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Analysis of Thiodiglycol: Validation of Semi-Volatile Analysis by HPLC-MS/MS by EPA Method MS777

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Disclaimer

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

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TECHNICAL REPORT

LLNL-TR-######

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Overview and Objectives

The Environmental Protection Agency's (EPA) Region 5 Chicago Regional Laboratory (CRL) developed a method for the analysis of thiodiglycol, the breakdown product of the sulfur mustard HD, in water by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), titled *Method EPA MS777* (hereafter referred to as EPA CRL SOP MS777). This draft standard operating procedure (SOP) was distributed to multiple EPA laboratories and to Lawrence Livermore National Laboratory, which was tasked to serve as a reference laboratory for EPA's Environmental Reference Laboratory Network (ERLN) and to develop and validate analytical procedures.

The primary objective of this study was to verify the analytical procedures described in MS777 for analysis of thiodiglycol in aqueous samples. The gathered data from this study will be used to: 1) demonstrate analytical method performance; 2) generate quality control acceptance criteria; and 3) revise the SOP to provide a validated method that would be available for use during a homeland security event. The data contained in this report will be compiled, by EPA CRL, with data generated by other EPA Regional laboratories so that performance metrics of *Method EPA MS777* can be determined.

Materials and Methods

Chemicals and Reagents

Thiodiglycol (TDG; CAS No. 111-48-8) was purchased from Sigma-Aldrich (St. Louis, MO) at 99.9% purity. The surrogate standard, 3,3'-thiodipropanol (TDP; CAS No. 10595-09-2) was also obtained from Sigma-Aldrich at 98% purity. Stock solutions, spiking standards, infusion standards, and calibration standards were prepared without adjustment for purity.

HPLC mobile phase modifier ammonium formate was also from Fluka of Sigma-Aldrich (MS purity grade) and formic acid was from Acros Organics. Acetonitrile was from Fisher Scientific (Fair Lawn, NJ), as were all other chemicals unless specified otherwise.

Sample Preparation and HPLC Consumables

Millipore Millex PVDF filters (33 mm diameter, 0.45 µm pore size) were used for filtering spiked reagent and surface water samples prior to analysis. HPLC autosampler vials and caps were from Alltech Associates (Deerfield, IL).

Owing to problems with TDG and TDP chromatography and poor peak shape caused by HPLC system contamination, the sample introduction system of the HPLC was re-built or replaced using manufacturer-specified parts for the Waters 2795 HPLC system (purchased from Waters Corp., Milford, MA). Parts included: 500 µL syringe, needle assembly and housing unit, injector valve, sample loop, inlet filter, injector port seat washer, injector port seal washer and all ferrules and PEEK tubing (0.005 inch i.d.) throughout the entire system.

The analytical column was a Primesep SB™ column (150 x 2.1 mm i.d., 5 µm, 100 Å particle; SIELC Technologies, Prospect Heights, IL).

LLNL Verification of Procedures

Task 1: Verification of Instrument Conditions

A Waters Micromass Quattro *api* triple quadrupole mass spectrometer (serial number QAA594) coupled to a Waters 2795 liquid chromatograph was utilized for the verification of thiodiglycol analysis in aqueous samples. Individual standards of TDG and the surrogate standard, TDP, were each prepared at a concentration of 100 µg/mL (ppm) in 50/45/5 acetonitrile/water/water containing 500 mM ammonium formate and 2% formic acid (v/v/v).

These standards were infused at 20 µL/min using an external Hamilton syringe pump. The MS tune file settings described in *EPA SOP MS777* and our optimized settings are listed in Table 1. All instrument conditions, including voltages (capillary, cone, extractor, and RF lens), temperature (source, desolvation), gas flows (desolvation, cone), energies (ion, entrance, collision, and exit), resolutions (for low and high mass), multipliers, reaction mode and optimal ions for analysis were optimized and recorded.

Table 1: MS Tune File Parameters Optimized for Thiodiglycol and Surrogate 3,3'-Thiodipropanol

	EPA MS777		LLNL-optimized	
Parameter	**TDG**	**TDP**	**TDG**	**TDP**
Ion transitions	123.1--> 104.9	151.2--> 133.1	123.1--> 104.9	151.2--> 133.1
Mode and Polarity	pos ESI	pos ESI	pos ESI	pos ESI
Capillary voltage (kV)	3.5	3.5	**4.0**	**4.0**
Cone voltage (V)	18	19	18	19
Extractor voltage (V)	2	2	2	2
RF lens (V)	0.2	0.2	0.2	0.2
Source Temp (C)	120	120	120	120
Desolvation Temp (C)	300	300	**350**	**350**
Desolvation gas flow (L/h)	500	500	500	500
Cone gas flow (L/h)	25	25	25	25
Low mass resolution 1	14.5	14.5	14.5	14.5
High mass resolution 1	14.5	14.5	14.5	14.5
Ion energy 1	0.5	0.5	0.5	0.5
Entrance energy (eV)	-1	-1	-1	-1
Collison energy (eV)	5	8	5	8
Exit energy (eV)	2	2	2	2
Low mass resolution 2	15	15	15	15
High mass resolution 2	15	15	15	15
Ion energy 2	0.5	0.5	**1.5**	**1.5**
Multiplier	650	650	650	650
Inter-channel delay (s)	0.02	0.02	0.02	0.02
Inter-scan delay (s)	0.1	0.1	0.1	0.1
Repeats	1	1	1	1
Span (Da)	0	0	0	0
Dwell (s)	0.1	0.1	0.1	0.1

Bold values indicate parameters that were changed from the EPA CRL MS777 SOP listed conditions.

Chromatographic conditions were then established after conditioning the analytical HPLC column for 6 hours using the initial mobile phase of 95% water and 5% of the 500 mM ammonium formate/2% formic acid in water solution. A standard curve was then prepared by injecting 50 µL each of standards Levels 7 – 1 (10,000 µg/L to 100 µg/L) using the gradient table and flow rate described in EPA CRL SOP MS777. The signal to noise (S/N) ratio of the TDG peak (for the *m/z* 123.1 to *m/z* 104.9 transition) at Level 7 (10,000 µg/L) was 15.2 and the width (min) of the base of the peak was 1.2 min. Chromatographic parameters, including flow rate, mobile phase composition, injection volume, column heater temperature, and injector wash system solvents were all adjusted in hopes of improving the chromatography. Improvement in instrument response was seen with a lowering of the flow rate to 0.150 mL/min (Figure 1) and an adjustment of column temperature from 30 °C to 45 °C.

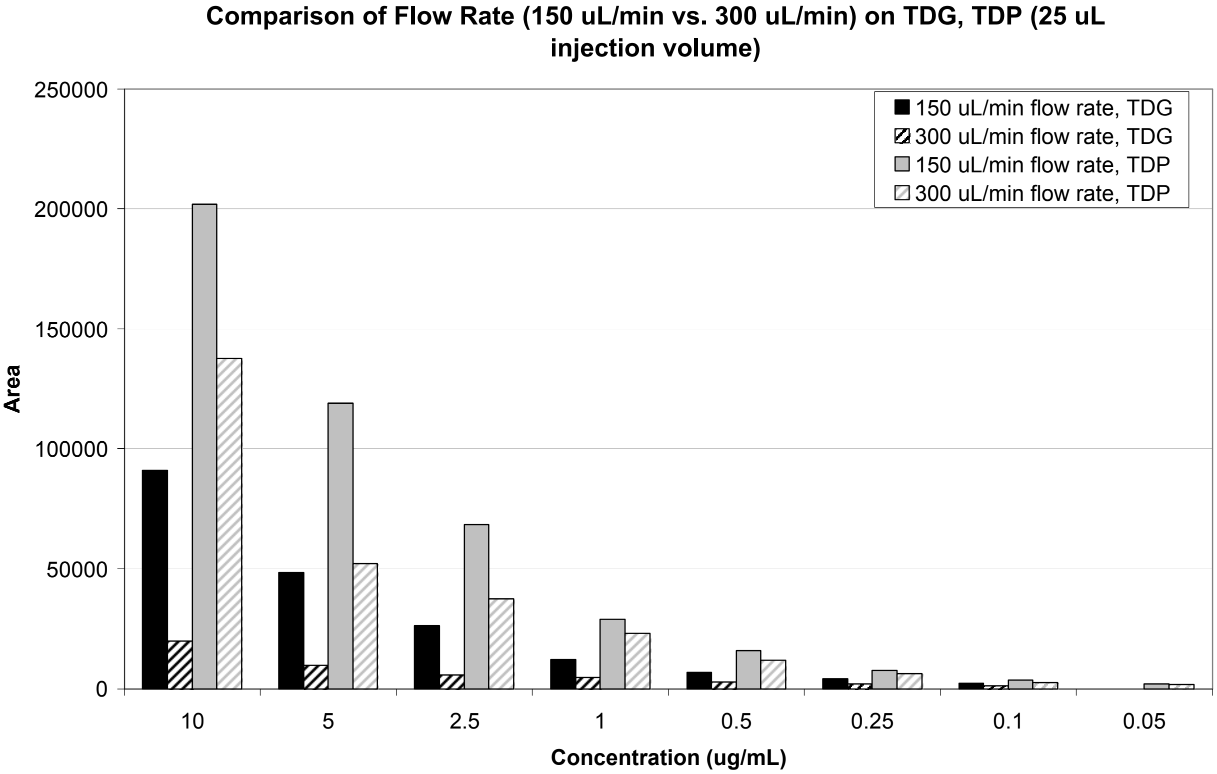


Figure 1: Optimization of flow rate for improved analyte response.

However, the peak shape and width at the base of the peak continued to worsen, despite efforts to improve the chromatography of the system. In response, the column was taken off-line and the entire system was cleaned for two days with isopropanol/methanol/acetonitrile/water + 1.0% formic acid at 0.150 mL/min. With the column still removed from the system, a section of PEEK tubing was filled with Level 7 standard. The section of PEEK tubing was moved backward from the detector to the injector valve to isolate the source of contamination affecting the peak shape of TDG. The injector was identified as the source of the poor peak shape, so this was re-built and all other parts of the sample introduction system of the HPLC were replaced.

A new Primesep SB column was then also installed and allowed to equilibrate for 1 day before use. Initial chromatographic conditions were changed to 0.100 mL/min with 50/45/5 water/acetonitrile/water containing 500 mM ammonium formate + 2.0% formic acid (v/v/v) as the initial mobile phase composition. Using this newly optimized and cleaned system, TDG eluted at 5.11 min and width at the base of the peak was 0.5 min (Figure 2) and TDP eluted at 5.12 min with a width of 0.50 min (Figure 3).

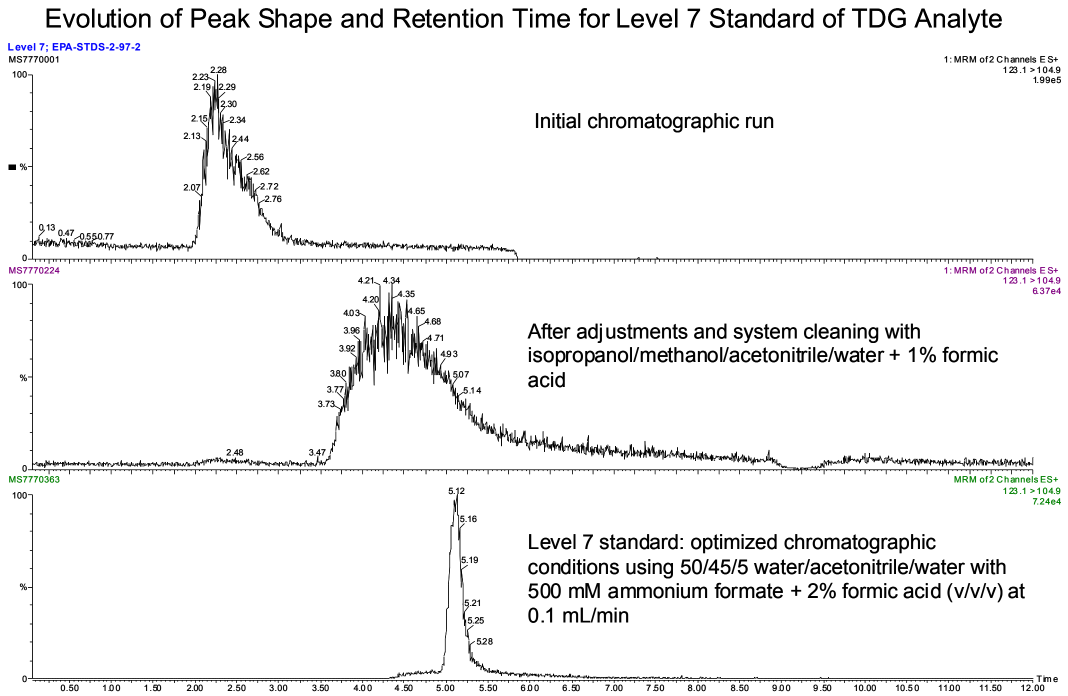
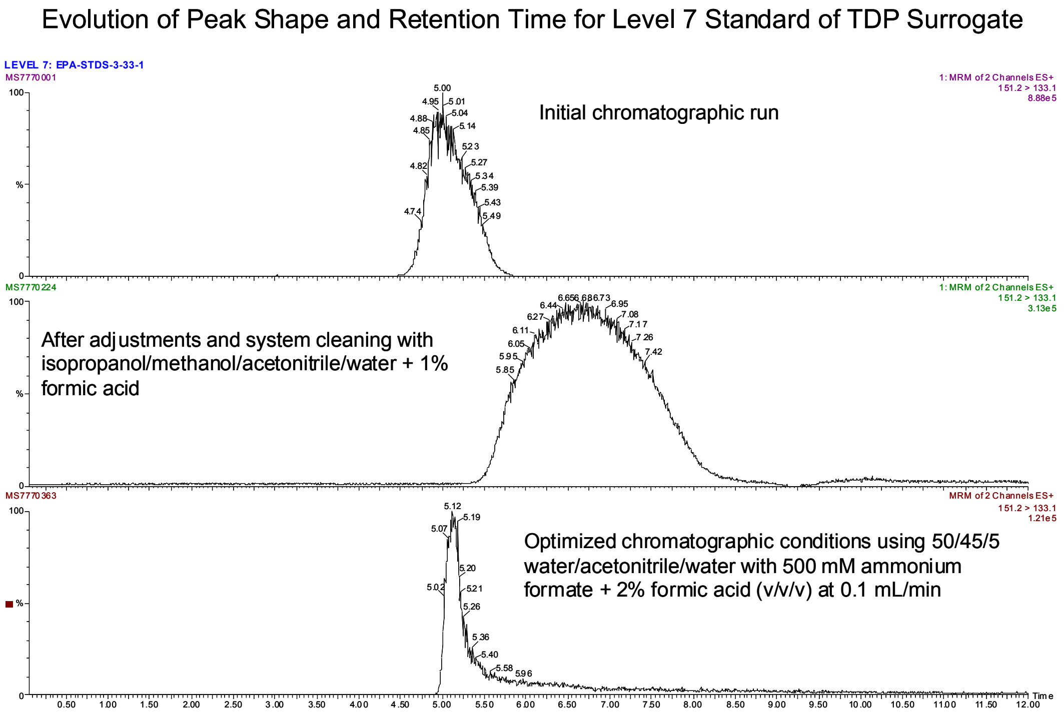


Figure 2: Optimization of TDG analyte peak shape and retention time


Evolution of Peak Shape and Retention Time for Level 7 Standard of TDP Surrogate

Initial chromatographic run

After adjustments and system cleaning with isopropanol/methanol/acetonitrile/water + 1% formic acid

Optimized chromatographic conditions using 50/45/5 water/acetonitrile/water with 500 mM ammonium formate + 2% formic acid (v/v/v) at 0.1 mL/min

Figure 3: Optimization of TDP surrogate peak shape and retention time.

The optimized HPLC conditions utilized were as follows:

◊ Inject 50 µL

◊ Autosampler compartment at 15 °C

◊ Mobile phase A: Water + 0.1% formic acid; mobile phase B: Acetonitrile + 0.1% formic acid; mobile phase D: Water containing 500 mM ammonium formate + 2.0% formic acid

◊ Flow rate throughout chromatographic run time was 0.100 mL/min

◊ Gradient conditions were as follows: 0 min (50% A, 45% B, 5% D), hold for 2.5 min. At 6 min, mobile phase composition is (0% A, 95% B, 5% D), hold for 4 min. At 12 min, mobile phase composition has returned to initial conditions (50% A, 45% B, 5% D), hold for 4 min. Column equilibration time between runs was 2.0 min.

◊ Column heater at 45 °C

Task 2: Determination of calibration curve data

Analytical standards were prepared to EPA CRL SOP MS777. The concentration of TDG and TDP each ranged from 100 µg/L to 10,000 µg/L. Additional standards of 25 µg/L and 50 µg/L were prepared to determine the limit of detection. The lowest level included in the curve was 100 µg/L (signal to noise ratio, S/N = 10.3 for TDG and 9.51 for TDP). The S/N ratio at 25 µg/L was 5.19 for TDG and 5.08 for TDP. The calibration curves were fit with a quadratic regression line with 1/X weighting. R^2 values for the calibration curves were 0.9968 for TDG and 0.9906 for TDP.

Task 3: Precision and Bias Study

Precision and bias were determined across the calibration ranges by including four replicate samples of reagent water at four different fortification levels (100 µg/mL, 250 µg/L, 2500 µg/L and 7500 µg/L) and duplicate samples of surface water at these same fortification levels. The results of the precision and bias study are shown in Table 2 (reagent water) and Table 3 (surface water).

Surface water was collected from the Zone 7 Water Agency Water Quality Laboratory located in Livermore, CA. Water collected was sampled from the South Bay Aqueduct. The water carried in the South Bay Aqueduct is from the Sacramento River delta, which carries snow melt water from the northern Sierra Nevada Mountains. The water was collected at a tap before any chemical pre-treatment by the facility. Five 1-L, pre-cleaned, amber, I-CHEM glass bottles were filled. The water temperature was 24 °C and the pH was 7.00 ± 0.025. The water samples were stored at 4 °C prior to sample preparation and analysis.

With the exception of TDG at 100 µg/L in reagent water, the recoveries of TDG and TDP were reasonable and within the range established in the quality control acceptance criteria (**Table 2** of EPA CRL SOP MS777). The listed % recovery ranges in EPA CRL SOP MS777 for TDG were from 80 to 140% and from 74 to 134% in reagent and surface waters, respectively. For TDP, the recovery range was 82 to 142% and 93 to 153%, in reagent and surface waters, respectively.

In our hands, mean recoveries (relative standard deviation, RSD in %) of TDG and TDP, respectively, at 100 µg/L were 168 (15.6%) and 114.5 (9.6%) in reagent water and 43 % and 85.5%, respectively, in surface water for a relative percent difference (RPD) of -74% and -25% for TDG and TDP. At 250 µg/L, recoveries (RSD, %) were 102.3% (8.0) and 103.8% (9.2) in reagent water, and 107.5% and 90.5% in surface water for TDG and TDP, respectively. The RPDs between reagent and surface water samples were 5.1% and -12.8% for TDG and TDP.

At the mid-calibration fortification level of 2500 µg/L, the recovery (RSD, %) in reagent water for analyte and surrogate was 120.8% (10.6) and 123% (8.7) versus 89.5% (RPD of -26%) and 85% (RPD of -31%), respectively, in surface water. At the highest fortification level of 7500 µg/L, the recovery (RSD, %) in reagent water was 128.3% (8.9) and 119% (1.2%) and in surface water, the mean recovery (RPD, %) for TDG and TDP was 108% (-16%) and 90.5 (-24%).

Blank samples for reagent and surface waters were included throughout the analysis to evaluate the potential of any contamination or interferences. These blank samples were spiked only with the surrogate standard TDP (data shown in **Tables 2** and **3**). The TDG analyte was detected in the blank reagent or blank surface water samples. Additionally, blank water samples from the calibration standard preparation were included in the sample list. These samples are simply called, ‘blank’ whereas the reagent water blanks and surface water blanks are specifically noted. The instrument sequence list is provided in **Appendix 1** for reference.

Table 2: Results of precision and bias study in reagent water at four fortification levels with four replicates at each level.

Laboratory: LLNL

Instrument ID: Waters Quattro *micro* API Micromass Triple Quadrupole MS (SN QAA594) with 2795 HPLC system

Surface Water Description: South Bay Aqueduct water (carries snowmelt from Sierra Nevada Mountains to San Diego, CA)

Date of Analysis: 07/23/2008

Data Reporting Form 3a. (Thiodiglycol) Precision and Bias in Reagent Water

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation (RSD)
Thiodiglycol	100	0	0	201	201	176	176	150	150	144	144	168.0	15.6
3,3'-Thiodipropanol	2500	2500	2270.48	2568.52	103	2690.51	108	2992.77	120	3170.77	127	114.5	9.6

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation (RSD)
Thiodiglycol	250	0	0	274.4	110	227.6	91	254.1	102	266.00	106	102.3	8.0
3,3'-Thiodipropanol	2500	2500	2796.68	2780.76	111	2726.1	109	2625.77	105	2240.41	90	103.8	9.2

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation (RSD)
Thiodiglycol	2500	0	0	2879.76	115	3366	135	3180	127	2653	106	120.8	10.6
3,3'-Thiodipropanol	2500	2500	2925	2894.24	116	3361	134	3242	130	2797	112	123	8.7

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation (RSD)
Thiodiglycol	7500	0	0	9423.0	126	10717	143	9655	129	8656.4	115	128.3	8.9
3,3'-Thiodipropanol	2500	2500	2855.34	2984.31	119	2950.2	118	3003.3	121	2957.0	118	119.0	1.2

Laboratory: LLNL

Instrument ID: Waters Quattro *micro* API Micromass Triple Quadrupole MS (SN QAA594) with 2795 HPLC system

Surface Water Description: South Bay Aqueduct water (carries snowmelt from Sierra Nevada Mountains to San Diego, CA)

Date of Analysis: 07/23/2008

Data Form 3b. (Thiodiglycol) Precision and Bias in Local Surface Water

			Surface Water Blank	Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
Thiodiglycol	100	0	0	48.83	49	37.37	37	43	-74.4
3,3'-Thiodipropanol	2500	2500	1951.23	2277.33	91	2002.32	80	85.5	-25.3

			Surface Water Blank	Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
Thiodiglycol	250	0	0	273.91	110	261.43	105	107.5	5.1
3,3'-Thiodipropanol	2500	2500	2441.69	2289.06	92	2228.20	89	90.5	-12.8

			Surface Water Blank	Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
Thiodiglycol	2500	0	0	2164.17	87	2294.00	92	89.5	-25.9
3,3'-Thiodipropanol	2500	2500	2342.57	2134.99	85	2122.09	85	85	-30.9

			Surface Water Blank	Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
Thiodiglycol	7500	0	0	7372.77	98	8820.5	118	108	-15.8
3,3'-Thiodipropanol	2500	2500	2694.33	2347.80	94	2178.86	87	90.5	-23.9

Table 3: Results of precision and bias study in surfacewater at four for tification levels with duplicate samplesat each level.

Appendix 1: Sample List

Sample List: 072308.SPL under C:\MassLynx\Janel\MS777.PRO\

MS File: MS777.exp; Inlet file: MS777.wat; Tune file: MS777.ipr, and Injection volume: 50 uL

No.	File Name	File Text	Position
1	MS7770362	BLANK	3:1
2	MS7770363	LEVEL 7; EPA-STDS-3-33-1	3:2
3	MS7770364	LEVEL 6; EPA-STDS-3-34-1	3:3
4	MS7770365	LEVEL 5; EPA-STDS-3-34-2	3:4
5	MS7770366	LEVEL 4; EPA-STDS-3-35-1	3:5
6	MS7770367	LEVEL 3; EPA-STDS-3-35-2	3:6
7	MS7770368	LEVEL 2; EPA-STDS-3-35-3	3:7
8	MS7770369	LEVEL 1; EPA-STDS-3-36-1	3:8
9	MS7770370	50 PPB; EPA-STDS-3-36-2	3:9
10	MS7770371	25 PPB; EPA-STDS-3-37-1	3:10
11	MS7770372	REAGENT WATER (RW) BLANK-4	3:11
12	MS7770373	RW-7500PPB-1	3:12
13	MS7770374	RW-7500PPB-2	3:13
14	MS7770375	RW-7500PPB-3	3:14
15	MS7770376	RW-7500PPB-4	3:15
16	MS7770377	RWB-3	3:16
17	MS7770378	RW-2500PPB-1	3:17
18	MS7770379	RW-2500PPB-2	3:18
19	MS7770380	RW-2500PPB-3	3:19
20	MS7770381	RW-2500PPB-4	3:20
21	MS7770382	RWB-2	3:21
22	MS7770383	RW-250PPB-1	3:22
23	MS7770384	RW-250PPB-2	3:23
24	MS7770385	RW-250PPB-3	3:24
25	MS7770386	RW-250PPB-4	3:25
26	MS7770387	RWB-1	3:26
27	MS7770388	RW-100PPB-1	3:27
28	MS7770389	RW-100PPB-2	3:28
29	MS7770390	RW-100PPB-3	3:29
30	MS7770391	RW-100PPB-4	3:30
31	MS7770392	SURFACE WATER (SW) BLANK-4	3:31
32	MS7770393	SW-7500PPB-1	3:32
33	MS7770394	SW-7500PPB-2	3:33
34	MS7770395	SWB-3	3:34
35	MS7770396	SW-2500PPB-1	3:35
36	MS7770397	SW-2500PPB-2	3:36
37	MS7770398	SWB-2	3:37
38	MS7770399	SW-250PPB-1	3:38
39	MS7770400	SW-250PPB-2	3:39
40	MS7770401	SWB-1	3:40
41	MS7770402	SW-100PPB-1	3:41
42	MS7770403	SW-100PPB-2	3:42
43	MS7770404	BLANK	3:1
44	MS7770405	LEVEL 4; EPA-STDS-3-35-1	3:5