12.3 Identify and confirm the presence of target analytes in the samples by matching the retention time of the MRM.

Compare the retention time of the MRM with the retention time determined during the initial calibration. The retention times should not be more than 5% different from the initial calibration average.

12.4 Linear (external) calibration may be used if the $r^2 \geq 0.99$.

12.5 Water samples

$$\text{Final result } (\mu g/L\ ClO_4^-) = (C)(D)$$

Where:
C = Concentration or calibration curve (µg/L analyte) D = Dilution factor (if needed)

13 Method Performance

13.1 Method performance is evaluated based on the criteria in Table 2.

13.2 DOC accuracy and precision data and MDL study data are maintained in the OASQA Central QS files.

13.3 NQLs are listed in Section 1. There are no problematic compounds associated with this method.

14 Pollution Prevention

14.1 This method has been developed to generate 10 mL or less of waste per aqueous sample. As this SOP is routinely performed, the analyst will consider other methods to reduce the use and generation of hazardous chemicals/waste.

14.2 Resource Management: Water Conservation. Laboratory personnel should be mindful

of water consumption, and whenever possible, employ practices that minimize water use.

15 Waste Management

15.1 *Waste type code*: Will vary with sample. Record the WO # on sample waste containers.

15.2 All laboratory waste must be handled in accordance with guidelines established in the ESC Chemical Hygiene Plan (current revision).

15.3 The waste flow chart is on file with the SHEM Office.

15.4 *Amount of waste per sample*: Approximately 10mL or less of waste will be generated per sample.

16 References

16.1 SW-846 Method 8321B,Solvent-extractable nonvolatile compounds by high-performance liquid chromatography/thermospray mass spectrometry or ultraviolet detection (rev 2, Feb 2007)

16.2 SW-846 Method 8000C, Determinative Chromatographic Procedures. (rev 3, March 2003)

16.3 ASTMD7731-11^{E1}, Standard Test Method for Determination of Dipropylene Glycol Monobutyl Ether in Sea Water by Liquid Chromatography/Tandem Mass Spectrometry. (August 2011)

16.4 Waters ACQUITY TQD Empower 2154 customer Familiarization Guide. Waters Corp. (2008) Milford MA.

16.5 EPA Region 3 OASQA Laboratory Quality Manual (QM), Current Revision.

16.6 EPA Region 3 OASQA Chemical Hygiene Plan, Current Revision.

16.7 EPA Region 3 OASQA Occupant Emergency Plan, Current Revision.

16.8 EPA Region 3 OASQA, Laboratory Notebook Policy, Current Revision.

16.9 TQD Maintenance logbook: SNB 357.

16.10 Waters TQD System Run Log: PNB 207

16.11 Certificates of analysis notebook: SNB 114

16.12 R3-QA067. Procedures for Manual Integration, Current revision.

16.13 R3-QA054. Glassware Preparation for Organic Analyses. Current revision.

16.14 R3-QA065. Calibration, Verification and Maintenance of Laboratory Support Equipemt. Current revision.

16.15 NELAC Standard. Current revision

17 Tables, Diagrams, Flowcharts and Validation Data

17.1 Waste handling flow chart is on file with the SHEM office.

17.2 QA/QC data is on file with the OASQA Quality Assurance Officer.

17.3 Attachment 1. EPA Internal Technical Review Checklist

Attachment 1: **Glycols by LC/MS (R3-QA239) Technical Review Checklist (TRC) Checklist**

For Internal Use Only

Site Name:_____ WO#_____
Analyst: _____ Date given to Reviewer:_____
Matrix (circle): Aqueous / Other _____
Program (circle): Superfund / RCRA / WPD (NPDES) / SDWA / Other: _____

The signature below indicates the following:
- This data meets the needs of the customer according to the request.
- The analysis was performed as per the SOP, or exceptions documented.
- All documentation needed to recreate the analyses has been reviewed.
- Data Review status set to Peer Reviewed in Element.

Peer Reviewer signature _____ Date accepted_____
If any data for this case is stored with another case file, give Site Name and WO#_____

Peer Reviewer Completes Section Below:

General:

	YES	NO	N/A	Comments
Raw data is identified with sample IDs, site name, WO#, analyst name, date of analysis.	____	____	____	

Quality Control:

	Yes	No	n/a	comments
NaCsI cal according to mfg recommendation within year				
Initial calibration: $r^2 \geq 0.99$				
Holding time: 14 days to analysis				
Method Blank < NQL				
SCV ~~(old term: LVM)~~ (±20%)				
CCV (old:CLC) (±20% mid-range)				
BS Blank spike (±20% mid range)				
Reported + results for samples met RT requirement for primary MRM fragment?				
Reported + results for samples met RT requirement for 2ndary MRM fragment or explained in narrative.				
Manual integration as per R3QA067				
Matrix spike/dup: ±30% aq, 25% mid range spike				

Calculations/Report:

Calculations and transcriptions checked. _____ ____ ____ _____
Element Draft Report reviewed. _____ ____ ____ _____
Deviations and problems documented. _____ ____ ____ _____

Additional Comments by Peer Reviewer:

_____
_____
_____
_____

Analyst ensures that the data case file is complete and accurate as per SOP R3QA-066:

____ Bench sheet or Work Order list
____ Sample Prep logs
____ Instrument run log
____ Standard/Reagent Prep log
____ Raw data
____ Data status set to analyzed
____ Appropriate TV sheets / Certificates of Analysis
____ Element Peer Review report

Additional Comments by Analyst on data issues:

_____
_____
_____
_____
_____
_____
_____

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