From:	Seitz, Teresa A. (CDC/NIOSH/DSHEFS)
To:	Streicher, Robert P. (CDC/NIOSH/DART); Grote, Ardith A. (CDC/NIOSH/DART) (CTR)
Subject:	FW: EPA Dispersant Methods
Date:	Wednesday, July 21, 2010 2:52:53 PM
Attachments:	RAM-DOSS draft.doc
	(b)(5)

FYI. In case we decide to do any further analysis of the dispersant for 2-butoxyethanol.

Teresa

From: Spahr, James S. (CDC/NIOSH/OD)
Sent: Wednesday, July 21, 2010 2:08 PM
To: Seitz, Teresa A. (CDC/NIOSH/DSHEFS); Tepper, Allison L. (CDC/NIOSH/DSHEFS)
Cc: Delaney, Lisa (CDC/NIOSH/OD); Little, Joseph D. (CDC/NIOSH/OD)
Subject: FW: EPA Dispersant Methods

FYI – more "approved" lab methods

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Subject: FW: EPA Dispersant Methods

Thank you to David Neleigh for sharing. . .these as well all other relevant documents are on APHL's alternate markers sharepoint site:
http://www.aphlweb.org/aphl_departments/Operations/EOC/miscpages/amwp.aspx.

From: Neleigh.David@epamail.epa.gov [mailto:Neleigh.David@epamail.epa.gov]
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Subject: EPA Dispersant Methods

We finally officially posted the preferred methods list for dispersants on our web site.

http://epa.gov/bpspill/dispersant-methods.html

The actual LC/MSMS methods are attached. Note these are still draft and subject to change.

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Regional Analytical Method for Dioctyl Sulfosuccinate (RAM-DOSS, CAS 577-11-7) in Sea Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)[1]

July 12, 2010

1. Scope

1.1 This procedure details dioctyl sulfosuccinate (DOSS) determination in water by direct injection using liquid chromatography (LC) and detection with tandem mass spectrometry (MS/MS). This analyte is qualitatively and quantitatively determined by this method. This method adheres to single reaction monitoring (SRM) mass spectrometry.

1.2 Units— The values stated in SI units are to be regarded as standard. No other units of measurement are included in this method.

1.3 The Detection Verification Level (DVL) and Reporting Range for DOSS are listed in Table 1.

1.3.1 The DVL is required to be at a concentration at least 3 times below the Reporting Limit (RL) and have a signal/noise ratio greater than 3:1. Figure 1 and 2 display the signal/noise ratio of the single reaction monitoring (SRM) transition.

1.3.2 The reporting limit is the concentration of the Level 1 calibration standard as shown in Table 4 for DOSS, taking into account the 50% sample preparation dilution factor.

[1] This draft analytical procedure was developed by US EPA Region 5 Chicago Regional Laboratory (CRL) in collaboration with the Region 6 Houston Laboratory. It was first developed by CRL, July 12, 2010, and is open to improvement and discussion. Do not cite, quote, or distribute this procedure.

TABLE 1. Detection Verification Level and Reporting Range

Analyte	DVL (µg/L)	Reporting Range (µg/L)
DOSS	3	20-400

1.4 *This procedure does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 ASTM Standards:

D 1129 Terminology Relating to Water
D 1193 Specification for Reagent Water

2.2 Other Documents[2]

EPA publication SW-846, entitled *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*

3. Terminology

3.1 Definitions:

3.1.1 Detection Verification Level, DVL, n– a concentration that has a signal/noise ratio greater than 3:1 and is at least 3 times below the Reporting Limit (RL).

3.1.2 Reporting Limit, RL, n– the concentration of the lowest-level calibration standard used for quantification.

3.2 Abbreviations:

[2] Available from National Technical Information Service (NTIS), U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA, 22161 or at http://www.epa.gov/epawaste/hazard/testmethods/index.htm

3.2.1 CH_3CN- acetonitrile

3.2.2 ESI- Electrospray ionization

3.2.3 DOSS- dioctyl sulfosuccinate (CAS # 577-11-7)

3.2.4 LC/MS- liquid chromatography mass spectrometry

3.2.5 ppb– parts per billion, µg/L

3.2.6 mM– millimolar, 1 x 10^{-3} moles/L

3.2.7 NA– no addition

3.2.8 NH_4CO_2H- ammonium formate

3.2.9 ND– non-detect

4. Summary of Procedure

4.1 Two surrogates are being considered, DOSS-D34 and DOSS-^{13}C. Deuterated DOSS surrogate (DOSS-D34) recovery data are included in this draft, and will be complemented with DOSS-^{13}C when available. References to DOSS-^{13}C are included as a place holder and will be updated when available.

4.2 This is a performance based method, and modifications are allowed to improve performance.

4.3 For DOSS analysis, samples are shipped to the lab between 0°C and 6°C and analyzed within 5 days. In the lab, the entire collected 20 mL sample is spiked with surrogate (when available), ammonium formate buffer solution and brought to a volume of 40 mL with acetonitrile. This prepared sample is then filtered using a syringe driven filter unit, and analyzed by LC/MS/MS. If visible oil is present, the prepared sample is allowed to settle resulting in an oil layer at the top of the 40 mL solution. A portion of the aqueous (bottom) layer is filtered,

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leaving the oil layer behind, through a syringe driven filter assembly and analyzed by LC/MS/MS.

4.4 DOSS and its surrogate are identified by retention time and one SRM transitions. The target analyte and surrogate are quantitated using the SRM transitions. The final report issued for each sample lists the concentration of DOSS and the surrogate recovery.

5. Significance and Use

5.1 DOSS has been identified as a possible ingredient in dispersant agents used to treat oil.

5.2 This method has been investigated for use with reagent and sea water for DOSS.

6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other apparatus producing discrete artifacts or elevated baselines. All of these materials are demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as samples.

6.2 All glassware is washed in hot water with detergent and rinsed in hot water followed by distilled water. The glassware is then dried and heated in an oven at 250°C for 15 to 30 minutes. All glassware is subsequently cleaned with methanol and/or 50% acetonitrile/50% water.

6.3 System contamination and surface binding are problematic as DOSS is a surface active compound. Notable DOSS is associated with the surface of glass containers after one hour. It is important to thoroughly rinse sample containers with organic solvent to recovery DOSS from samples. Thorough rinsing of all lab equipment is necessary to reduce contamination. Carefully analyze blanks to ensure that the method minimizes DOSS carryover.

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6.4 All reagents and solvents should be pesticide residue purity or higher to minimize interference problems.

6.5 Matrix interferences may be caused by contaminants in the sample. The extent of matrix interferences can vary considerably from sample source depending on variations of the sample matrix.

6.6 Graduated cylinder- a 50 mL cylinder was used to measure the volume of the sample and total volume. An appropriate cylinder for different sample volumes should be used to accurately measure the sample and prepared sample volumes.

7. Apparatus

7.1 LC/MS/MS System

7.1.1 Liquid Chromatography System- A complete LC system is needed in order to analyze samples.[3] Any system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard may be used.

7.1.2 Analytical Column- *Waters* (186001377)- Atlantis™ dC18, 2.1 x 150 mm, 3 µm particle size was used to develop this test method. Any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and need to be monitored.

7.1.3 Tandem Mass Spectrometer System- A MS/MS system capable of MRM analysis.[4] Any system that is capable of performing at the requirements in this standard may be used. See Appendix B for Agilent instrument conditions.

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[3] A Waters ACQUITY UltraPerformance Liquid Chromatography (UPLC®) System was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

[4] A Waters Quattro Premier™ XE tandem quadrupole mass spectrometer was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

7.2 Filtration Device

7.2.1 Hypodermic syringe- A Lock Tip Glass Syringe capable of holding a Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm or similar may be used.

7.2.1.1 A Lock Tip Glass Syringe was used in this test method.

7.2.2 Filter– Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm (Millipore Corporation, Catalog # SLGV033NS) or similar may be used.

7.2.3 Glass Pipette- small volume pipette for sample container rinses and sample transfer

8. Reagents and Materials

8.1 Purity of Reagents- High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall conform to the Committee on Analytical Reagents of the American Chemical Society.[5] Other reagent grades may be used provided they are first determined to be of sufficiently high purity to permit their use without affecting the accuracy of the measurements.

8.2 Purity of Water– Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type 1 of Specification D 1193. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

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[5] Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, D.C. For Suggestions on the testing of reagents not listed by the American Chemical Society, see Annual Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, U.K., and the United States Pharmacopeia and National Formulators, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

8.3 Gases– Ultrapure nitrogen and argon.

8.4 Acetonitrile (CH_3CN, CAS # 75-05-8).

8.5 Methanol (CAS # 67-56-1).

8.6 Ammonium Formate (NH_4CO_2H, CAS # 540-69-2).

8.7 2-Propanol (CAS # 67-63-0).

8.8 Dioctyl sulfosuccinate sodium salt (DOSS) (CAS # 577-11-7).

8.9 Dioctyl sulfosuccinate sodium salt (DOSS-^{13}C) ^{13}C labeled.

8.10 Deuterated dioctyl sulfosuccinate sodium salt (DOSS-D34, - bis(2-ethylhexyl-D17) sulfosuccinate sodium salt)

9. Hazards

9.1 Normal laboratory safety applies to this method. Analysts should wear safety glasses, gloves, and lab coats when working in the lab. Analysts should review the Material Safety Data Sheets (MSDS) for all reagents used in this method.

10. Sampling

10.1 *Sampling and Preservation*– Grab samples should be collected in 20 mL pre-cleaned glass vials with Teflon® lined septa caps demonstrated to be free of interferences. This test method is based on a 20 mL sample size per analysis. Each sample must be collected in duplicate and a quadruplicate sample must be included with each sample batch of 10 for MS/MSD quality control analyses. Store samples between 0°C and 6°C from sample collection to sample preparation. Analyze the sample within 5 days of collection. After 7 and 10 days of storage between 0°C and 6°C DOSS recoveries notably decreased (Table 12). Oil in water

absorbs DOSS from water and affects surrogate analysis (Table 11). Avoid oil in samples if possible. Samples may be encountered that have more than one phase. Samples may be prepared for analysis using one or a multiple of the following options:

10.1.1 Whole Sample*: For a 20 mL sample, add 0.2 mL of 20 ppm DOSS surrogate to the sample in the sample container. Cap the container and mix to insure homogeneity. Transfer the contents of the sample container to a graduated cylinder record the sample volume. Subsequently, add 0.4 mL of 500 mM ammonium formate to the graduated cylinder. Rinse the sample collection vial twice with 6 to 8 mLs of acetonitrile, which is added to the prepared sample to compose ~ 50% acetonitrile solution to ensure quantitative sample transfer. Bring the prepared sample volume to 40 mL using acetonitrile, and then mix thoroughly. If different sample sizes are used, spiking solution, ammonium formate, and acetronitrile volume shall be adjusted proportionally. The sample must be thoroughly mixed and then allowed to settle. A portion of the aqueous fraction (bottom) should be transferred to a glass syringe for filtration. Filter the sample using a 0.22 µm PVDF filter into an autosampler vial, or another glass vial and then transfer to an autosampler vial. Due to the high concentration of DOSS detected in gulf oil, the filtered solution should be diluted for a preliminary analysis. Transfer 20 µL of the filtered-prepared sample into an autosampler vial, and add 1.98 mL of 5 mM ammonium formate in 50% acetonitrile / 50% water. If the DOSS result of the preliminary analysis is below the reporting limit, analyze the filtered-prepared sample directly or an appropriate dilution.

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10.1.2 Water Subsample*: Collect a 10 mL subsample of the water fraction (bottom) using a needle and a glass syringe. To reduce the oil exposure, invert the vial and tap gently to cause the oil move away from the septum. Insert needle through septum and collect 10 mL of the water layer; place the aliquot removed in a graduated cylinder. Then add 100 µL of 20 ppm DOSS surrogate to the sample in the graduated cylinder. Subsequently, add 0.2 mL of 500 mM ammonium formate to the graduated cylinder. Add 10 mL acetonitrile to the prepared sample to compose ~ 50% acetonitrile solution to ensure quantitative prepared sample transfer. If different sample sizes are used, spiking solution, ammonium formate, and acetronitrile volume shall be adjusted proportionally. Filter the sample using a 0.22 µm PVDF filter into an autosampler vial, or another glass vial and then transfer to an autosampler vial.

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10.1.3 Oil Subsample*: Add 1.5 mL of HPLC grade water to graduated cylinder. Collect a 0.5 mL subsample of oil (top), and place in a graduated cylinder. Then add 20 µL of 20 ppm surrogate to the sample in the graduated cylinder. Subsequently, add 40 µL of 500 mM ammonium formate to the graduated cylinder. Add 2 mL acetonitrile to the prepared sample to compose ~ 50% acetonitrile solution to ensure quantitative prepared sample transfer. If different sample sizes are used, spiking solution, ammonium formate, and acetonitrile volume shall be adjusted proportionally. The sample should be thoroughly mixed and then allowed to settle. A portion of the aqueous fraction (bottom) should be transferred to a glass syringe for filtration. Filter the sample using a 0.22 µm PVDF filter into another vial (filtered-prepared sample). Due to the high concentration of DOSS detected in gulf oil, the filtered solution should be diluted for a preliminary analysis. Transfer 20 µL of the filtered-prepared sample into an autosampler vial, and add 1.98 mL of 5 mM ammonium formate in 50% acetonitrile/50% water. If the DOSS result of the preliminary analysis is below the reporting limit, analyze the filtered-prepared sample directly or an appropriate dilution.

*Note- Subsamples of water and oil fractions quantitate only the DOSS in the subsample, the DOSS concentration of subsamples may underestimate the sample DOSS concentration as a result of partitioning (Table 10) and surface binding. DOSS surrogate added to the subsample rather than the received sample and container does not indicate DOSS recovery from the total sample. Additionally, oil in samples resulted in low DOSS recovery, but resulted in higher DOSS recoveries than subsamples (Table 11).

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11. Preparation of LC/MS/MS

11.1 LC Chromatograph Operating Conditions[5]

11.1.1 Injection volumes of all calibration standards and samples are made at 50 µL volume using a full loop injection. "Full loop" mode is the preferred technique when performing quantitative analyses. Multiple blank samples should be analyzed at the beginning of a run to remove residual DOSS from the system. The first sample analyzed after the calibration curve is a blank to ensure there is minimal DOSS carry-over. The gradient conditions for the liquid chromatograph are shown in Table 2A. Divert the column flow away from the electrospray source for 0 to 5 minutes after injection. Flow diversion to waste may be done using the mass spectrometer divert valve, divert tubing configurations vary from manual injection. Test the divert valve configuration and operation prior to analysis. Seawater samples contain nonvolatile salts; the elution from injection to 5 minutes after injection is diverted to waste in order to prevent mass spectrometer source contamination. If there is carry-over from one sample to another, greater than half the reporting limit, the initial percentage of acetonitrile should be raised as shown in Table 2B to try and remove the carry-over. This will shorten the elution time of DOSS approximately 1 minute; therefore it is necessary reduce the flow diversion and adjust the MRM time. Increasing the initial acetonitrile gradient concentration does not increase the DVL or reporting limit. LC/MS/MS conditions using Eclipse XBD C18 analytical column on an Agilent 6410 Triple Quad Mass Spectrometer are detailed as an alternative in Appendix B.

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TABLE 2A. Gradient Conditions for DOSS Liquid Chromatography

Time (min)	Flow (mL/min)	Percent 95% Water/ 5% CH_3CN, 5 mM NH_4CO_2H	Percent 95% CH_3CN/ 5% Water, 5 mM NH_4CO_2H
0.0	0.3	100	0
2.0	0.3	100	0
5.0	0.3	0	100
8.0	0.3	0	100
8.3	0.3	100	0
10.0	0.3	100	0

TABLE 2B. Gradient Conditions for DOSS Liquid Chromatography Starting with a Higher Acetonitrile Concentration

Time (min)	Flow (mL/min)	Percent 95% Water/ 5% CH_3CN, 5 mM NH_4CO_2H	Percent 95% CH_3CN/ 5% Water, 5 mM NH_4CO_2H
0.0	0.3	50	50
2.0	0.3	50	50
5.0	0.3	0	100
8.0	0.3	0	100
8.3	0.3	50	50
10.0	0.3	50	50

11.2 LC Sample Manager Conditions:

11.2.1 Wash Solvents- Weak wash is 4.0 mL of 50% water/50% acetonitrile. Strong wash is 2.0 mL of 60% acetonitrile/40% 2-propanol. The strong wash solvent is needed to eliminate carry-over between injections of DOSS samples. The weak wash is used to remove the strong wash solvent. Instrument manufacturer specifications should be followed in order to eliminate

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sample carry-over. Acquity Autosampler Firmware Version 4.1 results in an error with 4.0 mL weak needle wash and 2.0 mL strong wash. Adjusting the weak wash volume to 3.0 corrected this error, earlier and later Acquity Firmware versions do not have this issue.

11.2.2 Temperatures– Column, 35°C; Sample compartment, 15°C.

11.2.3 Seal Wash– Solvent: 50% acetonitrile/50% water; Time: 2 minutes.

11.3 Mass Spectrometer Parameters[6]:

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters may be optimized according to your instrument. Each peak requires at least 10 scans per peak for adequate quantitation. This procedure will contain one surrogate, which is isotopically labeled DOSS, and DOSS which are in one MRM acquisition function to optimize sensitivity. Due to the low pKa of the sulfonate and to avoid bias associated with adducts, DOSS analysis was performed using electrospray negative ionization. Variable parameters regarding retention times, SRM transitions, and cone and collision energies are shown in Table 3. Mass spectrometer parameters used in the development of this method are listed below:

The instrument is set in the Electrospray negative source setting.
Capillary Voltage: 3.5 kV
Cone: Variable depending on analyte (Table 3)
Extractor: 2 Volts
RF Lens: 0.3 Volts
Source Temperature: 120°C
Desolvation Temperature: 350°C
Desolvation Gas Flow: 800 L/hr
Cone Gas Flow: 25 L/hr
Low Mass Resolution 1: 14.0
High Mass Resolution 1: 14.0
Ion Energy 1: 0.8

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Entrance Energy: -1
Collision Energy: Variable depending on analyte (Table 3)
Exit Energy: 0
Low Mass Resolution 2: 14.0
High Mass resolution 2: 14.0
Ion Energy 2: 1.0
Multiplier: 650
Gas Cell Pirani Gauge: 7.0 x 10^{-3} Torr
Inter-Channel Delay : 0.02 seconds
Inter-Scan Delay: 0.01 seconds
Dwell: 0.1 seconds
Solvent Delay: 5 minutes

TABLE 3. Retention Times, MRM transitions, and DOSS-Specific Mass Spectrometer Parameters

Analyte	Retention time (min)	Cone Voltage (Volts)	Collision Energy (eV)	MRM Mass Transition (Parent > Product)
DOSS	6.44	36	24	421.1 > 80.6
DOSS-D34 (Surrogate)	6.16	37	26	455.3 > 80.6
DOSS-^{13}C (Surrogate)*	TBD	TBD	TBD	>

12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated per manufacturer specifications before analysis. In order to obtain accurate analytical values through using this test method within the confidence limits, the following procedures must be followed when performing the test method. Prepare all solutions in the lab using Class A volumetric glassware.

12.2 Calibration and Standardization– To calibrate the instrument, analyze six DOSS and surrogate calibration standards; the calibration standards nominal concentrations are detailed in

Table 4. A calibration solution is prepared from standard materials or certified solutions. Level 6 calibration solution containing the DOSS and surrogate is prepared and aliquots of that solution are diluted to prepare Levels 1 through 5 and the DVL. The following steps will produce standards with the concentration values shown in Table 4. The analyst is responsible for recording initial component weight, calculating dilutions and preparing appropriate solutions.

12.2.1 Prepare Level 6 calibration stock standard at 200 ppb by adding to a 10 mL volumetric flask individual solutions of the following: 100 µL of DOSS and DOSS (isotopic labeled) each at 20 ppm in 50% water/50% acetonitrile and dilute to 10 mL with a solution of 5 millimolar ammonium formate in 50% water/50% acetonitrile. The preparation of the stock standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory. Depending on the prepared stock concentrations, the solubility at that concentration will have to be ensured.

12.2.2 Aliquots of Level 6 calibration stock standard are then diluted with 5 millimolar acetonitrile in 50% water/50% acetonitrile to prepare the desired calibration levels in 2 mL amber glass autosampler vials. The calibration vials must be used within 24 hours to ensure optimum results. Stock calibration standards are routinely replaced every 7 days if not previously discarded for quality control failure. Calibration standards are not filtered.

12.2.3 Inject each standard and obtain its chromatogram. An external calibration technique is used to monitor the SRM transitions of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates using the SRM transition. The calibration software manual should be consulted to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppb units. Concentrations may be calculated

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using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin is not recommended.

12.2.4 Linear calibration may be used if the coefficient of determination, r^2, is >0.98 for the analyte (Figure 3). The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or low point causes the r^2 of the curve to be <0.98, this point must be re-injected or a new calibration curve must be regenerated. If the Level 1 or Level 6 calibration result is excluded, minimally a five point curve is acceptable but the reporting range must be modified to reflect this change.

12.2.5 Quadratic calibration may be used if the coefficient of determination, r^2, is >0.99 for the analyte. The point of origin is excluded, and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations (Figure 4). If one of the calibration standards causes the curve to be <0.99, this point must be re-injected or a new calibration curve must be regenerated. At least six calibration points are required for quadratic regression. Each calibration point used to generate the curve must have a calculated percent deviation less than 25% between the nominal concentration and the regression calculated result.

12.2.6 The retention time window of the SRM transitions must be within 5% of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calibration curve to determine if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

12.2.7 A calibration check standard (midpoint) must be analyzed at the end of each batch of 20 samples or within 24 hours after the initial calibration curve was generated (100 ppb). The

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end calibration check should be the same calibration standard that was used to generate the initial curve. The regression result from the end calibration check standard must have a percent deviation less than 35% from the target analyte and surrogate nominal concentration. If the results are not within these criteria, the problem must be corrected and either all samples in the batch must be re-analyzed against a new calibration curve or the affected results must be qualified with an indication that they are not within the performance criteria of the test method. If the analyst inspects the vial containing the end calibration check standards and notices that the samples evaporated affecting the concentration, a new end calibration check standard may be made and analyzed. If this new end calibration check standard has a percent deviation less than 35% from the calculated concentration for the target analyte and surrogate, the results may be reported unqualified.

TABLE 4. Concentrations of Calibration Standards (PPB)

Analyte/Surrogate	DVL	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6
DOSS	3	10	20	60	100	150	200
DOSS-D34 (Surrogate)	3	10	20	60	100	150	200
DOSS-^{13}C (Surrogate)	3	10	20	60	100	150	200

12.3 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study must be performed to demonstrate laboratory capability (Section 16).

12.3.1 Analyze at least four replicates of a sample solution containing the DOSS and surrogate at a concentration in the calibration range of Levels 3 to 5. The Level 4 concentration of the 6 point calibration curve was used to set the QC acceptance criteria in this method. The matrix and chemistry should be similar to the solution used in this test method. Each replicate

must be taken through the complete analytical test method including any sample pre-treatment steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of the QC acceptance criteria for the Initial Demonstration of Performance in Table 5.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 5.

12.3.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in Table 5 are preliminary until a more data and multi-laboratory study is completed. Data generated from a single-laboratory validation from reagent and sea water matrices are shown in the Precision and Bias Section 16. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard. A Reference on how to generate QC acceptance criteria is in Method 8000B in EPA publication SW-846.

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TABLE 5. Preliminary QC Acceptance Criteria

Analyte	Test Conc. (µg/L) in Reagent Water	Initial Demonstration of Performance			Lab Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit
DOSS	200	50	150	30	50	150
DOSS-D34 (Surrogate)	200	50	150	30	50	150
DOSS-^{13}C (Surrogate)	TBD	TBD	TBD	TBD	TBD	TBD

12.4 Surrogate Spiking Solution (This draft contains DOSS-D34 data, DOSS-^{13}C will be reported when available):

June 22, 2010, DOSS-^{13}C was in the synthesis stage and DOSS-D34 was commercially available. Notable different retention times were observed with DOSS and DOSS-D34 (Table 3). DOSS-D34 surrogate resulted a different elution time than DOSS (Table 3) and had apparent deuterium hydrogen exchange (similar distribution at 2 and 16 hours). ^{13}Carbon surrogates are not liable to mass change associated with deuterated surrogate and typically elute at the same time as their analytes, therefore DOSS-^{13}C surrogate will be purchased and tested.

A surrogate spiking 50% water/50% CH_3CN solution containing DOSS-^{13}C or - D34 is added to all samples. A stock surrogate spiking solution is prepared at 20 ppm. Spiking 200 µL of this spiking solution into a 20 mL water sample results in a concentration of 200 ppb of the surrogate in the sample. The result obtained for the surrogate recovery must fall within the limits of Table 5. If the limits are not met, the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method.

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12.5 Method Blank:

12.5.1 Analyze a reagent water blank with each batch of 20 or fewer samples. The DOSS measured in the blank should be less than half of the reporting limit. If the concentration of DOSS is found above this level, analysis of samples is halted until the contamination is eliminated, and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method. DOSS has been found to carry-over in LC systems. A rigorous washing of the injector with stronger wash solvents has been shown to work well as described in this method. If you have an older LC system or have carry-over problems the LC conditions listed in Table 2B should be investigated to remove background.

12.6 Laboratory Control Sample (LCS):

12.6.1 To ensure that the test method is in control, analyze a LCS prepared with the DOSS at a concentration in the calibration range of Levels 3 to 5. The LCS is prepared following the analytical method and analyzed with each batch of 20 samples or less. Prepare a stock matrix spiking solution in 50% water/50% acetonitrile containing the DOSS at 20 ppm. Spike 200 µL of this stock solution into 20 mL of water to yield a concentration of 200 ppb for the DOSS in the sample. The LCS result must be within the limits in Table 5. Matrix spiking solutions are routinely replaced every 7 days if not previously discarded for quality control failure.

12.6.2 If the LCS regression result is not within Table 5 limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be re-analyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7 *Matrix Spike/Matrix Spike Duplicate (M/MSDS):*

12.7.1 To check for interferences in the specific matrix being tested, perform a MS/MSD on at least one sample from each batch of 10 or fewer samples by spiking the sample with a known concentration of DOSS and following the analytical method. Prepare a stock matrix spiking solution in 50% water/50% acetonitrile containing the DOSS at 20 ppm. Spike 200 µL of this stock solution into 20 mL of water to yield a concentration of 200 ppb of the DOSS in the sample.

12.7.2 If the spiked concentration plus the background concentration exceeds that of the Level 6 calibration standard, the sample must be diluted to a level near the midpoint of the calibration curve.

12.7.3 Calculate the spike percent recovery (P) using Equation 1:

Equation 1

$$P = 100\frac{\left|A(Vs+V) - BVs\right|}{CV}$$

Where:
A = concentration found in spiked sample
B = concentration found in unspiked sample
C = concentration of analyte in spiking solution
V_s = volume of sample used
V = volume of spiking solution added
P = percent recovery

12.7.4 The percent recovery of the spike shall fall within the limits in Table 6. If the percent recovery is not within these limits, matrix interference may be present in the selected sample. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not

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affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7.5 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 6 are preliminary until more data is acquired. The data generated by a single-laboratory using sea water samples are in the Precision and Bias Section 16. The matrix variation between the different waters may have a tendency to generate significantly wider control limits than those generated by a single-laboratory in one water matrix. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard. Surrogates should be used to identify and measure matrix affect.

12.7.5.1 The laboratory should generate their own in-house QC acceptance criteria after the analysis of 15-20 matrix spike samples of a particular surface water matrix. References on how to generate QC acceptance criteria is in Method 8000B in EPA publication SW-846.

TABLE 6. Preliminary MS/MSD QC Acceptance Criteria

Analyte	Test Conc. (µg/L)	MS/MSD Recovery (%) Lower Limit	MS/MSD Recovery (%) Upper Limit	MS/MSD Precision Maximum RPD (%)
DOSS	200	50	150	30
DOSS-D34 (Surrogate)	200	50	150	30
DOSS-^{13}C (Surrogate)	TBD	TBD	TBD	TBD

12.8 *Duplicate:*

12.8.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the sample contains the analyte at a level greater than 5 times

the detection limit of the method, the sample and duplicate may be analyzed unspiked; otherwise, an MSD should be used.

12.8.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq. 2. Compare to the RPD limit in Table 6.

Equation 2

$$\text{RPD} = \frac{|\text{MSR - MSDR}|}{(MSR + MSDR) \div 2} \times 100$$

Where:
RPD= relative percent difference
MSR= matrix spike recovery
MSDR= matrix spike duplicate recovery

12.8.3 If the result exceeds the precision limit, the batch must be re-analyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

13. Procedure

13.1 This test method is based upon a 20 mL sample size per analysis. **Any sample size may be used such as a half filled VOA vial as long as the QC spikes and sample preparation volumes are adjusted accordingly.** Samples shall be analyzed within 5 days of collection. If the samples are above 6°C when received or during storage, or not analyzed within 5 days of collection, the data is noted in the case narrative that accompanies the data.

13.2 In the laboratory, the entire 20 mL sample, collected in a 20 mL glass collection vial, is poured into a 50 mL graduated cylinder. The surrogate as described in Section 12 (added to the

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sample in the original sample container or graduated cylinder depending on sampling described in Section 10.1) and NH_4CO_2H are added to the sample. The laboratory control and matrix spike samples are then spiked with the target compound as described in Section 12. The vial is rinsed with two 6 to 8 mL portions of acetonitrile to collect DOSS remaining in the collection vial. These 2 portions are added to the 50 mL graduated cylinder. The samples are then diluted to 40 mL final volume with acetonitrile and mixed thoroughly. The prepared sample is filtered through the syringe driven filter unit fitted with a PVDF filter cartridge into a glass storage vial.

13.3 For samples that are not biphasic, the entire 40 mL volume is filtered through the filtration device described in Section 7.2 into a pre-cleaned collection vial (such as a VOA vial). A portion of that filtered sample is added to an amber glass LC vial with a Teflon® lined cap which is analyzed. A new filter unit is used for each sample.

13.4 For biphasic samples, the lower aqueous layer is filtered through the filtration device described in Section 7.2 into a pre-cleaned collection vial (such as a VOA vial). The upper oil layer is left behind and is not added to the filtration device. A portion of that filtered sample is added to an amber glass LC vial with a Teflon® lined cap which is analyzed. A new filter unit is used for each sample.

13.5 The syringe must be cleaned between each filtration. It is the analyst's responsibility to ensure that the syringe is clean. A suggested method for cleaning the syringe between filtrations is to first rinse with at least 5 syringe volumes of water, followed by at least 3 volumes of 50% water/50% acetonitrile.

13.6 Once a passing calibration curve is generated the analysis of samples may begin. An order of analysis may be: method blank, laboratory control sample and duplicate, method blank,

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up to 20 samples, matrix spike sample(s) and duplicate followed by an end calibration check standard and a method blank.

14. Calculation or Interpretation of Results

14.1 For DOSS and surrogate analysis, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. Calibration curves are used to calculate the amounts of DOSS and surrogate. Calculate the concentration in µg/L (ppb) for each analyte. The sample concentration was diluted by two fold by the addition of surrogates, ammonium formate, acetonitrile, and target compound spike where applicable. The two fold dilution must be accounted for when reporting the concentration. DOSS may be reported if present at or above the reporting limit. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with 5 mM ammonium formate in 50% acetonitrile/50% water to obtain a concentration near the mid-point of the calibration range and re-analyzed. This method uses one surrogate, either DOSS 1-^{13}C, or DOSS-D34, to monitor performance and matrix affect. The surrogate recoveries are provided with all data generated from this test method.

14.1.1 A surrogate is used to monitor the performance of DOSS. If the surrogate meets the quality control criteria in this test method, the data may be reported unqualified for DOSS if all other quality control in this test method are acceptable. If the surrogate does not meet the quality control criteria of the test method, the data is qualified for DOSS.

15. Report

15.1 Determine the results in units of µg/L (ppb) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve. All data that does not meet the specifications in the test method must be appropriately qualified.

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16. Precision and Bias

16.1 The determination of precision and bias was conducted through US EPA Region 5 Chicago Regional Laboratory. **More than 50 seawater samples have been analyzed using this procedure without noticeable chromatography effect or increased pressure.**

16.2 This test method was tested by CRL on reagent and sea water. The samples were spiked with the DOSS to obtain a 200 ppb concentration of each as described in Section 12. Table 7 contains the recoveries and standard deviation (SD) for the target compound.

TABLE 7. Single-Laboratory Recovery Data in Reagent Water using chromatography conditions outlined in Table 2A and Table 2B

Precision and Accuracy Samples	Measured ppb from 200 ppb DOSS Spikes in Reagent Water					
	Table 2A			Table 2B		
	Retention time (min)	DOSS measured (ppb)	Percent Recovery	Retention time (min)	DOSS measured (ppb)	Percent Recovery
1	6.38	182.6	91.3%	5.41	196.1	98.0%
2	6.37	232.9	116.5%	5.41	232.8	116.4%
3	6.37	224.6	112.3%	5.41	225.8	112.9%
4	6.37	226.8	113.4%	5.41	238.9	119.5%
Average Recovery:		216.7	108.4%		223.4	111.7%
Standard Deviation:		23.0			19.0	
% Relative SD		11.5%			9.5%	

16.3 This test method was tested by CRL on Gulf of Mexico sea water. The samples were spiked with target compound as described in Section 12. Table 8 contains the recoveries for the target compound.

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TABLE 8. Single-Laboratory Recovery Data in Gulf of Mexico Sea Water using chromatography conditions outlined in Table 2A and Table 2B

Precision and Accuracy Samples	Measured ppb from 200 ppb DOSS Spikes in Gulf Water					
	Table 2A			Table 2B		
	Retention time (min)	DOSS measured (ppb)	Percent Recovery	Retention time (min)	DOSS measured (ppb)	Percent Recovery
1	6.37	189.1	94.6%	5.4	257.2	128.6%
2	6.37	251.4	125.7%	5.4	251.4	125.7%
3	6.37	253.2	126.6%	5.41	263.0	131.5%
4	6.37	257.0	128.5%	5.4	262.4	131.2%
5	6.37	250.7	125.4%	5.4	255.0	127.5%
Average Recovery:		240.3	120.1%		257.8	128.9%
Standard Deviation:		28.7			4.9	
% Relative SD		14.4%			2.5%	

17. Keywords

17.1 Dioctyl Sulfosuccinate; Liquid Chromatography; Mass Spectrometry; Water

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APPENDIX A. CALIBRATION AND SAMPLE MATRIX RAM-DOSS RESULTS

MEASURED BY WATERS LC-MS/MS

TABLE 9. Single-Laboratory DOSS Recovery Data from Gulf of Mexico Sea Water with and without DOSS Contaminated Crude Oil and DOSS Spikes.

DOSS Recovery Data in Gulf of Mexico Water Spiked with Oil	Gulf Seawater (mL)	Gulf oil (mL)	DOSS spike (ppb)	DOSS measured (ppb)	Recovery %
Gulf water1	1.0	NA	NA	ND	-
Gulf water2	1.0	NA	NA	ND	-
Gulf water3	1.0	NA	NA	ND	-
Gulf water4	1.0	NA	NA	ND	-
Gulf water DOSS addition1	4.0	NA	200	277.8	138.9%
Gulf water DOSS addition2	4.0	NA	200	189.8	94.9%
Gulf water oil addition1	3.6	0.4	NA	3,319	-
Gulf water oil addition2	3.6	0.4	NA	1,198	-
Gulf water oil and DOSS addition1	3.6	0.4	500	2,046	*
Gulf water oil and DOSS addition2	3.6	0.4	500	1,876	*

* DOSS detected in Gulf of Mexico water samples with DOSS contaminated oil addition was greater than the spike concentration.

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TABLE 10. Single-Laboratory **DOSS-D34 Recovery Data from Gulf of Mexico Water with DOSS Contaminated Gulf Oil Spiked with DOSS-D34.**

				3 mL Subsampled Water[1]	**Water and Oil Extraction[2]**
			DOSS-D34*	DOSS-D34	DOSS-D34
	Gulf water (mL)	Gulf oil (mL)	ng spiked	ng measured	ng measured
MB1	3.6	0.4	ND	ND	ND
MB2	3.6	0.4	ND	ND	ND
Gulf Oil and water1	3.6	0.4	800	ND	694
Sample4	3.6	0.4	800	ND	864

*Sigma 710652-SPEC DOSS spiked at 200ppb (calibration midpoint) and stored for 16 hours prior to sample preparation.

[1]Gulf water subsampled and prepared following Section 10.1.2

[2] Gulf water and oil sample prepared following Section 10.1.1

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TABLE 11. Single-Laboratory DOSS Recovery Data from Gulf of Mexico Water with Southern Louisiana Reference Crude Oil Spiked with 0, 20, 200 and 400 ppb DOSS then Stored 16 hours between 0 and 6°C.

	Gulf Water (mL)	Southern Louisiana Reference Crude Oil (mL)	Water Subsample[1]		Whole Sample[2]	
			DOSS	DOSS-D34	DOSS	DOSS-D34
Method Blank 1	19.8	0.2	ND	107.4%	ND	98.3%
Method Blank 2	19.8	0.2	ND	115.4%	ND	102.4%
Method Blank 3	19.8	0.2	ND	155.8%	ND	132.9%
20 ppb 1	19.8	0.2	-	-	ND	123.7%
20 ppb 2	19.8	0.2	-	-	ND	121.8%
20 ppb 3	19.8	0.2	-	-	ND	127.8%
200 ppb 1	19.8	0.2	26.6%	105.8%	51.3%	134.7%
200 ppb 2	19.8	0.2	21.3%	101.2%	46.3%	126.8%
200 ppb 3	19.8	0.2	20.1%	98.1%	44.4%	112.5%
400 ppb 1	19.8	0.2	24.2%	92.1%	47.1%	108.6%
400 ppb 2	19.8	0.2	28.0%	91.6%	45.1%	107.7%
400 ppb 3	19.8	0.2	27.4%	93.0%	40.6%	103.6%

[1]Gulf water subsampled and prepared following Section 10.1.2.

[2] Gulf water and oil sample prepared following Section 10.1.1.

DOSS additions were performed prior to oil addition and vortexed. After the oil was added the samples were stored between 0 and 6 for 16 hours. The 20 ppb water subsamples were not tested as low recoveries from 200 ppb and 400 ppb indicated that DOSS measurements would be below the method RL. Percent recoveries from whole samples were on average greater than subsamples.

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X1.1.1 A holding time study was performed by US EPA Region 5 Chicago Regional Laboratory on DOSS in Gulf of Mexico water. The Gulf of Mexico water was spiked with DOSS to obtain a 50 ppb concentration of DOSS.

TABLE 12. Single-Laboratory DOSS in Gulf of Mexico Water Holding Time Study

50 ppb DOSS Storage in Gulf of Mexico Water (4 ºC)	Percent Recovery			
	Day 0	Day 7	Day 10	
	DOSS	DOSS	DOSS	DOSS-D34*
1	93.3%	74.2%	51.8%	97.6%
2	95.3%	77.1%	51.4%	100.8%
3	97.9%	78.7%	48.4%	97.4%
Average Recovery	95.5%	76.7%	50.5%	98.6%
% Relative SD	0.023	0.022	0.019	0.019

*Sigma 710652-SPEC DOSS-D34 was spiked during Day 10 sample preparation (Section10.1.2). The DOSS-D34 surrogate was not available for Day 0 and Day 7 sample preparation.

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Figure 1. Detection Verification Level (3 ppb DOSS in calibration standard) Signal/Noise Ratio.

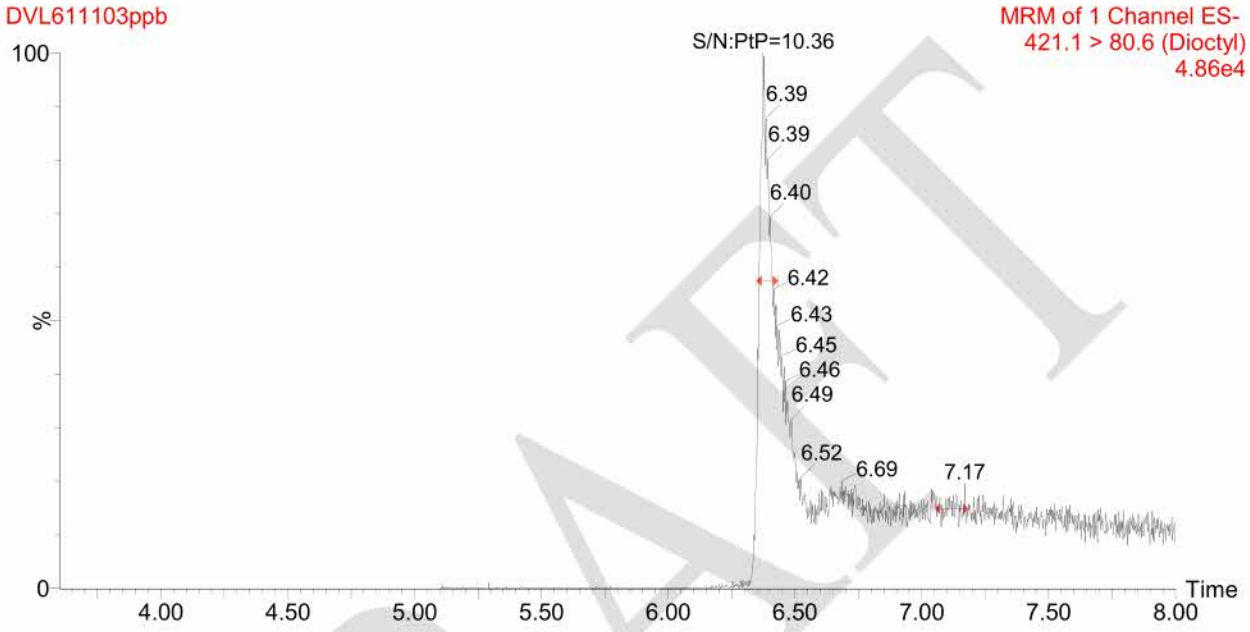

Figure 2. Reporting Level (10 ppb DOSS in calibration standard) Signal/Noise Ratio.

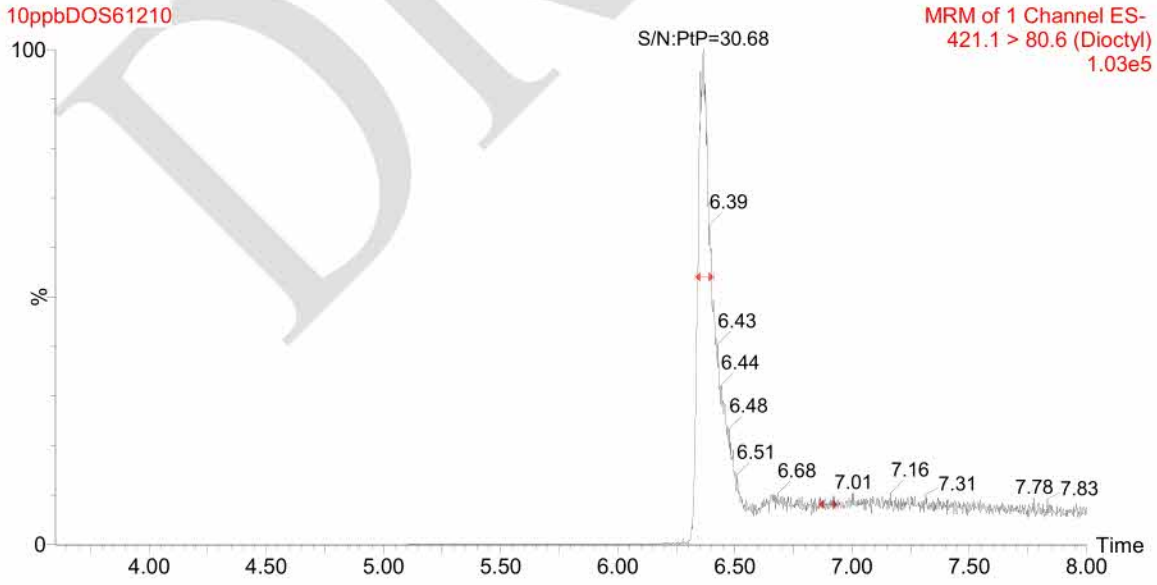

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Figure 3. Ten to 200 ppb DOSS Linear calibration curve (5 millimolar ammonium formate in 50% acetonitrile/50% water).

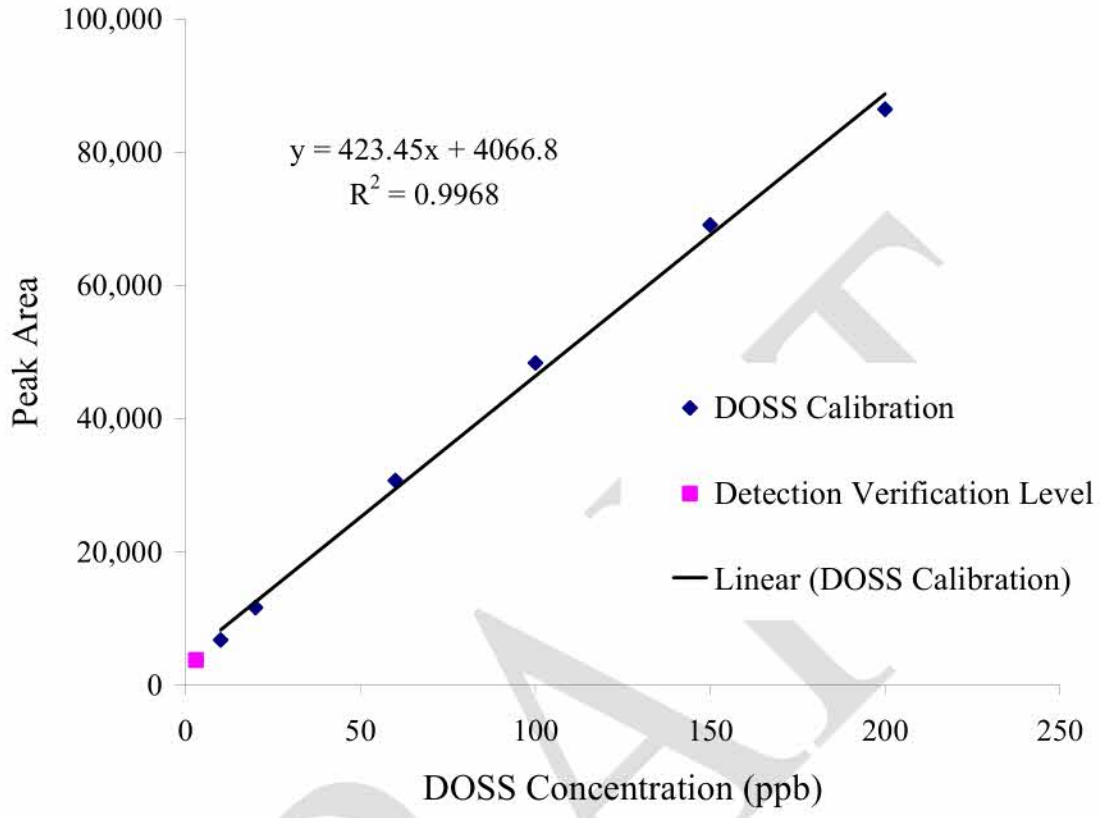

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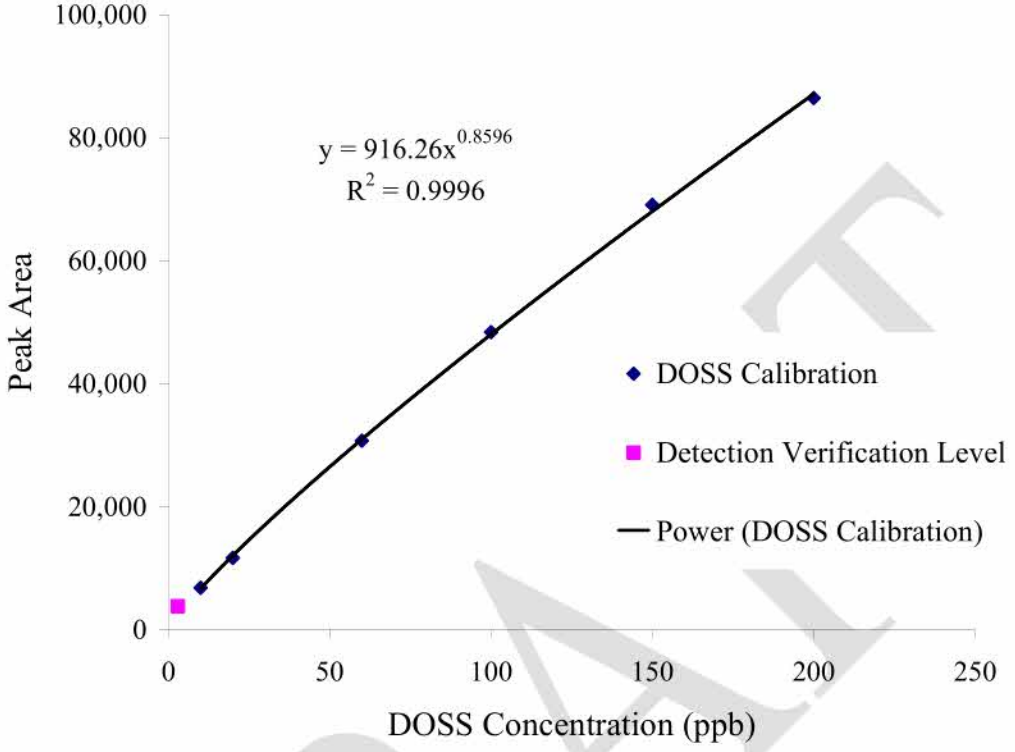
Figure 4. Ten to 200 ppb DOSS quadratic calibration curve (5 millimolar ammonium formate in 50% acetonitrile/50% water).



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APPENDIX B. RAM-DOSS BY AGILENT LC-MS/MS

LIQUID CHROMATOGRAPHY / MASS SPECTRAL IDENTIFICAITON (using AGILENT instrument)

Instrumentation

i. Agilent 6410 Triple Quad Mass Spectrometer with Mass Hunter Version v3.0 for system control and Data acquisition and processing
ii. Agilent 1200 Binary HPLC pump, Auto sampler, Column compartment and online Degasser.

b. LC conditions
   i. Zorbax Eclipse XBD C18 analytical column 2.1 x 50 mm, 3.5 micron particle size(part no.971700-902)
   ii. Eluent
      1. A: 99% water +1% ACN with 0.1% formic acid
      2. B: 99% ACN +1% water with 0.1% formic acid
   iii. Injection volume: 15uL
   iv. LC conditions (see table below)

Time (min)	Flow (mL/Min)	%A (water with 0.1% Formic)	%B (ACN with 0.1% Formic)
0-1	0.4	98	2
4	0.4	60	38
7	0.4	20	80
12	0.4	2	98
12.1	0.4	98	2

c. Mass Spectrometer Conditions
   i. Data Acquisition parameters(see table below)

Parameter	Settings
Scan Mode	negative
Capillary Voltage	4.0 kV
To Waste	Until 2.6 min
Segments	3
Gas Temperature	350 °C
Gas Flow	6 L/min
Nebulizer	15 psi

Compound	Ion	Segment	Prec. ion	Prod. ion	Frag (V)	CE (V)	Multiplier (V)
DOSS	M-Na	1	421	81	188	30	600
DOSS-D34	M-Na	1	445.4	81	188	30	600

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Region 5 Draft Procedure for the Determination of Dipropylene Glycol Monobutyl Ether (CAS # 29911-28-2) and Ethylene Glycol Monobutyl Ether (CAS# 111-76-2) in Sea Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)[1]

1. Scope

1.1 This procedure covers the determination of Dipropylene Glycol Monobutyl Ether (DPGBE) and Ethylene Glycol Monobutyl Ether (EGBE) in sea water by direct injection using liquid chromatography (LC) and detection with tandem mass spectrometry (MS/MS). This analyte is qualitatively and quantitatively determined by this method. This method adheres to single reaction monitoring (SRM) mass spectrometry.

1.2 *Units*— The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 The Detection Verification Level (DVL) and Reporting Range for DPGBE and EGBE are listed in Table 1.

1.3.1 The DVL is required to be at a concentration at least 3 times below the Reporting Limit (RL) and have a signal/noise ratio greater than 3:1. Figures 1 and 2 display the signal/noise ratio of the single reaction monitoring (SRM) transition.

1.3.2 The reporting limit is the concentration of the Level 1 calibration standard as shown in Table 4 for DGGBE and EGBE, taking into account the 20% sample preparation dilution factor.

[1] Do not cite, quote, or distribute this draft analytical procedure developed by US EPA Region 5 Chicago Regional Laboratory (CRL), it is the first procedure developed by CRL and is open to improvement and discussion. July 12, 2010.

TABLE 1. Detection Verification Level and Reporting Range

Analyte	DVL (µg/L)	Reporting Range (µg/L)
DPGBE	0.2	1-10
EGBE	25	125-1250

1.4 *This procedure does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water
D 1193 Specification for Reagent Water

2.2 *Other Documents*[2]

EPA publication SW-846, entitled *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*

3. Terminology

3.1 *Definitions:*

3.1.1 Detection Verification Level, DVL, n– a concentration that has a signal/noise ratio greater than 3:1 and is at least 3 times below the Reporting Limit (RL).

[2] Available from National Technical Information Service (NTIS), U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA, 22161 or at http://www.epa.gov/epawaste/hazard/testmethods/index.htm

3.1.2 Reporting Limit, RL, n– the concentration of the lowest-level calibration standard used for quantification.

3.2 *Abbreviations:*

3.2.1 ESI- Electrospray ionization

3.2.2 DPGBE- Dipropylene Glycol Monobutyl Ether (CAS # 29911-28-2)

3.2.3 EGBE- Ethylene Glycol Monobutyl Ether (CAS# 111-76-2)

3.2.4 *n*-NP2EO- normal- Nonylphenol Diethoxylate (CAS# Not available, Source: Cambridge Isotope Laboratories or Accustandard, Inc.)

3.2.5 LC/MS- liquid chromatography mass spectrometry

3.2.6 ppb- parts per billion, µg/L

3.2.7 ppt- parts per trillion, ng/L

3.2.8 mM- millimolar, 1 x 10^{-3} moles/L

3.2.9 NA- no addition

3.2.10 ND- non-detect

4. Summary of Procedure

4.1 This is a performance based method, and modifications are allowed to improve performance.

4.2 For DPGBE and EGBE analysis, samples are shipped to the lab between 0°C and 6°C and analyzed within 5 days of collection. The DOW MSDS sheet on DOWANOL* DPNB glycol ether (DPGBE) Issue Date: 06/18/2010 lists that the material is readily biodegradable. The OECD 302B Test lists 96% biodegradation in 28 days.

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4.3 In the lab, the entire collected 20 mL sample is spiked with surrogate and brought to a volume of 25 mL with acetonitrile. This prepared sample is then filtered using a syringe driven filter unit, and analyzed by LC/MS/MS. If visible oil is present, the prepared sample is allowed to settle resulting in an oil layer at the top of the 25 mL solution. A portion of the aqueous (bottom) layer is filtered, leaving the oil layer behind, through a syringe driven filter assembly and analyzed by LC/MS/MS.

4.4 DPGBE, EGBE and surrogate are identified by retention time and one SRM transition. The target analytes and surrogate are quantitated using the SRM transitions utilizing an external calibration. The final report issued for each sample lists the concentration of DPGBE, EGBE and the surrogate recovery.

5. Significance and Use

5.1 DPGBE and EGBE have been identified as possible ingredients in dispersant agents used to treat oil.

5.2 This method has been investigated for use with reagent and sea water.

6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other apparatus producing discrete artifacts or elevated baselines. All of these materials are demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as samples.

6.2 All glassware is washed in hot water with detergent and rinsed in hot water followed by distilled water. Detergents containing DPGBE or EGBE must not be used. The glassware is

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then dried and heated in an oven at 250°C for 15 to 30 minutes. All glassware is subsequently cleaned with acetone followed by methanol.

6.3 All reagents and solvents should be pesticide residue purity or higher to minimize interference problems.

6.4 Matrix interferences may be caused by contaminants in the sample. The extent of matrix interferences can vary considerably from sample source depending on variations of the sample matrix.

6.5 Graduated cylinder- a graduated cylinder was used to measure the volume of the sample and total volume. An appropriate cylinder for different sample volumes should be used to accurately measure the sample and prepared sample volumes.

7. Apparatus

7.1 LC/MS/MS System

7.1.1 *Liquid Chromatography System*- A complete LC system is needed in order to analyze samples.[3] Any system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard may be used.

7.1.2 *Analytical Column- Waters-* XBridge™, 2.1 x 150 mm, 3.5 µm particle size was used to develop this test method. Any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and need to be monitored.

7.1.3 *Tandem Mass Spectrometer System*- A MS/MS system capable of MRM analysis.[4] Any system that is capable of performing at the requirements in this procedure may be used.

[3] A Waters ACQUITY UltraPerformance Liquid Chromatography (UPLC®) System was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

7.2 Filtration Device

7.2.1 *Hypodermic syringe*- A Lock Tip Glass Syringe capable of holding a Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm or similar may be used.

7.2.1.1 A 10 or 25 mL Lock Tip Glass Syringe size was used in this test method.

7.2.2 *Filter*– Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm (Millipore Corporation, Catalog # SLGV033NS) or similar may be used.

7.2.3 Glass Pipette- small volume pipette for sample container rinses and sample transfer

8. Reagents and Materials

8.1 *Purity of Reagents*- High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall conform to the Committee on Analytical Reagents of the American Chemical Society.[5] Other reagent grades may be used provided they are first determined to be of sufficiently high purity to permit their use without affecting the accuracy of the measurements.

8.2 *Purity of Water*– Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Type 1 of Specification D 1193. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

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[4] A Waters Quattro Premier™ XE tandem quadrupole mass spectrometer was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

[5] Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, D.C. For Suggestions on the testing of reagents not listed by the American Chemical Society, see Annual Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, U.K., and the United States Pharmacopeia and National Formulators, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

8.3 *Gases*– Ultrapure nitrogen and argon.

8.4 Acetonitrile (CAS # 75-05-8).

8.5 Methanol (CAS # 67-56-1).

8.6 Formic Acid (CAS # 64-18-6).

8.7 2-Propanol (CAS # 67-63-0).

8.8 DPGBE- Dipropylene Glycol Monobutyl Ether (CAS # 29911-28-2)

8.9 EGBE- Ethylene Glycol Monobutyl Ether (CAS# 111-76-2)

8.10 *n*-NP2EO- normal- Nonylphenol Diethoxylate (CAS# Not available)

9. Hazards

9.1 Normal laboratory safety applies to this method. Analysts should wear safety glasses, gloves, and lab coats when working in the lab. Analysts should review the Material Safety Data Sheets (MSDS) for all reagents used in this method.

10. Sampling

10.1 *Sampling and Preservation*– Grab samples should be collected in 20 mL pre-cleaned glass vials with Teflon® lined septa caps demonstrated to be free of interferences. The vial should be filled to approximately 20 mL. This may be just below the neck of the vial, depending on the vial manufacturer. This test method is based on a 20 mL sample size per analysis. Each sample must be collected in duplicate and a quadruplicate sample must be included with each sample batch of 10 for MS/MSD quality control analyses. Store samples between 0°C and 6°C from sample collection to sample preparation. Analyze the sample within 5 days of collection.

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Samples may be encountered that have more than one phase. Samples may be prepared for analysis using one or multiple of the following options:

10.1.1 For a 20 mL water sample, add 0.1 mL of 2.4 ppm *n*-NP2EO surrogate spike solution to the sample in the sample container. Cap the container and mix to ensure homogeneity. Transfer the contents of the sample container to a graduated cylinder and record the sample volume. Rinse the sample collection vial twice with 2 mL portions of acetonitrile, which is added to the prepared sample and dilute to 25 mL with acetonitrile to compose ~ 20% acetonitrile solution to ensure quantitative sample transfer. If different sample sizes are used, spiking solution and acetonitrile volume shall be adjusted proportionally. (Note: Since an accurate sample volume may not be known prior to measurement into the graduated cylinder the appropriate spike may be added directly to the graduate cylinder instead of the collection vial. The collection vial must be rinsed with acetonitrile which is added to the graduated cylinder and this prepared sample must be thoroughly mixed.) The entire sample should be filtered through the syringe driven filter unit described in Section 7.2. If the sample is biphasic, due to a top oil layer, a portion of the aqueous fraction (bottom) should be transferred to a glass syringe for filtration (see section 10.1.1.1). Filter the sample using a 0.22 µm PVDF filter into a glass vial and then transfer to an autosampler vial. For samples that contain an oil layer, an aliquot of the filtered solution should be diluted with 80% water/20% acetonitrile for a preliminary analysis, a 100 fold dilution should be considered until the site samples are characterized. The concentration of the target analytes, especially DPGBE, may be very high in samples that contain an oil layer.

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10.1.1.1 *n*-NP2EO has been shown to be absorbed into the oil layer yielding a non-detect as a result. If oil is present in the sample the recovery of the *n*-NP2EO surrogate may be very low or not detected at or above the reporting limit.

10.1.2 Water Subsample*: Collect a 4 mL subsample of the water fraction (bottom) using a needle and a glass syringe. To reduce the oil exposure, invert the vial and tap gently to cause the oil move away from the septum. Insert needle through septum and collect 4 mL of the water layer; place the aliquot removed in a graduated cylinder. Then add 20 µL of 2.4 ppm *n*-NP2EO surrogate spike solution to the sample in the graduated cylinder. Add 1 mL of acetonitrile to the prepared sample to compose ~ 20% acetonitrile solution. If different sample sizes are used, surrogate spiking solution and acetronitrile volume shall be adjusted proportionally. Filter the sample using a 0.22 µm PVDF filter into a glass vial and then transfer to an autosampler vial.

*Note- Subsamples of water quantitates only the DPGBE and EGBE in the subsample, the DPGBE and EGBE concentration of subsamples may underestimate the sample DPGBE and EGBE concentration as a result of oil partitioning and surface binding.

11. Preparation of LC/MS/MS

11.1 Liquid Chromatograph Operating Conditions[5]

11.1.1 Injection volumes of all calibration standards and samples are made at 100 µL volume. The first sample analyzed after the calibration curve is a blank to ensure there is no carry-over. The gradient conditions for the liquid chromatograph are shown in Table 2. Divert the column flow away from the electrospray source for 0 to 5 minutes after injection. Flow

diversion to waste may be done using the mass spectrometer divert valve, divert tubing configurations vary from manual injection. Sea water samples contain nonvolatile salts, the first 5 minute elution is diverted in order to keep the mass spectrometer source clean.

TABLE 2. Gradient Conditions for Liquid Chromatography

Time (min)	Flow (mL/min)	Percent 95% Water/ 5% CH_3CN	Percent CH_3CN	Percent 2% Formic Acid 95% Water/ 5% CH_3CN
0.0	0.30	95	0	5
2.0	0.30	95	0	5
5.0	0.30	0	95	5
14.0	0.30	0	95	5
15.0	0.30	95	0	5
18.0	0.30	95	0	5

11.2 LC Conditions:

11.2.1 *Needle Wash Solvent*- 60% Acetonitrile/40% 2-propanol

11.2.2 *Temperatures*– Column, 30°C; Sample compartment, 15°C.

11.2.3 *Seal Wash*– 60% Acetonitrile/40% 2-propanol.

11.3 Mass Spectrometer Parameters[6]:

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters may be optimized according to your instrument. Each peak requires at least 10 scans per peak for adequate quantitation. This procedure contains DPGBE, EGBE and one surrogate which are in three MRM acquisition functions to optimize sensitivity. Variable parameters regarding retention times, SRM transitions, and cone and

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collision energies are shown in Table 3. Mass spectrometer parameters used in the development of this method are listed below:

The instrument is set in the Electrospray positive source setting.
Capillary Voltage: 3.5 kV
Cone: Variable depending on analyte (Table 3)
Extractor: 2 Volts
RF Lens: 0.2 Volts
Source Temperature: 120°C
Desolvation Temperature: 350°C
Desolvation Gas Flow: 800 L/hr
Cone Gas Flow: 25 L/hr
Low Mass Resolution 1: 14.5
High Mass Resolution 1: 14.5
Ion Energy 1: 0.5
Entrance Energy: -1
Collision Energy: Variable depending on analyte (Table 3)
Exit Energy: 1
Low Mass Resolution 2: 14.5
High Mass resolution 2: 14.5
Ion Energy 2: 0.8
Multiplier: 650
Gas Cell Pirani Gauge: 7.0×10^{-3} Torr
Inter-Channel Delay : 0.1 seconds
Inter-Scan Delay: 0.1 seconds
Dwell: 0.1 seconds
Solvent Delay: 5 minutes

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TABLE 3. Retention Times, MRM transitions, and Specific Mass Spectrometer Parameters

Analyte	Retention time (min)	Cone Voltage (Volts)	Collision Energy (eV)	MRM Mass Transition (Precursor > Product)
DPGBE	8.5	19	7	191.3 > 115.1
EGBE	7.6	13	5	119.1> 62.9
n-NP2EO (Surrogate)	11.2	28	10	309.3> 89.0
EGBE-D_4	TBD	TBD	TBD	TBD

12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated per manufacturer specifications before analysis. In order to obtain accurate analytical values through using this test method within the confidence limits, the following procedures must be followed when performing the test method. Prepare all solutions in the lab using Class A volumetric glassware.

12.2 *Calibration and Standardization*– To calibrate the instrument, analyze six calibration standards containing (nominal concentrations in Table 4) DPGBE, EGBE and *n*-NP2EO. A calibration solution is prepared from standard materials or they are purchased as certified solutions. Level 6 calibration solution containing the targets and surrogate is prepared and aliquots of that solution are diluted to prepare Levels 1 through 5 and the DVL. The following steps will produce standards with the concentration values shown in Table 4. The analyst is responsible for recording initial component weights correctly calculating and preparing appropriate dilution calculations.

12.2.1 Prepare Level 6 calibration stock standard at 1000 ppb for EGBE, 8 ppb for DPGBE and 40 ppb for *n*-NP2EO in 80% water/20% acetonitrile. The EGBE and DPGBE concentrated stock solutions were prepared in methanol at approximately 2 g/L concentration and the *n*-

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NP2EO surrogate concentrated stock solution was prepared in acetonitrile at approximately 0.5 g/L. The preparation of the stock standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory. Depending on the prepared stock concentrations, the solubility at that concentration will have to be ensured.

12.2.2 Aliquots of Level 6 calibration stock standard are then diluted with 80% water/20% acetonitrile to prepare the desired calibration levels in 2 mL amber glass autosampler vials. The calibration vials must be used within 24 hours to ensure optimum results. Stock calibration standards are routinely replaced every 7 days if not previously discarded for quality control failure. Calibration standards are not filtered.

12.2.3 Inject each standard and obtain its chromatogram. An external calibration technique is used to monitor the SRM transitions of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates using the SRM transition. The calibration software manual should be consulted to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppb units. Concentrations may be calculated using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin is not recommended.

12.2.4 Linear calibration may be used if the coefficient of determination, r^2, is >0.98 for the analyte. The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or low point causes the r^2 of the curve to be <0.98, this point must be re-injected or a new calibration curve must be regenerated. If the low and/or high point is excluded, minimally a five point curve is acceptable but the reporting range must be modified to reflect this change.

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12.2.5 Quadratic calibration may be used if the coefficient of determination, r^2, is >0.99 for the analyte. The point of origin is excluded, and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards causes the curve to be <0.99, this point must be re-injected or a new calibration curve must be regenerated. Minimally a six point curve is acceptable using a quadratic fit. Each calibration point used to generate the curve must have a calculated percent deviation less than 25% from the generated curve.

12.2.6 The retention time window of the SRM transitions must be within 5% of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calibration curve to determine if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

12.2.7 A calibration midpoint check standard must be analyzed at the end of each batch of 20 samples or within 24 hours after the initial calibration curve was generated. This end calibration check should be the same calibration standard that was used to generate the initial curve. The results from the end calibration check standard must have a percent deviation less than 35% from the calculated concentration for the target analytes and surrogates. If the results are not within these criteria, the problem must be corrected and either all samples in the batch must be re-analyzed against a new calibration curve or the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method. If the analyst inspects the vial containing the end calibration check standards and notices that the samples evaporated affecting the concentration, a new end calibration check standard may be made and analyzed. If this new end calibration check standard has a percent deviation less than 35% from

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the calculated concentration for the target analyte and surrogate, the results may be reported unqualified.

TABLE 4. Concentrations of Calibration Standards (PPB)

Analyte/Surrogate	DVL	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6
DPGBE	0.20	0.80	1.6	2.4	3.2	4.0	8.0
EGBE	25	100	200	300	400	500	1000
n-NP2EO (Surrogate)	1.0	4.0	8.0	12	16	20	40
EGBE-D_4	TBD	TBD	TBD	TBD	TBD	TBD	TBD

12.3 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study must be performed to demonstrate laboratory capability (Section 16).

12.3.1 Analyze at least four replicates of a sample solution containing the targets and surrogate at a concentration in the calibration range of Levels 3 to 5. The Level 3 concentration of the 6 point calibration curve was used to set the QC acceptance criteria in this method. The matrix and chemistry should be similar to the solution used in this test method. Each replicate must be taken through the complete analytical test method including any sample pre-treatment steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of the QC acceptance criteria for the Initial Demonstration of Performance in Table 5.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 5.

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12.3.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in Table 5 is preliminary until more data and multi-laboratory study is completed. Data generated from a single-laboratory validation from reagent and sea water matrices are shown in the Precision and Bias Section 16. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard. A reference on how to generate QC acceptance criteria is in Method 8000B in EPA publication SW-846.

TABLE 5. Preliminary QC Acceptance Criteria

Analyte	Test Conc. (µg/L) in Reagent Water	Initial Demonstration of Performance			Lab Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit
DPGBE	2.4	50	150	30	50	150
EGBE	300	50	150	30	50	150
n-NP2EO (Surrogate)	12	25	125	30	25	125
EGBE-D_4	TBD	TBD	TBD	TBD	TBD	TBD

12.4 *Surrogate Spiking Solution:*

12.4.1 A surrogate spiking methanol solution containing *n*-NP2EO is added to all samples. A stock surrogate spiking solution is prepared at 2 .4 ppm. Spiking 100 µL of this spiking solution into a 20 mL water sample results in a concentration of 12 ppb of the surrogate in the sample. The result obtained for the surrogate recovery must fall within the limits of Table 5. If

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the limits are not met, the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.4.1.1 *n*-NP2EO has been shown to be absorbed into the oil layer yielding a non-detect as a result. If oil is present in the sample, the recovery of the *n*-NP2EO surrogate may be very low or not detected at or above the reporting limit. A better surrogate alternative is EGBE-D_4 which is being procured from Cambridge Isotope Laboratories. ^{13}C or ^{2}H labeled DPGBE surrogates are not currently commercially available.

12.5 *Method Blank*:

12.5.1 Analyze a reagent water blank with each batch of 20 or fewer samples. The concentration of the DPGBE and EGBE found in the blank should be 2 times below the reporting limit. If the concentration of DPGBE or EGBE is found above this level, analysis of samples is halted until the contamination is eliminated, and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method. If DPGBE or EGBE are found in a method blank at greater than 2 times below the reporting limit the reporting limit must be raised to at least 2 times the concentration of the DPGBE and EGBE found in the blank. This may occur if samples are encountered that have a high concentration of DPGBE, a water blank between samples may be required to remove carry-over between samples.

12.6 *Laboratory Control Sample (LCS):*

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12.6.1 To ensure that the test method is in control, analyze a LCS prepared with the target analytes at a concentration in the calibration range of Levels 3 to 5. The LCS is prepared following the analytical method and analyzed with each batch of 20 samples or less. Prepare a stock matrix spiking solution in methanol containing the DPGBE at 0.48 ppm and EGBE at 60 ppm. Spike 100 µL of this stock solution into 20 mL of water to yield a concentration of 2.4 ppb for the DPGBE and 300 ppb for EGBE in the sample. The LCS result must be within the limits in Table 5. Matrix spiking solutions are routinely replaced every 7 days if not previously discarded for quality control failure.

12.6.2 If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be re-analyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7 *Matrix Spike/Matrix Spike Duplicate (MS/MSD):*

12.7.1 To check for interferences in the specific matrix being tested, perform a MS/MSD on at least one sample from each batch of 10 or fewer samples by spiking the sample with a known concentration of DPGBE and EGBE and following the analytical method. Prepare a stock matrix spiking solution in methanol containing the DPGBE at 0.48 ppm and EGBE at 60 ppm. Spike 100 µL of this stock solution into 20 mL of water to yield a concentration of 2.4 ppb for the DPGBE and 300 ppb for EGBE in the sample. The result obtained for the MS/MSD must fall within the limits in Table 6. Matrix spiking solutions are routinely replaced every 7 days if not previously discarded for quality control failure.

12.7.2 If the spiked concentration plus the background concentration exceeds that of the Level 6 calibration standard, the sample must be diluted to a level near the midpoint of the calibration curve.

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12.7.3 Calculate the percent recovery of the spike (P) using Equation 1:

Equation 1

$$P = 100\frac{\left|A(Vs+V) - BVs\right|}{CV}$$

Where:

A = concentration found in spiked sample

B = concentration found in unspiked sample

C = concentration of analyte in spiking solution

V_s = volume of sample used

V = volume of spiking solution added

P = percent recovery

12.7.4 The percent recovery of the spike shall fall within the limits in Table 6. If the percent recovery is not within these limits, matrix interference may be present in the selected sample. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7.5 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 6 are preliminary until more data is acquired. The data generated by a single-laboratory using sea water samples are in the Precision and Bias Section 16. The matrix variation between the different waters may have a tendency to generate significantly wider control limits than those generated by a single-laboratory in one water matrix. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard.

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12.7.5.1 The laboratory should generate their own in-house QC acceptance criteria after the analysis of 15-20 matrix spike samples of a particular surface water matrix. References on how to generate QC acceptance criteria is in Method 8000B in EPA publication SW-846.

TABLE 6. Preliminary MS/MSD QC Acceptance Criteria

Analyte	Test Conc. (µg/L)	MS/MSD		
		Recovery (%)		Precision
		Lower Limit	Upper Limit	Maximum RPD (%)
DPGBE	2.4	50	150	30
EGBE	300	50	150	30
n-NP2EO (Surrogate)	12	25	150	30
EGBE-D_4	TBD	TBD	TBD	TBD

12.8 *Duplicate:*

12.8.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the sample contains the analyte at a level greater than 5 times

the detection limit of the method, the sample and duplicate may be analyzed unspiked; otherwise, an MSD should be used.

12.8.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq. 2. Compare to the RPD limit in Table 6.

Equation 2

$$\text{RPD} = \frac{|\text{MSR - MSDR}|}{(MSR + MSDR) \div 2} \times 100$$

Where:
RPD= relative percent difference
MSR= matrix spike recovery
MSDR= matrix spike duplicate recovery

12.8.3 If the result exceeds the precision limit, the batch must be re-analyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

13. Procedure

13.1 This test method is based upon a 20 mL sample size per analysis. **Any sample size may be used such as a half filled VOA vial as long as the QC spikes and sample preparation volumes are adjusted accordingly.** The samples must be analyzed within 5 days of collection. If the samples are above 6°C when received or during storage, or not analyzed within 5 days of collection, the data is qualified estimated and noted in the case narrative that accompanies the data.

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13.2 In the laboratory, the entire 20 mL sample that was collected in a 20 mL VOA vial is poured into a 50 mL graduated cylinder. Every sample is spiked with the surrogate as described in Section 12. The laboratory control and matrix spike samples are then spiked with the target compounds as described in Section 12. The spiking solutions are added to the sample before transfer to the 50 mL graduated cylinder and that volume is subtracted from the measured amount. The exact volume of the sample size is recorded in order to calculate the exact final concentration of DPGBE, EGBE and surrogate. The vial is rinsed with two 2 mL portions of CH_3CN to remove any residual DPGBE, EGBE and surrogate adhered to the collection vial. These 2 portions are added to the 50 mL graduated cylinder. The samples are then diluted to 25 mL final volume with CH_3CN, shaken, filtered through the syringe driven filter unit fitted with a PVDF filter cartridge into glass storage vials and then aliquoted into 2 mL amber glass LC vials for analysis.

13.3 For samples that are homogeneous, the entire 25 mL volume is filtered through the filtration device described in Section 7.2 into a pre-cleaned collection vial (such as a VOA vial). A portion of that filtered sample is added to an amber glass LC vial with a Teflon® lined cap which is analyzed. A new filter unit is used for each sample.

13.4 For biphasic samples, a portion of the lower aqueous layer is filtered through the filtration device described in Section 7.2 into a pre-cleaned collection vial (such as a VOA vial). The upper oil layer is left behind and is not added to the filtration device. A portion of that filtered sample is added to an amber glass LC vial with a Teflon® lined cap which is analyzed. A new filter unit is used for each sample.

13.5 The syringe must be cleaned between each filtration. It is the analyst's responsibility to ensure that the syringe is clean. A suggested method for cleaning the syringe between filtrations

is to first rinse with at least 5 syringe volumes of water, followed by at least 3 volumes of 50% water/50% CH_3CN.

13.6 Once a passing calibration curve is generated the analysis of samples may begin. An order of analysis may be: method blank, laboratory control sample and duplicate, up to 20 samples, matrix spike sample(s) and duplicate followed by an end calibration check which includes a midpoint calibration check standard and a method blank.

14. Calculation or Interpretation of Results

14.1 For quantitative analysis of DPGBE, EGBE and surrogate, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. External calibration curves are used to calculate the amounts of DPGBE, EGBE and surrogate. Calculate the concentration in µg/L (ppb) for each analyte. The sample concentration was diluted by 20% by the addition of surrogates and CH_3CN and target compound spike where applicable, this dilution must be accounted for when reporting the concentration. DPGBE and EGBE may be reported if present at or above the reporting limit. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with reagent water to obtain a concentration near the mid-point of the calibration range and re-analyzed. This method uses one surrogate, *n*-NP2EO, to monitor performance. The surrogate recoveries are provided with all data generated from this test method.

14.1.1 A surrogate is used to monitor the performance of DPGBE and EGBE. If the surrogate meets the quality control criteria in this test method, the data may be reported unqualified for DPGBE and EGBE if all other quality control in this test method are acceptable.

If the surrogate does not meet the quality control criteria of the test method, the data is qualified for DPGBE and EGBE.

15. Report

15.1 Determine the results in units of µg/L (ppb) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve generated. All data that does not meet the specifications in the test method must be appropriately qualified.

16. Precision and Bias

16.1 The determination of precision and bias was conducted through US EPA Region 5 Chicago Regional Laboratory.

16.2 This test method was tested by CRL on reagent water. The samples were spiked with the DPGBE, EGBE and *n*-NP2EO to obtain a 2.4 ppb concentration of DPGBE, 300 ppb EGBE and 12 ppb concentration of *n*-NP2EO each as described in Section 12. Table 7 contains the recoveries and standard deviation (SD) for the target compounds and surrogate in reagent water.

TABLE 7. Single-Laboratory Recovery Data in Reagent Water

Precision and Accuracy Samples	Analyte Measured (ppb)		
	DPGBE	EGBE	n-NP2EO
1	2.22	330	8.90
2	2.10	382	9.09
3	2.08	334	8.14
4	2.23	352	8.66
5	2.18	327	9.02
6	2.01	331	8.84
Spike Concentration (ppb)	2.40	300	12.00
Average Recovery:	2.14	343	8.78
Average Percent Recovery	89.0	114	73.1
Standard Deviation:	0.09	21.2	0.35
% Relative SD	4.09	6.19	3.93

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16.3 This test method was tested by CRL on Gulf of Mexico sea water. The samples were spiked with target compounds and surrogate as described in Section 12. Table 8 contains the recoveries and standard deviation (SD) for the target compounds and surrogate in sea water.

TABLE 8. Single-Laboratory Recovery Data in Gulf of Mexico Sea Water

Precision and Accuracy Samples	Analyte Measured (ppb)		
	DPGBE	EGBE	n-NP2EO
Method Blank Sea Water 1	ND	ND	7.83
Method Blank Sea Water 2	ND	ND	8.45
Sea Water 1	1.96	353	9.09
Sea Water 2	1.77	361	8.56
Sea Water 3	2.12	303	7.64
Sea Water 4	2.14	293	8.57
Sea Water 5	2.85	429	8.41
Spike Concentration (ppb)	2.4	300	12
Average Recovery:	2.17	348	8.36
Average Percent Recovery	90.3	116	69.7
Standard Deviation:	0.41	54.3	0.52
% Relative SD	18.9	15.6	6.3

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16.4 This test method was tested by CRL on Gulf of Mexico sea water containing crude oil. The samples were spiked with target compounds as described in Section 12. Table 9 contains the recoveries for the target compounds in reagent water, whole sample and subsample.

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TABLE 9. Single-Laboratory Recovery Data in Gulf of Mexico Sea Water Containing Oil

Sample	Reagent Water (Whole Sample)		Gulf Sea Water with 1% Crude Oil (Whole Sample)		Gulf Sea Water with 1% Crude Oil (Water Subsample)	
	Analyte Measured (ppb)		Analyte Measured (ppb)		Analyte Measured (ppb)	
	DPGBE	EGBE	DPGBE	EGBE	DPGBE	EGBE
Method Blank	ND	ND	ND	ND	Not Analyzed	Not Analyzed
Sample 1	2.04	284	1.34	240	1.20	263
Sample 2	2.04	284	1.39	239	1.18	272
Sample 3	Not Analyzed	Not Analyzed	1.35	251	1.32	239
Spike Concentration (ppb)	2.4	300	2.4	300	2.4	300
Average Recovery:	2.0	284.0	1.36	243.3	1.23	258.0
Average Percent Recovery	85.0	94.7	56.7	81.1	51.4	86.0
Target compound spike was added to the entire whole sample before subsampling.						

17. Keywords

17.1 Dipropylene Glycol Monobutyl Ether, Ethylene Glycol Monobutyl Ether (EGBE); Liquid Chromatography; Mass Spectrometry; Water

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APPENDIX

Figure 1. Detection Verification Level Signal/Noise Ratio.

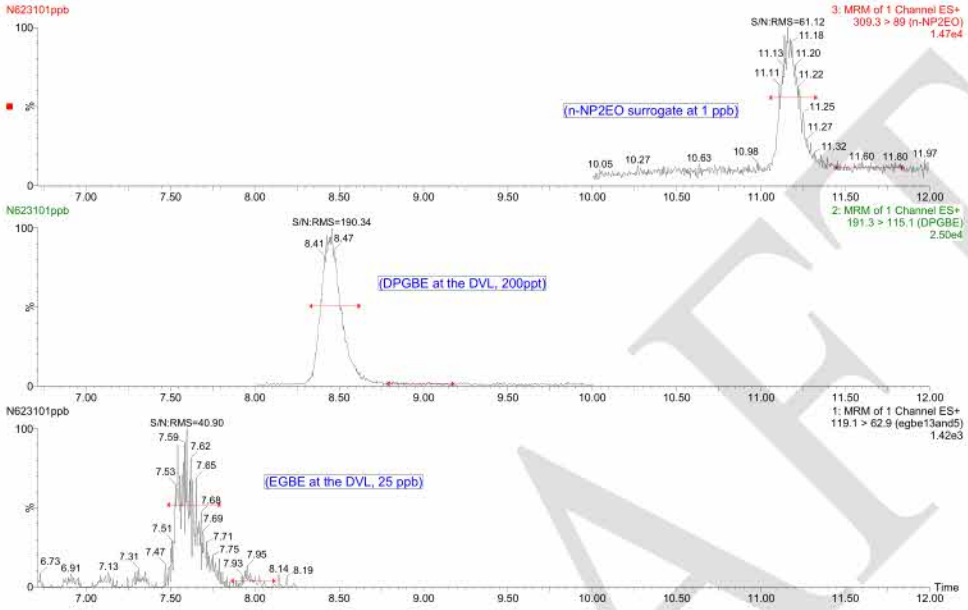

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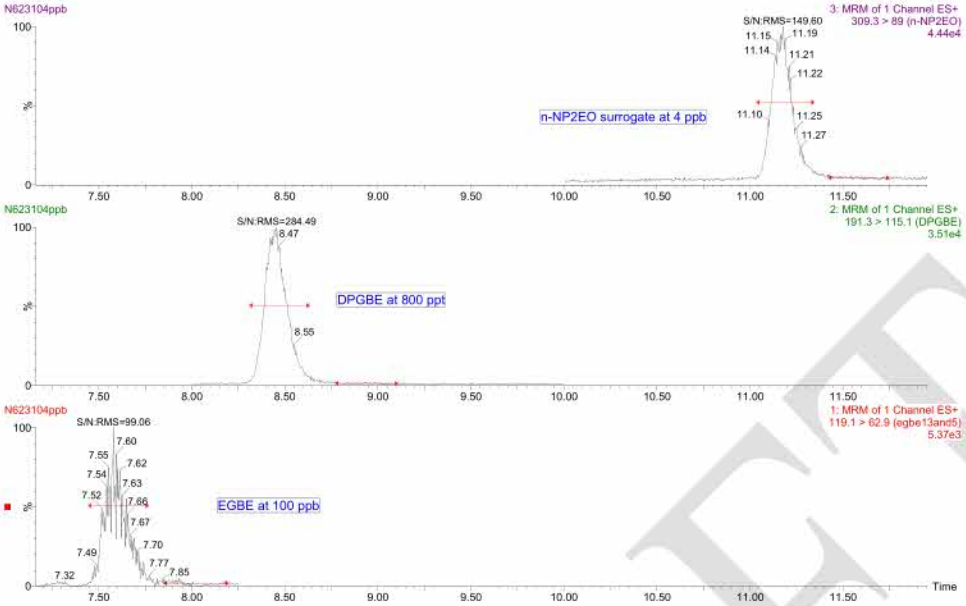
Figure 2. Reporting Level (Calibration standard) Signal/Noise Ratio.



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Region 5 Draft Procedure for the Determination of Dipropylene Glycol Monobutyl Ether (CAS # 29911-28-2) and Ethylene Glycol Monobutyl Ether (CAS# 111-76-2) in Sea Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)[1]

1. Scope

1.1 This procedure covers the determination of Dipropylene Glycol Monobutyl Ether (DPGBE) and Ethylene Glycol Monobutyl Ether (EGBE) in sea water by direct injection using liquid chromatography (LC) and detection with tandem mass spectrometry (MS/MS). This analyte is qualitatively and quantitatively determined by this method. This method adheres to single reaction monitoring (SRM) mass spectrometry.

1.2 *Units*— The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 The Detection Verification Level (DVL) and Reporting Range for DPGBE and EGBE are listed in Table 1.

1.3.1 The DVL is required to be at a concentration at least 3 times below the Reporting Limit (RL) and have a signal/noise ratio greater than 3:1. Figures 1 and 2 display the signal/noise ratio of the single reaction monitoring (SRM) transition.

1.3.2 The reporting limit is the concentration of the Level 1 calibration standard as shown in Table 4 for DGGBE and EGBE, taking into account the 20% sample preparation dilution factor.

[1] Do not cite, quote, or distribute this draft analytical procedure developed by US EPA Region 5 Chicago Regional Laboratory (CRL), it is the first procedure developed by CRL and is open to improvement and discussion. July 12, 2010.

TABLE 1. Detection Verification Level and Reporting Range

Analyte	DVL (µg/L)	Reporting Range (µg/L)
DPGBE	0.2	1-10
EGBE	25	125-1250

1.4 *This procedure does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water
D 1193 Specification for Reagent Water

2.2 *Other Documents*[2]

EPA publication SW-846, entitled *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*

3. Terminology

3.1 *Definitions:*

3.1.1 Detection Verification Level, DVL, n– a concentration that has a signal/noise ratio greater than 3:1 and is at least 3 times below the Reporting Limit (RL).

[2] Available from National Technical Information Service (NTIS), U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA, 22161 or at http://www.epa.gov/epawaste/hazard/testmethods/index.htm

3.1.2 Reporting Limit, RL, n– the concentration of the lowest-level calibration standard used for quantification.

3.2 *Abbreviations:*

3.2.1 ESI- Electrospray ionization

3.2.2 DPGBE- Dipropylene Glycol Monobutyl Ether (CAS # 29911-28-2)

3.2.3 EGBE- Ethylene Glycol Monobutyl Ether (CAS# 111-76-2)

3.2.4 *n*-NP2EO- normal- Nonylphenol Diethoxylate (CAS# Not available, Source: Cambridge Isotope Laboratories or Accustandard, Inc.)

3.2.5 LC/MS- liquid chromatography mass spectrometry

3.2.6 ppb- parts per billion, µg/L

3.2.7 ppt- parts per trillion, ng/L

3.2.8 mM- millimolar, 1 x 10^{-3} moles/L

3.2.9 NA- no addition

3.2.10 ND- non-detect

4. Summary of Procedure

4.1 This is a performance based method, and modifications are allowed to improve performance.

4.2 For DPGBE and EGBE analysis, samples are shipped to the lab between 0°C and 6°C and analyzed within 5 days of collection. The DOW MSDS sheet on DOWANOL* DPNB glycol ether (DPGBE) Issue Date: 06/18/2010 lists that the material is readily biodegradable. The OECD 302B Test lists 96% biodegradation in 28 days.

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4.3 In the lab, the entire collected 20 mL sample is spiked with surrogate and brought to a volume of 25 mL with acetonitrile. This prepared sample is then filtered using a syringe driven filter unit, and analyzed by LC/MS/MS. If visible oil is present, the prepared sample is allowed to settle resulting in an oil layer at the top of the 25 mL solution. A portion of the aqueous (bottom) layer is filtered, leaving the oil layer behind, through a syringe driven filter assembly and analyzed by LC/MS/MS.

4.4 DPGBE, EGBE and surrogate are identified by retention time and one SRM transition. The target analytes and surrogate are quantitated using the SRM transitions utilizing an external calibration. The final report issued for each sample lists the concentration of DPGBE, EGBE and the surrogate recovery.

5. Significance and Use

5.1 DPGBE and EGBE have been identified as possible ingredients in dispersant agents used to treat oil.

5.2 This method has been investigated for use with reagent and sea water.

6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other apparatus producing discrete artifacts or elevated baselines. All of these materials are demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as samples.

6.2 All glassware is washed in hot water with detergent and rinsed in hot water followed by distilled water. Detergents containing DPGBE or EGBE must not be used. The glassware is

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then dried and heated in an oven at 250°C for 15 to 30 minutes. All glassware is subsequently cleaned with acetone followed by methanol.

6.3 All reagents and solvents should be pesticide residue purity or higher to minimize interference problems.

6.4 Matrix interferences may be caused by contaminants in the sample. The extent of matrix interferences can vary considerably from sample source depending on variations of the sample matrix.

6.5 Graduated cylinder- a graduated cylinder was used to measure the volume of the sample and total volume. An appropriate cylinder for different sample volumes should be used to accurately measure the sample and prepared sample volumes.

7. Apparatus

7.1 LC/MS/MS System

7.1.1 *Liquid Chromatography System*- A complete LC system is needed in order to analyze samples.[3] Any system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard may be used.

7.1.2 *Analytical Column- Waters-* XBridge™, 2.1 x 150 mm, 3.5 µm particle size was used to develop this test method. Any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and need to be monitored.

7.1.3 *Tandem Mass Spectrometer System*- A MS/MS system capable of MRM analysis.[4] Any system that is capable of performing at the requirements in this procedure may be used.

[3] A Waters ACQUITY UltraPerformance Liquid Chromatography (UPLC®) System was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

7.2 Filtration Device

7.2.1 *Hypodermic syringe*- A Lock Tip Glass Syringe capable of holding a Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm or similar may be used.

7.2.1.1 A 10 or 25 mL Lock Tip Glass Syringe size was used in this test method.

7.2.2 *Filter*– Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm (Millipore Corporation, Catalog # SLGV033NS) or similar may be used.

7.2.3 Glass Pipette- small volume pipette for sample container rinses and sample transfer

8. Reagents and Materials

8.1 *Purity of Reagents*- High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall conform to the Committee on Analytical Reagents of the American Chemical Society.[5] Other reagent grades may be used provided they are first determined to be of sufficiently high purity to permit their use without affecting the accuracy of the measurements.

8.2 *Purity of Water*– Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to ASTM Type 1 of Specification D 1193. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

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[4] A Waters Quattro Premier™ XE tandem quadrupole mass spectrometer was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

[5] Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, D.C. For Suggestions on the testing of reagents not listed by the American Chemical Society, see Annual Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, U.K., and the United States Pharmacopeia and National Formulators, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

8.3 *Gases*– Ultrapure nitrogen and argon.

8.4 Acetonitrile (CAS # 75-05-8).

8.5 Methanol (CAS # 67-56-1).

8.6 Formic Acid (CAS # 64-18-6).

8.7 2-Propanol (CAS # 67-63-0).

8.8 DPGBE- Dipropylene Glycol Monobutyl Ether (CAS # 29911-28-2)

8.9 EGBE- Ethylene Glycol Monobutyl Ether (CAS# 111-76-2)

8.10 *n*-NP2EO- normal- Nonylphenol Diethoxylate (CAS# Not available)

9. Hazards

9.1 Normal laboratory safety applies to this method. Analysts should wear safety glasses, gloves, and lab coats when working in the lab. Analysts should review the Material Safety Data Sheets (MSDS) for all reagents used in this method.

10. Sampling

10.1 *Sampling and Preservation*– Grab samples should be collected in 20 mL pre-cleaned glass vials with Teflon® lined septa caps demonstrated to be free of interferences. The vial should be filled to approximately 20 mL. This may be just below the neck of the vial, depending on the vial manufacturer. This test method is based on a 20 mL sample size per analysis. Each sample must be collected in duplicate and a quadruplicate sample must be included with each sample batch of 10 for MS/MSD quality control analyses. Store samples between 0°C and 6°C from sample collection to sample preparation. Analyze the sample within 5 days of collection.

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Samples may be encountered that have more than one phase. Samples may be prepared for analysis using one or multiple of the following options:

10.1.1 For a 20 mL water sample, add 0.1 mL of 2.4 ppm *n*-NP2EO surrogate spike solution to the sample in the sample container. Cap the container and mix to ensure homogeneity. Transfer the contents of the sample container to a graduated cylinder and record the sample volume. Rinse the sample collection vial twice with 2 mL portions of acetonitrile, which is added to the prepared sample and dilute to 25 mL with acetonitrile to compose ~ 20% acetonitrile solution to ensure quantitative sample transfer. If different sample sizes are used, spiking solution and acetonitrile volume shall be adjusted proportionally. (Note: Since an accurate sample volume may not be known prior to measurement into the graduated cylinder the appropriate spike may be added directly to the graduate cylinder instead of the collection vial. The collection vial must be rinsed with acetonitrile which is added to the graduated cylinder and this prepared sample must be thoroughly mixed.) The entire sample should be filtered through the syringe driven filter unit described in Section 7.2. If the sample is biphasic, due to a top oil layer, a portion of the aqueous fraction (bottom) should be transferred to a glass syringe for filtration (see section 10.1.1.1). Filter the sample using a 0.22 µm PVDF filter into a glass vial and then transfer to an autosampler vial. For samples that contain an oil layer, an aliquot of the filtered solution should be diluted with 80% water/20% acetonitrile for a preliminary analysis, a 100 fold dilution should be considered until the site samples are characterized. The concentration of the target analytes, especially DPGBE, may be very high in samples that contain an oil layer.

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10.1.1.1 *n*-NP2EO has been shown to be absorbed into the oil layer yielding a non-detect as a result. If oil is present in the sample the recovery of the *n*-NP2EO surrogate may be very low or not detected at or above the reporting limit.

10.1.2 Water Subsample*: Collect a 4 mL subsample of the water fraction (bottom) using a needle and a glass syringe. To reduce the oil exposure, invert the vial and tap gently to cause the oil move away from the septum. Insert needle through septum and collect 4 mL of the water layer; place the aliquot removed in a graduated cylinder. Then add 20 µL of 2.4 ppm *n*-NP2EO surrogate spike solution to the sample in the graduated cylinder. Add 1 mL of acetonitrile to the prepared sample to compose ~ 20% acetonitrile solution. If different sample sizes are used, surrogate spiking solution and acetronitrile volume shall be adjusted proportionally. Filter the sample using a 0.22 µm PVDF filter into a glass vial and then transfer to an autosampler vial.

*Note- Subsamples of water quantitates only the DPGBE and EGBE in the subsample, the DPGBE and EGBE concentration of subsamples may underestimate the sample DPGBE and EGBE concentration as a result of oil partitioning and surface binding.

11. Preparation of LC/MS/MS

11.1 Liquid Chromatograph Operating Conditions[5]

11.1.1 Injection volumes of all calibration standards and samples are made at 100 µL volume. The first sample analyzed after the calibration curve is a blank to ensure there is no carry-over. The gradient conditions for the liquid chromatograph are shown in Table 2. Divert the column flow away from the electrospray source for 0 to 5 minutes after injection. Flow

diversion to waste may be done using the mass spectrometer divert valve, divert tubing configurations vary from manual injection. Sea water samples contain nonvolatile salts, the first 5 minute elution is diverted in order to keep the mass spectrometer source clean.

TABLE 2. Gradient Conditions for Liquid Chromatography

Time (min)	Flow (mL/min)	Percent 95% Water/ 5% CH_3CN	Percent CH_3CN	Percent 2% Formic Acid 95% Water/ 5% CH_3CN
0.0	0.30	95	0	5
2.0	0.30	95	0	5
5.0	0.30	0	95	5
14.0	0.30	0	95	5
15.0	0.30	95	0	5
18.0	0.30	95	0	5

11.2 LC Conditions:

11.2.1 *Needle Wash Solvent*- 60% Acetonitrile/40% 2-propanol

11.2.2 *Temperatures*– Column, 30°C; Sample compartment, 15°C.

11.2.3 *Seal Wash*– 60% Acetonitrile/40% 2-propanol.

11.3 Mass Spectrometer Parameters[6]:

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters may be optimized according to your instrument. Each peak requires at least 10 scans per peak for adequate quantitation. This procedure contains DPGBE, EGBE and one surrogate which are in three MRM acquisition functions to optimize sensitivity. Variable parameters regarding retention times, SRM transitions, and cone and

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collision energies are shown in Table 3. Mass spectrometer parameters used in the development of this method are listed below:

The instrument is set in the Electrospray positive source setting.
Capillary Voltage: 3.5 kV
Cone: Variable depending on analyte (Table 3)
Extractor: 2 Volts
RF Lens: 0.2 Volts
Source Temperature: 120°C
Desolvation Temperature: 350°C
Desolvation Gas Flow: 800 L/hr
Cone Gas Flow: 25 L/hr
Low Mass Resolution 1: 14.5
High Mass Resolution 1: 14.5
Ion Energy 1: 0.5
Entrance Energy: -1
Collision Energy: Variable depending on analyte (Table 3)
Exit Energy: 1
Low Mass Resolution 2: 14.5
High Mass resolution 2: 14.5
Ion Energy 2: 0.8
Multiplier: 650
Gas Cell Pirani Gauge: 7.0×10^{-3} Torr
Inter-Channel Delay : 0.1 seconds
Inter-Scan Delay: 0.1 seconds
Dwell: 0.1 seconds
Solvent Delay: 5 minutes

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TABLE 3. Retention Times, MRM transitions, and Specific Mass Spectrometer Parameters

Analyte	Retention time (min)	Cone Voltage (Volts)	Collision Energy (eV)	MRM Mass Transition (Precursor > Product)
DPGBE	8.5	19	7	191.3 > 115.1
EGBE	7.6	13	5	119.1> 62.9
n-NP2EO (Surrogate)	11.2	28	10	309.3> 89.0
EGBE-D_4	TBD	TBD	TBD	TBD

12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated per manufacturer specifications before analysis. In order to obtain accurate analytical values through using this test method within the confidence limits, the following procedures must be followed when performing the test method. Prepare all solutions in the lab using Class A volumetric glassware.

12.2 *Calibration and Standardization*– To calibrate the instrument, analyze six calibration standards containing (nominal concentrations in Table 4) DPGBE, EGBE and *n*-NP2EO. A calibration solution is prepared from standard materials or they are purchased as certified solutions. Level 6 calibration solution containing the targets and surrogate is prepared and aliquots of that solution are diluted to prepare Levels 1 through 5 and the DVL. The following steps will produce standards with the concentration values shown in Table 4. The analyst is responsible for recording initial component weights correctly calculating and preparing appropriate dilution calculations.

12.2.1 Prepare Level 6 calibration stock standard at 1000 ppb for EGBE, 8 ppb for DPGBE and 40 ppb for *n*-NP2EO in 80% water/20% acetonitrile. The EGBE and DPGBE concentrated stock solutions were prepared in methanol at approximately 2 g/L concentration and the *n*-

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NP2EO surrogate concentrated stock solution was prepared in acetonitrile at approximately 0.5 g/L. The preparation of the stock standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory. Depending on the prepared stock concentrations, the solubility at that concentration will have to be ensured.

12.2.2 Aliquots of Level 6 calibration stock standard are then diluted with 80% water/20% acetonitrile to prepare the desired calibration levels in 2 mL amber glass autosampler vials. The calibration vials must be used within 24 hours to ensure optimum results. Stock calibration standards are routinely replaced every 7 days if not previously discarded for quality control failure. Calibration standards are not filtered.

12.2.3 Inject each standard and obtain its chromatogram. An external calibration technique is used to monitor the SRM transitions of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates using the SRM transition. The calibration software manual should be consulted to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppb units. Concentrations may be calculated using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin is not recommended.

12.2.4 Linear calibration may be used if the coefficient of determination, r^2, is >0.98 for the analyte. The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or low point causes the r^2 of the curve to be <0.98, this point must be re-injected or a new calibration curve must be regenerated. If the low and/or high point is excluded, minimally a five point curve is acceptable but the reporting range must be modified to reflect this change.

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12.2.5 Quadratic calibration may be used if the coefficient of determination, r^2, is >0.99 for the analyte. The point of origin is excluded, and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards causes the curve to be <0.99, this point must be re-injected or a new calibration curve must be regenerated. Minimally a six point curve is acceptable using a quadratic fit. Each calibration point used to generate the curve must have a calculated percent deviation less than 25% from the generated curve.

12.2.6 The retention time window of the SRM transitions must be within 5% of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calibration curve to determine if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

12.2.7 A calibration midpoint check standard must be analyzed at the end of each batch of 20 samples or within 24 hours after the initial calibration curve was generated. This end calibration check should be the same calibration standard that was used to generate the initial curve. The results from the end calibration check standard must have a percent deviation less than 35% from the calculated concentration for the target analytes and surrogates. If the results are not within these criteria, the problem must be corrected and either all samples in the batch must be re-analyzed against a new calibration curve or the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method. If the analyst inspects the vial containing the end calibration check standards and notices that the samples evaporated affecting the concentration, a new end calibration check standard may be made and analyzed. If this new end calibration check standard has a percent deviation less than 35% from

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the calculated concentration for the target analyte and surrogate, the results may be reported unqualified.

TABLE 4. Concentrations of Calibration Standards (PPB)

Analyte/Surrogate	DVL	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6
DPGBE	0.20	0.80	1.6	2.4	3.2	4.0	8.0
EGBE	25	100	200	300	400	500	1000
n-NP2EO (Surrogate)	1.0	4.0	8.0	12	16	20	40
EGBE-D_4	TBD	TBD	TBD	TBD	TBD	TBD	TBD

12.3 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study must be performed to demonstrate laboratory capability (Section 16).

12.3.1 Analyze at least four replicates of a sample solution containing the targets and surrogate at a concentration in the calibration range of Levels 3 to 5. The Level 3 concentration of the 6 point calibration curve was used to set the QC acceptance criteria in this method. The matrix and chemistry should be similar to the solution used in this test method. Each replicate must be taken through the complete analytical test method including any sample pre-treatment steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of the QC acceptance criteria for the Initial Demonstration of Performance in Table 5.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 5.

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12.3.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in Table 5 is preliminary until more data and multi-laboratory study is completed. Data generated from a single-laboratory validation from reagent and sea water matrices are shown in the Precision and Bias Section 16. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard. A reference on how to generate QC acceptance criteria is in Method 8000B in EPA publication SW-846.

TABLE 5. Preliminary QC Acceptance Criteria

Analyte	Test Conc. (µg/L) in Reagent Water	Initial Demonstration of Performance			Lab Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit
DPGBE	2.4	50	150	30	50	150
EGBE	300	50	150	30	50	150
n-NP2EO (Surrogate)	12	25	125	30	25	125
EGBE-D_4	TBD	TBD	TBD	TBD	TBD	TBD

12.4 *Surrogate Spiking Solution:*

12.4.1 A surrogate spiking methanol solution containing *n*-NP2EO is added to all samples. A stock surrogate spiking solution is prepared at 2 .4 ppm. Spiking 100 µL of this spiking solution into a 20 mL water sample results in a concentration of 12 ppb of the surrogate in the sample. The result obtained for the surrogate recovery must fall within the limits of Table 5. If

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the limits are not met, the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.4.1.1 *n*-NP2EO has been shown to be absorbed into the oil layer yielding a non-detect as a result. If oil is present in the sample, the recovery of the *n*-NP2EO surrogate may be very low or not detected at or above the reporting limit. A better surrogate alternative is EGBE-D_4 which is being procured from Cambridge Isotope Laboratories. ^{13}C or ^{2}H labeled DPGBE surrogates are not currently commercially available.

12.5 *Method Blank*:

12.5.1 Analyze a reagent water blank with each batch of 20 or fewer samples. The concentration of the DPGBE and EGBE found in the blank should be 2 times below the reporting limit. If the concentration of DPGBE or EGBE is found above this level, analysis of samples is halted until the contamination is eliminated, and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method. If DPGBE or EGBE are found in a method blank at greater than 2 times below the reporting limit the reporting limit must be raised to at least 2 times the concentration of the DPGBE and EGBE found in the blank. This may occur if samples are encountered that have a high concentration of DPGBE, a water blank between samples may be required to remove carry-over between samples.

12.6 *Laboratory Control Sample (LCS):*

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12.6.1 To ensure that the test method is in control, analyze a LCS prepared with the target analytes at a concentration in the calibration range of Levels 3 to 5. The LCS is prepared following the analytical method and analyzed with each batch of 20 samples or less. Prepare a stock matrix spiking solution in methanol containing the DPGBE at 0.48 ppm and EGBE at 60 ppm. Spike 100 µL of this stock solution into 20 mL of water to yield a concentration of 2.4 ppb for the DPGBE and 300 ppb for EGBE in the sample. The LCS result must be within the limits in Table 5. Matrix spiking solutions are routinely replaced every 7 days if not previously discarded for quality control failure.

12.6.2 If the result is not within these limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be re-analyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7 *Matrix Spike/Matrix Spike Duplicate (MS/MSD):*

12.7.1 To check for interferences in the specific matrix being tested, perform a MS/MSD on at least one sample from each batch of 10 or fewer samples by spiking the sample with a known concentration of DPGBE and EGBE and following the analytical method. Prepare a stock matrix spiking solution in methanol containing the DPGBE at 0.48 ppm and EGBE at 60 ppm. Spike 100 µL of this stock solution into 20 mL of water to yield a concentration of 2.4 ppb for the DPGBE and 300 ppb for EGBE in the sample. The result obtained for the MS/MSD must fall within the limits in Table 6. Matrix spiking solutions are routinely replaced every 7 days if not previously discarded for quality control failure.

12.7.2 If the spiked concentration plus the background concentration exceeds that of the Level 6 calibration standard, the sample must be diluted to a level near the midpoint of the calibration curve.

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12.7.3 Calculate the percent recovery of the spike (P) using Equation 1:

Equation 1

$$P = 100\frac{\left|A(Vs+V) - BVs\right|}{CV}$$

Where:

A = concentration found in spiked sample

B = concentration found in unspiked sample

C = concentration of analyte in spiking solution

V_s = volume of sample used

V = volume of spiking solution added

P = percent recovery

12.7.4 The percent recovery of the spike shall fall within the limits in Table 6. If the percent recovery is not within these limits, matrix interference may be present in the selected sample. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7.5 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 6 are preliminary until more data is acquired. The data generated by a single-laboratory using sea water samples are in the Precision and Bias Section 16. The matrix variation between the different waters may have a tendency to generate significantly wider control limits than those generated by a single-laboratory in one water matrix. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard.

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12.7.5.1 The laboratory should generate their own in-house QC acceptance criteria after the analysis of 15-20 matrix spike samples of a particular surface water matrix. References on how to generate QC acceptance criteria is in Method 8000B in EPA publication SW-846.

TABLE 6. Preliminary MS/MSD QC Acceptance Criteria

Analyte	Test Conc. (µg/L)	MS/MSD		
		Recovery (%)		Precision
		Lower Limit	Upper Limit	Maximum RPD (%)
DPGBE	2.4	50	150	30
EGBE	300	50	150	30
n-NP2EO (Surrogate)	12	25	150	30
EGBE-D_4	TBD	TBD	TBD	TBD

12.8 *Duplicate:*

12.8.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the sample contains the analyte at a level greater than 5 times

the detection limit of the method, the sample and duplicate may be analyzed unspiked; otherwise, an MSD should be used.

12.8.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq. 2. Compare to the RPD limit in Table 6.

Equation 2

$$\text{RPD} = \frac{|\text{MSR - MSDR}|}{(MSR + MSDR) \div 2} \times 100$$

Where:
RPD= relative percent difference
MSR= matrix spike recovery
MSDR= matrix spike duplicate recovery

12.8.3 If the result exceeds the precision limit, the batch must be re-analyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

13. Procedure

13.1 This test method is based upon a 20 mL sample size per analysis. **Any sample size may be used such as a half filled VOA vial as long as the QC spikes and sample preparation volumes are adjusted accordingly.** The samples must be analyzed within 5 days of collection. If the samples are above 6°C when received or during storage, or not analyzed within 5 days of collection, the data is qualified estimated and noted in the case narrative that accompanies the data.

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13.2 In the laboratory, the entire 20 mL sample that was collected in a 20 mL VOA vial is poured into a 50 mL graduated cylinder. Every sample is spiked with the surrogate as described in Section 12. The laboratory control and matrix spike samples are then spiked with the target compounds as described in Section 12. The spiking solutions are added to the sample before transfer to the 50 mL graduated cylinder and that volume is subtracted from the measured amount. The exact volume of the sample size is recorded in order to calculate the exact final concentration of DPGBE, EGBE and surrogate. The vial is rinsed with two 2 mL portions of CH_3CN to remove any residual DPGBE, EGBE and surrogate adhered to the collection vial. These 2 portions are added to the 50 mL graduated cylinder. The samples are then diluted to 25 mL final volume with CH_3CN, shaken, filtered through the syringe driven filter unit fitted with a PVDF filter cartridge into glass storage vials and then aliquoted into 2 mL amber glass LC vials for analysis.

13.3 For samples that are homogeneous, the entire 25 mL volume is filtered through the filtration device described in Section 7.2 into a pre-cleaned collection vial (such as a VOA vial). A portion of that filtered sample is added to an amber glass LC vial with a Teflon® lined cap which is analyzed. A new filter unit is used for each sample.

13.4 For biphasic samples, a portion of the lower aqueous layer is filtered through the filtration device described in Section 7.2 into a pre-cleaned collection vial (such as a VOA vial). The upper oil layer is left behind and is not added to the filtration device. A portion of that filtered sample is added to an amber glass LC vial with a Teflon® lined cap which is analyzed. A new filter unit is used for each sample.

13.5 The syringe must be cleaned between each filtration. It is the analyst's responsibility to ensure that the syringe is clean. A suggested method for cleaning the syringe between filtrations

is to first rinse with at least 5 syringe volumes of water, followed by at least 3 volumes of 50% water/50% CH_3CN.

13.6 Once a passing calibration curve is generated the analysis of samples may begin. An order of analysis may be: method blank, laboratory control sample and duplicate, up to 20 samples, matrix spike sample(s) and duplicate followed by an end calibration check which includes a midpoint calibration check standard and a method blank.

14. Calculation or Interpretation of Results

14.1 For quantitative analysis of DPGBE, EGBE and surrogate, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. External calibration curves are used to calculate the amounts of DPGBE, EGBE and surrogate. Calculate the concentration in µg/L (ppb) for each analyte. The sample concentration was diluted by 20% by the addition of surrogates and CH_3CN and target compound spike where applicable, this dilution must be accounted for when reporting the concentration. DPGBE and EGBE may be reported if present at or above the reporting limit. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with reagent water to obtain a concentration near the mid-point of the calibration range and re-analyzed. This method uses one surrogate, *n*-NP2EO, to monitor performance. The surrogate recoveries are provided with all data generated from this test method.

14.1.1 A surrogate is used to monitor the performance of DPGBE and EGBE. If the surrogate meets the quality control criteria in this test method, the data may be reported unqualified for DPGBE and EGBE if all other quality control in this test method are acceptable.

If the surrogate does not meet the quality control criteria of the test method, the data is qualified for DPGBE and EGBE.

15. Report

15.1 Determine the results in units of µg/L (ppb) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve generated. All data that does not meet the specifications in the test method must be appropriately qualified.

16. Precision and Bias

16.1 The determination of precision and bias was conducted through US EPA Region 5 Chicago Regional Laboratory.

16.2 This test method was tested by CRL on reagent water. The samples were spiked with the DPGBE, EGBE and *n*-NP2EO to obtain a 2.4 ppb concentration of DPGBE, 300 ppb EGBE and 12 ppb concentration of *n*-NP2EO each as described in Section 12. Table 7 contains the recoveries and standard deviation (SD) for the target compounds and surrogate in reagent water.

TABLE 7. Single-Laboratory Recovery Data in Reagent Water

Precision and Accuracy Samples	Analyte Measured (ppb)		
	DPGBE	EGBE	n-NP2EO
1	2.22	330	8.90
2	2.10	382	9.09
3	2.08	334	8.14
4	2.23	352	8.66
5	2.18	327	9.02
6	2.01	331	8.84
Spike Concentration (ppb)	2.40	300	12.00
Average Recovery:	2.14	343	8.78
Average Percent Recovery	89.0	114	73.1
Standard Deviation:	0.09	21.2	0.35
% Relative SD	4.09	6.19	3.93

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16.3 This test method was tested by CRL on Gulf of Mexico sea water. The samples were spiked with target compounds and surrogate as described in Section 12. Table 8 contains the recoveries and standard deviation (SD) for the target compounds and surrogate in sea water.

TABLE 8. Single-Laboratory Recovery Data in Gulf of Mexico Sea Water

Precision and Accuracy Samples	Analyte Measured (ppb)		
	DPGBE	EGBE	n-NP2EO
Method Blank Sea Water 1	ND	ND	7.83
Method Blank Sea Water 2	ND	ND	8.45
Sea Water 1	1.96	353	9.09
Sea Water 2	1.77	361	8.56
Sea Water 3	2.12	303	7.64
Sea Water 4	2.14	293	8.57
Sea Water 5	2.85	429	8.41
Spike Concentration (ppb)	2.4	300	12
Average Recovery:	2.17	348	8.36
Average Percent Recovery	90.3	116	69.7
Standard Deviation:	0.41	54.3	0.52
% Relative SD	18.9	15.6	6.3

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16.4 This test method was tested by CRL on Gulf of Mexico sea water containing crude oil. The samples were spiked with target compounds as described in Section 12. Table 9 contains the recoveries for the target compounds in reagent water, whole sample and subsample.

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TABLE 9. Single-Laboratory Recovery Data in Gulf of Mexico Sea Water Containing Oil

Sample	Reagent Water (Whole Sample)		Gulf Sea Water with 1% Crude Oil (Whole Sample)		Gulf Sea Water with 1% Crude Oil (Water Subsample)	
	Analyte Measured (ppb)		Analyte Measured (ppb)		Analyte Measured (ppb)	
	DPGBE	EGBE	DPGBE	EGBE	DPGBE	EGBE
Method Blank	ND	ND	ND	ND	Not Analyzed	Not Analyzed
Sample 1	2.04	284	1.34	240	1.20	263
Sample 2	2.04	284	1.39	239	1.18	272
Sample 3	Not Analyzed	Not Analyzed	1.35	251	1.32	239
Spike Concentration (ppb)	2.4	300	2.4	300	2.4	300
Average Recovery:	2.0	284.0	1.36	243.3	1.23	258.0
Average Percent Recovery	85.0	94.7	56.7	81.1	51.4	86.0
Target compound spike was added to the entire whole sample before subsampling.						

17. Keywords

17.1 Dipropylene Glycol Monobutyl Ether, Ethylene Glycol Monobutyl Ether (EGBE); Liquid Chromatography; Mass Spectrometry; Water

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APPENDIX

Figure 1. Detection Verification Level Signal/Noise Ratio.

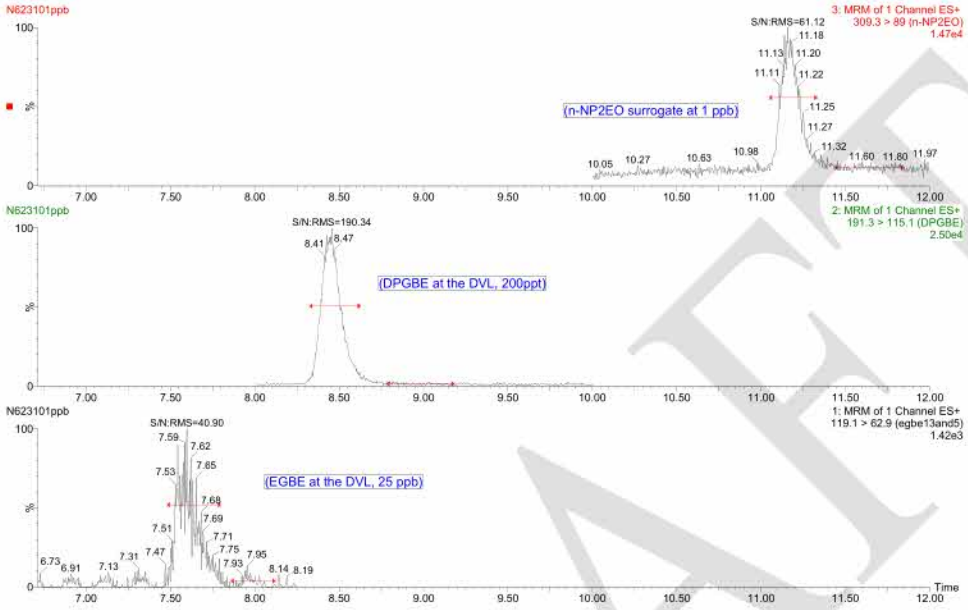

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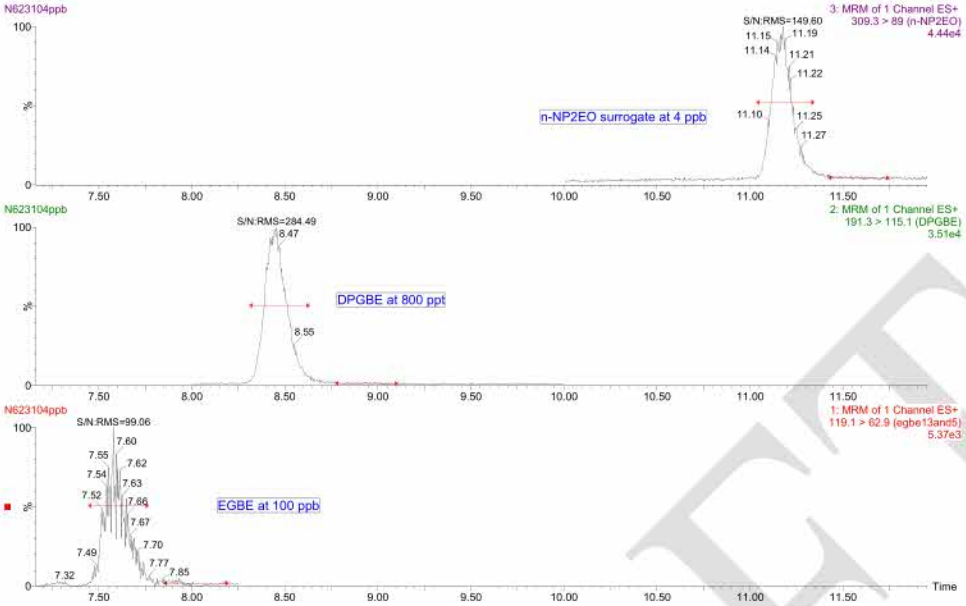
Figure 2. Reporting Level (Calibration standard) Signal/Noise Ratio.



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Regional Analytical Method for Dioctyl Sulfosuccinate (RAM-DOSS, CAS 577-11-7) in Sea Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)[1]

July 12, 2010

1. Scope

1.1 This procedure details dioctyl sulfosuccinate (DOSS) determination in water by direct injection using liquid chromatography (LC) and detection with tandem mass spectrometry (MS/MS). This analyte is qualitatively and quantitatively determined by this method. This method adheres to single reaction monitoring (SRM) mass spectrometry.

1.2 Units— The values stated in SI units are to be regarded as standard. No other units of measurement are included in this method.

1.3 The Detection Verification Level (DVL) and Reporting Range for DOSS are listed in Table 1.

1.3.1 The DVL is required to be at a concentration at least 3 times below the Reporting Limit (RL) and have a signal/noise ratio greater than 3:1. Figure 1 and 2 display the signal/noise ratio of the single reaction monitoring (SRM) transition.

1.3.2 The reporting limit is the concentration of the Level 1 calibration standard as shown in Table 4 for DOSS, taking into account the 50% sample preparation dilution factor.

[1] This draft analytical procedure was developed by US EPA Region 5 Chicago Regional Laboratory (CRL) in collaboration with the Region 6 Houston Laboratory. It was first developed by CRL, July 12, 2010, and is open to improvement and discussion. Do not cite, quote, or distribute this procedure.

TABLE 1. Detection Verification Level and Reporting Range

Analyte	DVL (µg/L)	Reporting Range (µg/L)
DOSS	3	20-400

1.4 *This procedure does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 ASTM Standards:

D 1129 Terminology Relating to Water
D 1193 Specification for Reagent Water

2.2 Other Documents[2]

EPA publication SW-846, entitled *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*

3. Terminology

3.1 Definitions:

3.1.1 Detection Verification Level, DVL, n– a concentration that has a signal/noise ratio greater than 3:1 and is at least 3 times below the Reporting Limit (RL).

3.1.2 Reporting Limit, RL, n– the concentration of the lowest-level calibration standard used for quantification.

3.2 Abbreviations:

[2] Available from National Technical Information Service (NTIS), U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA, 22161 or at http://www.epa.gov/epawaste/hazard/testmethods/index.htm

3.2.1 CH_3CN- acetonitrile

3.2.2 ESI- Electrospray ionization

3.2.3 DOSS- dioctyl sulfosuccinate (CAS # 577-11-7)

3.2.4 LC/MS- liquid chromatography mass spectrometry

3.2.5 ppb– parts per billion, µg/L

3.2.6 mM– millimolar, 1 x 10^{-3} moles/L

3.2.7 NA– no addition

3.2.8 NH_4CO_2H- ammonium formate

3.2.9 ND– non-detect

4. Summary of Procedure

4.1 Two surrogates are being considered, DOSS-D34 and DOSS-^{13}C. Deuterated DOSS surrogate (DOSS-D34) recovery data are included in this draft, and will be complemented with DOSS-^{13}C when available. References to DOSS-^{13}C are included as a place holder and will be updated when available.

4.2 This is a performance based method, and modifications are allowed to improve performance.

4.3 For DOSS analysis, samples are shipped to the lab between 0°C and 6°C and analyzed within 5 days. In the lab, the entire collected 20 mL sample is spiked with surrogate (when available), ammonium formate buffer solution and brought to a volume of 40 mL with acetonitrile. This prepared sample is then filtered using a syringe driven filter unit, and analyzed by LC/MS/MS. If visible oil is present, the prepared sample is allowed to settle resulting in an oil layer at the top of the 40 mL solution. A portion of the aqueous (bottom) layer is filtered,

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leaving the oil layer behind, through a syringe driven filter assembly and analyzed by LC/MS/MS.

4.4 DOSS and its surrogate are identified by retention time and one SRM transitions. The target analyte and surrogate are quantitated using the SRM transitions. The final report issued for each sample lists the concentration of DOSS and the surrogate recovery.

5. Significance and Use

5.1 DOSS has been identified as a possible ingredient in dispersant agents used to treat oil.

5.2 This method has been investigated for use with reagent and sea water for DOSS.

6. Interferences

6.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other apparatus producing discrete artifacts or elevated baselines. All of these materials are demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as samples.

6.2 All glassware is washed in hot water with detergent and rinsed in hot water followed by distilled water. The glassware is then dried and heated in an oven at 250°C for 15 to 30 minutes. All glassware is subsequently cleaned with methanol and/or 50% acetonitrile/50% water.

6.3 System contamination and surface binding are problematic as DOSS is a surface active compound. Notable DOSS is associated with the surface of glass containers after one hour. It is important to thoroughly rinse sample containers with organic solvent to recovery DOSS from samples. Thorough rinsing of all lab equipment is necessary to reduce contamination. Carefully analyze blanks to ensure that the method minimizes DOSS carryover.

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6.4 All reagents and solvents should be pesticide residue purity or higher to minimize interference problems.

6.5 Matrix interferences may be caused by contaminants in the sample. The extent of matrix interferences can vary considerably from sample source depending on variations of the sample matrix.

6.6 Graduated cylinder- a 50 mL cylinder was used to measure the volume of the sample and total volume. An appropriate cylinder for different sample volumes should be used to accurately measure the sample and prepared sample volumes.

7. Apparatus

7.1 LC/MS/MS System

7.1.1 Liquid Chromatography System- A complete LC system is needed in order to analyze samples.[3] Any system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard may be used.

7.1.2 Analytical Column- *Waters* (186001377)- Atlantis™ dC18, 2.1 x 150 mm, 3 µm particle size was used to develop this test method. Any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and need to be monitored.

7.1.3 Tandem Mass Spectrometer System- A MS/MS system capable of MRM analysis.[4] Any system that is capable of performing at the requirements in this standard may be used. See Appendix B for Agilent instrument conditions.

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[3] A Waters ACQUITY UltraPerformance Liquid Chromatography (UPLC®) System was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

[4] A Waters Quattro Premier™ XE tandem quadrupole mass spectrometer was used to develop this test method. All parameters in this test method are based on this system and may vary depending on your instrument.

7.2 Filtration Device

7.2.1 Hypodermic syringe- A Lock Tip Glass Syringe capable of holding a Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm or similar may be used.

7.2.1.1 A Lock Tip Glass Syringe was used in this test method.

7.2.2 Filter– Millex® HV Syringe Driven Filter Unit PVDF 0.22 µm (Millipore Corporation, Catalog # SLGV033NS) or similar may be used.

7.2.3 Glass Pipette- small volume pipette for sample container rinses and sample transfer

8. Reagents and Materials

8.1 Purity of Reagents- High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall conform to the Committee on Analytical Reagents of the American Chemical Society.[5] Other reagent grades may be used provided they are first determined to be of sufficiently high purity to permit their use without affecting the accuracy of the measurements.

8.2 Purity of Water– Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type 1 of Specification D 1193. It must be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

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[5] Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, D.C. For Suggestions on the testing of reagents not listed by the American Chemical Society, see Annual Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, U.K., and the United States Pharmacopeia and National Formulators, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

8.3 Gases– Ultrapure nitrogen and argon.

8.4 Acetonitrile (CH_3CN, CAS # 75-05-8).

8.5 Methanol (CAS # 67-56-1).

8.6 Ammonium Formate (NH_4CO_2H, CAS # 540-69-2).

8.7 2-Propanol (CAS # 67-63-0).

8.8 Dioctyl sulfosuccinate sodium salt (DOSS) (CAS # 577-11-7).

8.9 Dioctyl sulfosuccinate sodium salt (DOSS-^{13}C) ^{13}C labeled.

8.10 Deuterated dioctyl sulfosuccinate sodium salt (DOSS-D34, - bis(2-ethylhexyl-D17) sulfosuccinate sodium salt)

9. Hazards

9.1 Normal laboratory safety applies to this method. Analysts should wear safety glasses, gloves, and lab coats when working in the lab. Analysts should review the Material Safety Data Sheets (MSDS) for all reagents used in this method.

10. Sampling

10.1 *Sampling and Preservation*– Grab samples should be collected in 20 mL pre-cleaned glass vials with Teflon® lined septa caps demonstrated to be free of interferences. This test method is based on a 20 mL sample size per analysis. Each sample must be collected in duplicate and a quadruplicate sample must be included with each sample batch of 10 for MS/MSD quality control analyses. Store samples between 0°C and 6°C from sample collection to sample preparation. Analyze the sample within 5 days of collection. After 7 and 10 days of storage between 0°C and 6°C DOSS recoveries notably decreased (Table 12). Oil in water

absorbs DOSS from water and affects surrogate analysis (Table 11). Avoid oil in samples if possible. Samples may be encountered that have more than one phase. Samples may be prepared for analysis using one or a multiple of the following options:

10.1.1 Whole Sample*: For a 20 mL sample, add 0.2 mL of 20 ppm DOSS surrogate to the sample in the sample container. Cap the container and mix to insure homogeneity. Transfer the contents of the sample container to a graduated cylinder record the sample volume. Subsequently, add 0.4 mL of 500 mM ammonium formate to the graduated cylinder. Rinse the sample collection vial twice with 6 to 8 mLs of acetonitrile, which is added to the prepared sample to compose ~ 50% acetonitrile solution to ensure quantitative sample transfer. Bring the prepared sample volume to 40 mL using acetonitrile, and then mix thoroughly. If different sample sizes are used, spiking solution, ammonium formate, and acetronitrile volume shall be adjusted proportionally. The sample must be thoroughly mixed and then allowed to settle. A portion of the aqueous fraction (bottom) should be transferred to a glass syringe for filtration. Filter the sample using a 0.22 µm PVDF filter into an autosampler vial, or another glass vial and then transfer to an autosampler vial. Due to the high concentration of DOSS detected in gulf oil, the filtered solution should be diluted for a preliminary analysis. Transfer 20 µL of the filtered-prepared sample into an autosampler vial, and add 1.98 mL of 5 mM ammonium formate in 50% acetonitrile / 50% water. If the DOSS result of the preliminary analysis is below the reporting limit, analyze the filtered-prepared sample directly or an appropriate dilution.

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10.1.2 Water Subsample*: Collect a 10 mL subsample of the water fraction (bottom) using a needle and a glass syringe. To reduce the oil exposure, invert the vial and tap gently to cause the oil move away from the septum. Insert needle through septum and collect 10 mL of the water layer; place the aliquot removed in a graduated cylinder. Then add 100 µL of 20 ppm DOSS surrogate to the sample in the graduated cylinder. Subsequently, add 0.2 mL of 500 mM ammonium formate to the graduated cylinder. Add 10 mL acetonitrile to the prepared sample to compose ~ 50% acetonitrile solution to ensure quantitative prepared sample transfer. If different sample sizes are used, spiking solution, ammonium formate, and acetronitrile volume shall be adjusted proportionally. Filter the sample using a 0.22 µm PVDF filter into an autosampler vial, or another glass vial and then transfer to an autosampler vial.

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10.1.3 Oil Subsample*: Add 1.5 mL of HPLC grade water to graduated cylinder. Collect a 0.5 mL subsample of oil (top), and place in a graduated cylinder. Then add 20 µL of 20 ppm surrogate to the sample in the graduated cylinder. Subsequently, add 40 µL of 500 mM ammonium formate to the graduated cylinder. Add 2 mL acetonitrile to the prepared sample to compose ~ 50% acetonitrile solution to ensure quantitative prepared sample transfer. If different sample sizes are used, spiking solution, ammonium formate, and acetonitrile volume shall be adjusted proportionally. The sample should be thoroughly mixed and then allowed to settle. A portion of the aqueous fraction (bottom) should be transferred to a glass syringe for filtration. Filter the sample using a 0.22 µm PVDF filter into another vial (filtered-prepared sample). Due to the high concentration of DOSS detected in gulf oil, the filtered solution should be diluted for a preliminary analysis. Transfer 20 µL of the filtered-prepared sample into an autosampler vial, and add 1.98 mL of 5 mM ammonium formate in 50% acetonitrile/50% water. If the DOSS result of the preliminary analysis is below the reporting limit, analyze the filtered-prepared sample directly or an appropriate dilution.

*Note- Subsamples of water and oil fractions quantitate only the DOSS in the subsample, the DOSS concentration of subsamples may underestimate the sample DOSS concentration as a result of partitioning (Table 10) and surface binding. DOSS surrogate added to the subsample rather than the received sample and container does not indicate DOSS recovery from the total sample. Additionally, oil in samples resulted in low DOSS recovery, but resulted in higher DOSS recoveries than subsamples (Table 11).

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11. Preparation of LC/MS/MS

11.1 LC Chromatograph Operating Conditions[5]

11.1.1 Injection volumes of all calibration standards and samples are made at 50 µL volume using a full loop injection. "Full loop" mode is the preferred technique when performing quantitative analyses. Multiple blank samples should be analyzed at the beginning of a run to remove residual DOSS from the system. The first sample analyzed after the calibration curve is a blank to ensure there is minimal DOSS carry-over. The gradient conditions for the liquid chromatograph are shown in Table 2A. Divert the column flow away from the electrospray source for 0 to 5 minutes after injection. Flow diversion to waste may be done using the mass spectrometer divert valve, divert tubing configurations vary from manual injection. Test the divert valve configuration and operation prior to analysis. Seawater samples contain nonvolatile salts; the elution from injection to 5 minutes after injection is diverted to waste in order to prevent mass spectrometer source contamination. If there is carry-over from one sample to another, greater than half the reporting limit, the initial percentage of acetonitrile should be raised as shown in Table 2B to try and remove the carry-over. This will shorten the elution time of DOSS approximately 1 minute; therefore it is necessary reduce the flow diversion and adjust the MRM time. Increasing the initial acetonitrile gradient concentration does not increase the DVL or reporting limit. LC/MS/MS conditions using Eclipse XBD C18 analytical column on an Agilent 6410 Triple Quad Mass Spectrometer are detailed as an alternative in Appendix B.

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TABLE 2A. Gradient Conditions for DOSS Liquid Chromatography

Time (min)	Flow (mL/min)	Percent 95% Water/ 5% CH_3CN, 5 mM NH_4CO_2H	Percent 95% CH_3CN/ 5% Water, 5 mM NH_4CO_2H
0.0	0.3	100	0
2.0	0.3	100	0
5.0	0.3	0	100
8.0	0.3	0	100
8.3	0.3	100	0
10.0	0.3	100	0

TABLE 2B. Gradient Conditions for DOSS Liquid Chromatography Starting with a Higher Acetonitrile Concentration

Time (min)	Flow (mL/min)	Percent 95% Water/ 5% CH_3CN, 5 mM NH_4CO_2H	Percent 95% CH_3CN/ 5% Water, 5 mM NH_4CO_2H
0.0	0.3	50	50
2.0	0.3	50	50
5.0	0.3	0	100
8.0	0.3	0	100
8.3	0.3	50	50
10.0	0.3	50	50

11.2 LC Sample Manager Conditions:

11.2.1 Wash Solvents- Weak wash is 4.0 mL of 50% water/50% acetonitrile. Strong wash is 2.0 mL of 60% acetonitrile/40% 2-propanol. The strong wash solvent is needed to eliminate carry-over between injections of DOSS samples. The weak wash is used to remove the strong wash solvent. Instrument manufacturer specifications should be followed in order to eliminate

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sample carry-over. Acquity Autosampler Firmware Version 4.1 results in an error with 4.0 mL weak needle wash and 2.0 mL strong wash. Adjusting the weak wash volume to 3.0 corrected this error, earlier and later Acquity Firmware versions do not have this issue.

11.2.2 Temperatures– Column, 35°C; Sample compartment, 15°C.

11.2.3 Seal Wash– Solvent: 50% acetonitrile/50% water; Time: 2 minutes.

11.3 Mass Spectrometer Parameters[6]:

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters may be optimized according to your instrument. Each peak requires at least 10 scans per peak for adequate quantitation. This procedure will contain one surrogate, which is isotopically labeled DOSS, and DOSS which are in one MRM acquisition function to optimize sensitivity. Due to the low pKa of the sulfonate and to avoid bias associated with adducts, DOSS analysis was performed using electrospray negative ionization. Variable parameters regarding retention times, SRM transitions, and cone and collision energies are shown in Table 3. Mass spectrometer parameters used in the development of this method are listed below:

The instrument is set in the Electrospray negative source setting.
Capillary Voltage: 3.5 kV
Cone: Variable depending on analyte (Table 3)
Extractor: 2 Volts
RF Lens: 0.3 Volts
Source Temperature: 120°C
Desolvation Temperature: 350°C
Desolvation Gas Flow: 800 L/hr
Cone Gas Flow: 25 L/hr
Low Mass Resolution 1: 14.0
High Mass Resolution 1: 14.0
Ion Energy 1: 0.8

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Entrance Energy: -1
Collision Energy: Variable depending on analyte (Table 3)
Exit Energy: 0
Low Mass Resolution 2: 14.0
High Mass resolution 2: 14.0
Ion Energy 2: 1.0
Multiplier: 650
Gas Cell Pirani Gauge: 7.0 x 10^{-3} Torr
Inter-Channel Delay : 0.02 seconds
Inter-Scan Delay: 0.01 seconds
Dwell: 0.1 seconds
Solvent Delay: 5 minutes

TABLE 3. Retention Times, MRM transitions, and DOSS-Specific Mass Spectrometer Parameters

Analyte	Retention time (min)	Cone Voltage (Volts)	Collision Energy (eV)	MRM Mass Transition (Parent > Product)
DOSS	6.44	36	24	421.1 > 80.6
DOSS-D34 (Surrogate)	6.16	37	26	455.3 > 80.6
DOSS-^{13}C (Surrogate)*	TBD	TBD	TBD	>

12. Calibration and Standardization

12.1 The mass spectrometer must be calibrated per manufacturer specifications before analysis. In order to obtain accurate analytical values through using this test method within the confidence limits, the following procedures must be followed when performing the test method. Prepare all solutions in the lab using Class A volumetric glassware.

12.2 Calibration and Standardization– To calibrate the instrument, analyze six DOSS and surrogate calibration standards; the calibration standards nominal concentrations are detailed in

Table 4. A calibration solution is prepared from standard materials or certified solutions. Level 6 calibration solution containing the DOSS and surrogate is prepared and aliquots of that solution are diluted to prepare Levels 1 through 5 and the DVL. The following steps will produce standards with the concentration values shown in Table 4. The analyst is responsible for recording initial component weight, calculating dilutions and preparing appropriate solutions.

12.2.1 Prepare Level 6 calibration stock standard at 200 ppb by adding to a 10 mL volumetric flask individual solutions of the following: 100 µL of DOSS and DOSS (isotopic labeled) each at 20 ppm in 50% water/50% acetonitrile and dilute to 10 mL with a solution of 5 millimolar ammonium formate in 50% water/50% acetonitrile. The preparation of the stock standard can be accomplished using different volumes and concentrations of stock solutions as is accustomed in the individual laboratory. Depending on the prepared stock concentrations, the solubility at that concentration will have to be ensured.

12.2.2 Aliquots of Level 6 calibration stock standard are then diluted with 5 millimolar acetonitrile in 50% water/50% acetonitrile to prepare the desired calibration levels in 2 mL amber glass autosampler vials. The calibration vials must be used within 24 hours to ensure optimum results. Stock calibration standards are routinely replaced every 7 days if not previously discarded for quality control failure. Calibration standards are not filtered.

12.2.3 Inject each standard and obtain its chromatogram. An external calibration technique is used to monitor the SRM transitions of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates using the SRM transition. The calibration software manual should be consulted to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppb units. Concentrations may be calculated

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using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin is not recommended.

12.2.4 Linear calibration may be used if the coefficient of determination, r^2, is >0.98 for the analyte (Figure 3). The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or low point causes the r^2 of the curve to be <0.98, this point must be re-injected or a new calibration curve must be regenerated. If the Level 1 or Level 6 calibration result is excluded, minimally a five point curve is acceptable but the reporting range must be modified to reflect this change.

12.2.5 Quadratic calibration may be used if the coefficient of determination, r^2, is >0.99 for the analyte. The point of origin is excluded, and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations (Figure 4). If one of the calibration standards causes the curve to be <0.99, this point must be re-injected or a new calibration curve must be regenerated. At least six calibration points are required for quadratic regression. Each calibration point used to generate the curve must have a calculated percent deviation less than 25% between the nominal concentration and the regression calculated result.

12.2.6 The retention time window of the SRM transitions must be within 5% of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calibration curve to determine if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

12.2.7 A calibration check standard (midpoint) must be analyzed at the end of each batch of 20 samples or within 24 hours after the initial calibration curve was generated (100 ppb). The

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end calibration check should be the same calibration standard that was used to generate the initial curve. The regression result from the end calibration check standard must have a percent deviation less than 35% from the target analyte and surrogate nominal concentration. If the results are not within these criteria, the problem must be corrected and either all samples in the batch must be re-analyzed against a new calibration curve or the affected results must be qualified with an indication that they are not within the performance criteria of the test method. If the analyst inspects the vial containing the end calibration check standards and notices that the samples evaporated affecting the concentration, a new end calibration check standard may be made and analyzed. If this new end calibration check standard has a percent deviation less than 35% from the calculated concentration for the target analyte and surrogate, the results may be reported unqualified.

TABLE 4. Concentrations of Calibration Standards (PPB)

Analyte/Surrogate	DVL	LV 1	LV 2	LV 3	LV 4	LV 5	LV 6
DOSS	3	10	20	60	100	150	200
DOSS-D34 (Surrogate)	3	10	20	60	100	150	200
DOSS-^{13}C (Surrogate)	3	10	20	60	100	150	200

12.3 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., a precision and bias study must be performed to demonstrate laboratory capability (Section 16).

12.3.1 Analyze at least four replicates of a sample solution containing the DOSS and surrogate at a concentration in the calibration range of Levels 3 to 5. The Level 4 concentration of the 6 point calibration curve was used to set the QC acceptance criteria in this method. The matrix and chemistry should be similar to the solution used in this test method. Each replicate

must be taken through the complete analytical test method including any sample pre-treatment steps.

12.3.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of the QC acceptance criteria for the Initial Demonstration of Performance in Table 5.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 5.

12.3.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in Table 5 are preliminary until a more data and multi-laboratory study is completed. Data generated from a single-laboratory validation from reagent and sea water matrices are shown in the Precision and Bias Section 16. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard. A Reference on how to generate QC acceptance criteria is in Method 8000B in EPA publication SW-846.

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TABLE 5. Preliminary QC Acceptance Criteria

Analyte	Test Conc. (µg/L) in Reagent Water	Initial Demonstration of Performance			Lab Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Limit	Upper Limit
DOSS	200	50	150	30	50	150
DOSS-D34 (Surrogate)	200	50	150	30	50	150
DOSS-^{13}C (Surrogate)	TBD	TBD	TBD	TBD	TBD	TBD

12.4 Surrogate Spiking Solution (This draft contains DOSS-D34 data, DOSS-^{13}C will be reported when available):

June 22, 2010, DOSS-^{13}C was in the synthesis stage and DOSS-D34 was commercially available. Notable different retention times were observed with DOSS and DOSS-D34 (Table 3). DOSS-D34 surrogate resulted a different elution time than DOSS (Table 3) and had apparent deuterium hydrogen exchange (similar distribution at 2 and 16 hours). ^{13}Carbon surrogates are not liable to mass change associated with deuterated surrogate and typically elute at the same time as their analytes, therefore DOSS-^{13}C surrogate will be purchased and tested.

A surrogate spiking 50% water/50% CH_3CN solution containing DOSS-^{13}C or - D34 is added to all samples. A stock surrogate spiking solution is prepared at 20 ppm. Spiking 200 µL of this spiking solution into a 20 mL water sample results in a concentration of 200 ppb of the surrogate in the sample. The result obtained for the surrogate recovery must fall within the limits of Table 5. If the limits are not met, the affected results must be qualified with an indication that they do not fall within the performance criteria of the test method.

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12.5 Method Blank:

12.5.1 Analyze a reagent water blank with each batch of 20 or fewer samples. The DOSS measured in the blank should be less than half of the reporting limit. If the concentration of DOSS is found above this level, analysis of samples is halted until the contamination is eliminated, and a blank shows no contamination at or above this level, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method. DOSS has been found to carry-over in LC systems. A rigorous washing of the injector with stronger wash solvents has been shown to work well as described in this method. If you have an older LC system or have carry-over problems the LC conditions listed in Table 2B should be investigated to remove background.

12.6 Laboratory Control Sample (LCS):

12.6.1 To ensure that the test method is in control, analyze a LCS prepared with the DOSS at a concentration in the calibration range of Levels 3 to 5. The LCS is prepared following the analytical method and analyzed with each batch of 20 samples or less. Prepare a stock matrix spiking solution in 50% water/50% acetonitrile containing the DOSS at 20 ppm. Spike 200 µL of this stock solution into 20 mL of water to yield a concentration of 200 ppb for the DOSS in the sample. The LCS result must be within the limits in Table 5. Matrix spiking solutions are routinely replaced every 7 days if not previously discarded for quality control failure.

12.6.2 If the LCS regression result is not within Table 5 limits, analysis of samples is halted until the problem is corrected, and either all samples in the batch must be re-analyzed, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7 *Matrix Spike/Matrix Spike Duplicate (M/MSDS):*

12.7.1 To check for interferences in the specific matrix being tested, perform a MS/MSD on at least one sample from each batch of 10 or fewer samples by spiking the sample with a known concentration of DOSS and following the analytical method. Prepare a stock matrix spiking solution in 50% water/50% acetonitrile containing the DOSS at 20 ppm. Spike 200 µL of this stock solution into 20 mL of water to yield a concentration of 200 ppb of the DOSS in the sample.

12.7.2 If the spiked concentration plus the background concentration exceeds that of the Level 6 calibration standard, the sample must be diluted to a level near the midpoint of the calibration curve.

12.7.3 Calculate the spike percent recovery (P) using Equation 1:

Equation 1

$$P = 100\frac{\left|A(Vs+V) - BVs\right|}{CV}$$

Where:
A = concentration found in spiked sample
B = concentration found in unspiked sample
C = concentration of analyte in spiking solution
V_s = volume of sample used
V = volume of spiking solution added
P = percent recovery

12.7.4 The percent recovery of the spike shall fall within the limits in Table 6. If the percent recovery is not within these limits, matrix interference may be present in the selected sample. Under these circumstances, one of the following remedies must be employed: the matrix interference must be removed, all samples in the batch must be analyzed by a test method not

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affected by the matrix interference, or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

12.7.5 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 6 are preliminary until more data is acquired. The data generated by a single-laboratory using sea water samples are in the Precision and Bias Section 16. The matrix variation between the different waters may have a tendency to generate significantly wider control limits than those generated by a single-laboratory in one water matrix. It is recommended that the laboratory generate their own in-house QC acceptance criteria which meets or exceeds the criteria in this standard. Surrogates should be used to identify and measure matrix affect.

12.7.5.1 The laboratory should generate their own in-house QC acceptance criteria after the analysis of 15-20 matrix spike samples of a particular surface water matrix. References on how to generate QC acceptance criteria is in Method 8000B in EPA publication SW-846.

TABLE 6. Preliminary MS/MSD QC Acceptance Criteria

Analyte	Test Conc. (µg/L)	MS/MSD Recovery (%) Lower Limit	MS/MSD Recovery (%) Upper Limit	MS/MSD Precision Maximum RPD (%)
DOSS	200	50	150	30
DOSS-D34 (Surrogate)	200	50	150	30
DOSS-^{13}C (Surrogate)	TBD	TBD	TBD	TBD

12.8 *Duplicate:*

12.8.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the sample contains the analyte at a level greater than 5 times

the detection limit of the method, the sample and duplicate may be analyzed unspiked; otherwise, an MSD should be used.

12.8.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq. 2. Compare to the RPD limit in Table 6.

Equation 2

$$\text{RPD} = \frac{|\text{MSR - MSDR}|}{(MSR + MSDR) \div 2} \times 100$$

Where:
RPD= relative percent difference
MSR= matrix spike recovery
MSDR= matrix spike duplicate recovery

12.8.3 If the result exceeds the precision limit, the batch must be re-analyzed or the results must be qualified with an indication that they do not fall within the performance criteria of the test method.

13. Procedure

13.1 This test method is based upon a 20 mL sample size per analysis. **Any sample size may be used such as a half filled VOA vial as long as the QC spikes and sample preparation volumes are adjusted accordingly.** Samples shall be analyzed within 5 days of collection. If the samples are above 6°C when received or during storage, or not analyzed within 5 days of collection, the data is noted in the case narrative that accompanies the data.

13.2 In the laboratory, the entire 20 mL sample, collected in a 20 mL glass collection vial, is poured into a 50 mL graduated cylinder. The surrogate as described in Section 12 (added to the

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sample in the original sample container or graduated cylinder depending on sampling described in Section 10.1) and NH_4CO_2H are added to the sample. The laboratory control and matrix spike samples are then spiked with the target compound as described in Section 12. The vial is rinsed with two 6 to 8 mL portions of acetonitrile to collect DOSS remaining in the collection vial. These 2 portions are added to the 50 mL graduated cylinder. The samples are then diluted to 40 mL final volume with acetonitrile and mixed thoroughly. The prepared sample is filtered through the syringe driven filter unit fitted with a PVDF filter cartridge into a glass storage vial.

13.3 For samples that are not biphasic, the entire 40 mL volume is filtered through the filtration device described in Section 7.2 into a pre-cleaned collection vial (such as a VOA vial). A portion of that filtered sample is added to an amber glass LC vial with a Teflon® lined cap which is analyzed. A new filter unit is used for each sample.

13.4 For biphasic samples, the lower aqueous layer is filtered through the filtration device described in Section 7.2 into a pre-cleaned collection vial (such as a VOA vial). The upper oil layer is left behind and is not added to the filtration device. A portion of that filtered sample is added to an amber glass LC vial with a Teflon® lined cap which is analyzed. A new filter unit is used for each sample.

13.5 The syringe must be cleaned between each filtration. It is the analyst's responsibility to ensure that the syringe is clean. A suggested method for cleaning the syringe between filtrations is to first rinse with at least 5 syringe volumes of water, followed by at least 3 volumes of 50% water/50% acetonitrile.

13.6 Once a passing calibration curve is generated the analysis of samples may begin. An order of analysis may be: method blank, laboratory control sample and duplicate, method blank,

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up to 20 samples, matrix spike sample(s) and duplicate followed by an end calibration check standard and a method blank.

14. Calculation or Interpretation of Results

14.1 For DOSS and surrogate analysis, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. Calibration curves are used to calculate the amounts of DOSS and surrogate. Calculate the concentration in µg/L (ppb) for each analyte. The sample concentration was diluted by two fold by the addition of surrogates, ammonium formate, acetonitrile, and target compound spike where applicable. The two fold dilution must be accounted for when reporting the concentration. DOSS may be reported if present at or above the reporting limit. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with 5 mM ammonium formate in 50% acetonitrile/50% water to obtain a concentration near the mid-point of the calibration range and re-analyzed. This method uses one surrogate, either DOSS 1-^{13}C, or DOSS-D34, to monitor performance and matrix affect. The surrogate recoveries are provided with all data generated from this test method.

14.1.1 A surrogate is used to monitor the performance of DOSS. If the surrogate meets the quality control criteria in this test method, the data may be reported unqualified for DOSS if all other quality control in this test method are acceptable. If the surrogate does not meet the quality control criteria of the test method, the data is qualified for DOSS.

15. Report

15.1 Determine the results in units of µg/L (ppb) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve. All data that does not meet the specifications in the test method must be appropriately qualified.

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16. Precision and Bias

16.1 The determination of precision and bias was conducted through US EPA Region 5 Chicago Regional Laboratory. **More than 50 seawater samples have been analyzed using this procedure without noticeable chromatography effect or increased pressure.**

16.2 This test method was tested by CRL on reagent and sea water. The samples were spiked with the DOSS to obtain a 200 ppb concentration of each as described in Section 12. Table 7 contains the recoveries and standard deviation (SD) for the target compound.

TABLE 7. Single-Laboratory Recovery Data in Reagent Water using chromatography conditions outlined in Table 2A and Table 2B

Precision and Accuracy Samples	Measured ppb from 200 ppb DOSS Spikes in Reagent Water					
	Table 2A			Table 2B		
	Retention time (min)	DOSS measured (ppb)	Percent Recovery	Retention time (min)	DOSS measured (ppb)	Percent Recovery
1	6.38	182.6	91.3%	5.41	196.1	98.0%
2	6.37	232.9	116.5%	5.41	232.8	116.4%
3	6.37	224.6	112.3%	5.41	225.8	112.9%
4	6.37	226.8	113.4%	5.41	238.9	119.5%
Average Recovery:		216.7	108.4%		223.4	111.7%
Standard Deviation:		23.0			19.0	
% Relative SD		11.5%			9.5%	

16.3 This test method was tested by CRL on Gulf of Mexico sea water. The samples were spiked with target compound as described in Section 12. Table 8 contains the recoveries for the target compound.

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TABLE 8. Single-Laboratory Recovery Data in Gulf of Mexico Sea Water using chromatography conditions outlined in Table 2A and Table 2B

Precision and Accuracy Samples	Measured ppb from 200 ppb DOSS Spikes in Gulf Water					
	Table 2A			Table 2B		
	Retention time (min)	DOSS measured (ppb)	Percent Recovery	Retention time (min)	DOSS measured (ppb)	Percent Recovery
1	6.37	189.1	94.6%	5.4	257.2	128.6%
2	6.37	251.4	125.7%	5.4	251.4	125.7%
3	6.37	253.2	126.6%	5.41	263.0	131.5%
4	6.37	257.0	128.5%	5.4	262.4	131.2%
5	6.37	250.7	125.4%	5.4	255.0	127.5%
Average Recovery:		240.3	120.1%		257.8	128.9%
Standard Deviation:		28.7			4.9	
% Relative SD		14.4%			2.5%	

17. Keywords

17.1 Dioctyl Sulfosuccinate; Liquid Chromatography; Mass Spectrometry; Water

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APPENDIX A. CALIBRATION AND SAMPLE MATRIX RAM-DOSS RESULTS

MEASURED BY WATERS LC-MS/MS

TABLE 9. Single-Laboratory DOSS Recovery Data from Gulf of Mexico Sea Water with and without DOSS Contaminated Crude Oil and DOSS Spikes.

DOSS Recovery Data in Gulf of Mexico Water Spiked with Oil	Gulf Seawater (mL)	Gulf oil (mL)	DOSS spike (ppb)	DOSS measured (ppb)	Recovery %
Gulf water1	1.0	NA	NA	ND	-
Gulf water2	1.0	NA	NA	ND	-
Gulf water3	1.0	NA	NA	ND	-
Gulf water4	1.0	NA	NA	ND	-
Gulf water DOSS addition1	4.0	NA	200	277.8	138.9%
Gulf water DOSS addition2	4.0	NA	200	189.8	94.9%
Gulf water oil addition1	3.6	0.4	NA	3,319	-
Gulf water oil addition2	3.6	0.4	NA	1,198	-
Gulf water oil and DOSS addition1	3.6	0.4	500	2,046	*
Gulf water oil and DOSS addition2	3.6	0.4	500	1,876	*

* DOSS detected in Gulf of Mexico water samples with DOSS contaminated oil addition was greater than the spike concentration.

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TABLE 10. Single-Laboratory **DOSS-D34 Recovery Data from Gulf of Mexico Water with DOSS Contaminated Gulf Oil Spiked with DOSS-D34.**

				3 mL Subsampled Water[1]	**Water and Oil Extraction[2]**
			DOSS-D34*	DOSS-D34	DOSS-D34
	Gulf water (mL)	Gulf oil (mL)	ng spiked	ng measured	ng measured
MB1	3.6	0.4	ND	ND	ND
MB2	3.6	0.4	ND	ND	ND
Gulf Oil and water1	3.6	0.4	800	ND	694
Sample4	3.6	0.4	800	ND	864

*Sigma 710652-SPEC DOSS spiked at 200ppb (calibration midpoint) and stored for 16 hours prior to sample preparation.

[1]Gulf water subsampled and prepared following Section 10.1.2

[2] Gulf water and oil sample prepared following Section 10.1.1

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TABLE 11. Single-Laboratory DOSS Recovery Data from Gulf of Mexico Water with Southern Louisiana Reference Crude Oil Spiked with 0, 20, 200 and 400 ppb DOSS then Stored 16 hours between 0 and 6°C.

	Gulf Water (mL)	Southern Louisiana Reference Crude Oil (mL)	Water Subsample[1]		Whole Sample[2]	
			DOSS	DOSS-D34	DOSS	DOSS-D34
Method Blank 1	19.8	0.2	ND	107.4%	ND	98.3%
Method Blank 2	19.8	0.2	ND	115.4%	ND	102.4%
Method Blank 3	19.8	0.2	ND	155.8%	ND	132.9%
20 ppb 1	19.8	0.2	-	-	ND	123.7%
20 ppb 2	19.8	0.2	-	-	ND	121.8%
20 ppb 3	19.8	0.2	-	-	ND	127.8%
200 ppb 1	19.8	0.2	26.6%	105.8%	51.3%	134.7%
200 ppb 2	19.8	0.2	21.3%	101.2%	46.3%	126.8%
200 ppb 3	19.8	0.2	20.1%	98.1%	44.4%	112.5%
400 ppb 1	19.8	0.2	24.2%	92.1%	47.1%	108.6%
400 ppb 2	19.8	0.2	28.0%	91.6%	45.1%	107.7%
400 ppb 3	19.8	0.2	27.4%	93.0%	40.6%	103.6%

[1]Gulf water subsampled and prepared following Section 10.1.2.

[2] Gulf water and oil sample prepared following Section 10.1.1.

DOSS additions were performed prior to oil addition and vortexed. After the oil was added the samples were stored between 0 and 6 for 16 hours. The 20 ppb water subsamples were not tested as low recoveries from 200 ppb and 400 ppb indicated that DOSS measurements would be below the method RL. Percent recoveries from whole samples were on average greater than subsamples.

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X1.1.1 A holding time study was performed by US EPA Region 5 Chicago Regional Laboratory on DOSS in Gulf of Mexico water. The Gulf of Mexico water was spiked with DOSS to obtain a 50 ppb concentration of DOSS.

TABLE 12. Single-Laboratory DOSS in Gulf of Mexico Water Holding Time Study

50 ppb DOSS Storage in Gulf of Mexico Water (4 ºC)	Percent Recovery			
	Day 0	Day 7	Day 10	
	DOSS	DOSS	DOSS	DOSS-D34*
1	93.3%	74.2%	51.8%	97.6%
2	95.3%	77.1%	51.4%	100.8%
3	97.9%	78.7%	48.4%	97.4%
Average Recovery	95.5%	76.7%	50.5%	98.6%
% Relative SD	0.023	0.022	0.019	0.019

*Sigma 710652-SPEC DOSS-D34 was spiked during Day 10 sample preparation (Section10.1.2). The DOSS-D34 surrogate was not available for Day 0 and Day 7 sample preparation.

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Figure 1. Detection Verification Level (3 ppb DOSS in calibration standard) Signal/Noise Ratio.

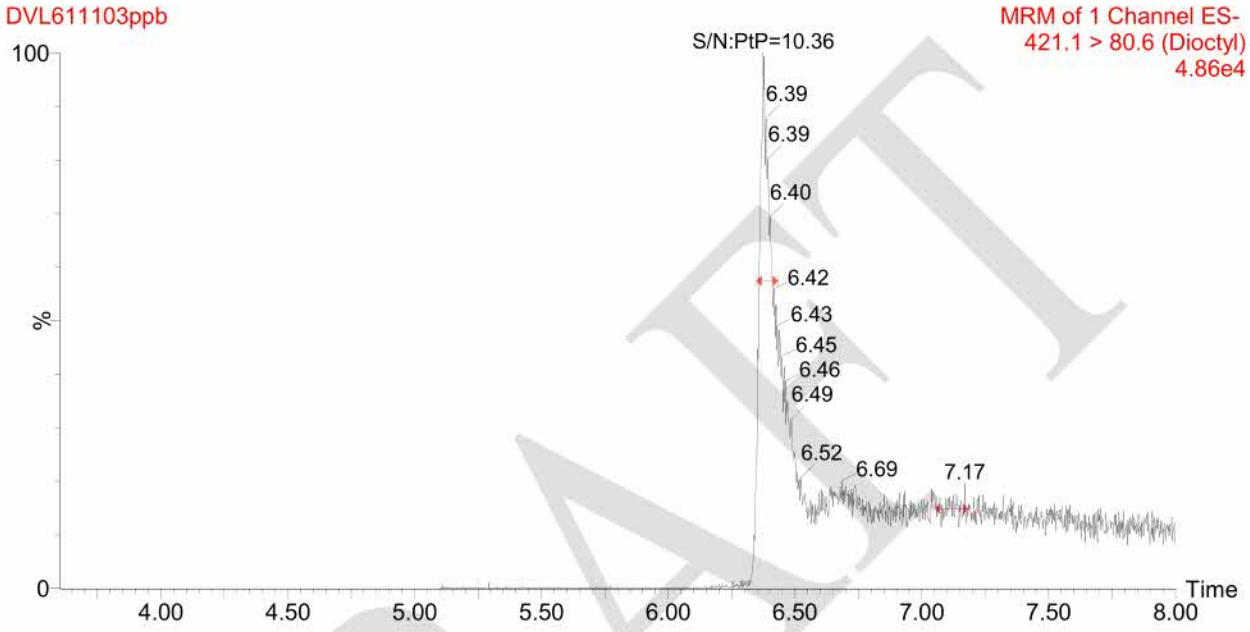

Figure 2. Reporting Level (10 ppb DOSS in calibration standard) Signal/Noise Ratio.

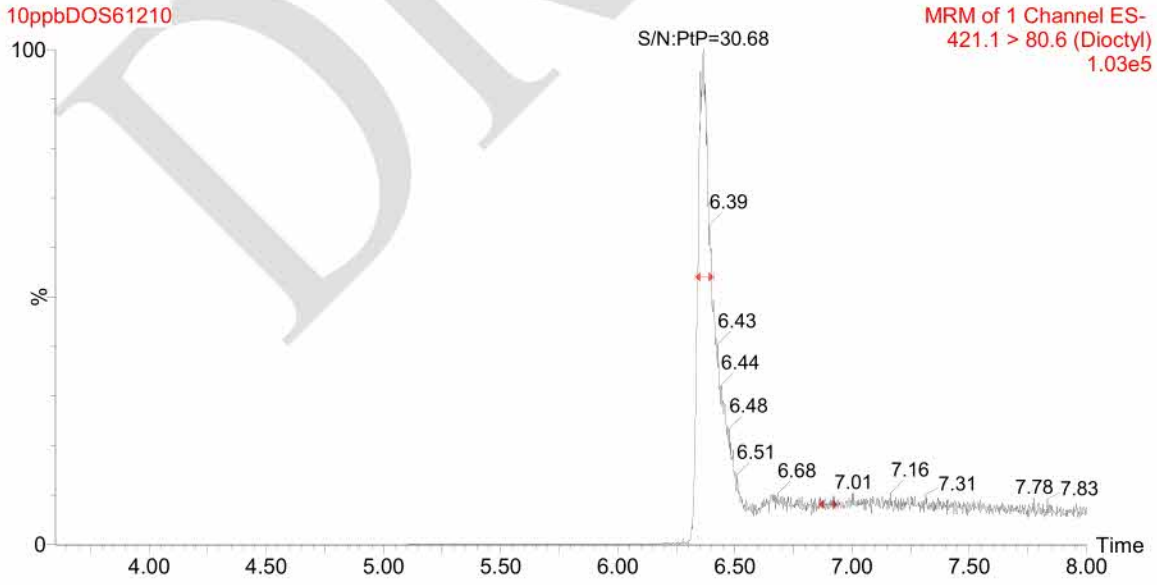

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Figure 3. Ten to 200 ppb DOSS Linear calibration curve (5 millimolar ammonium formate in 50% acetonitrile/50% water).

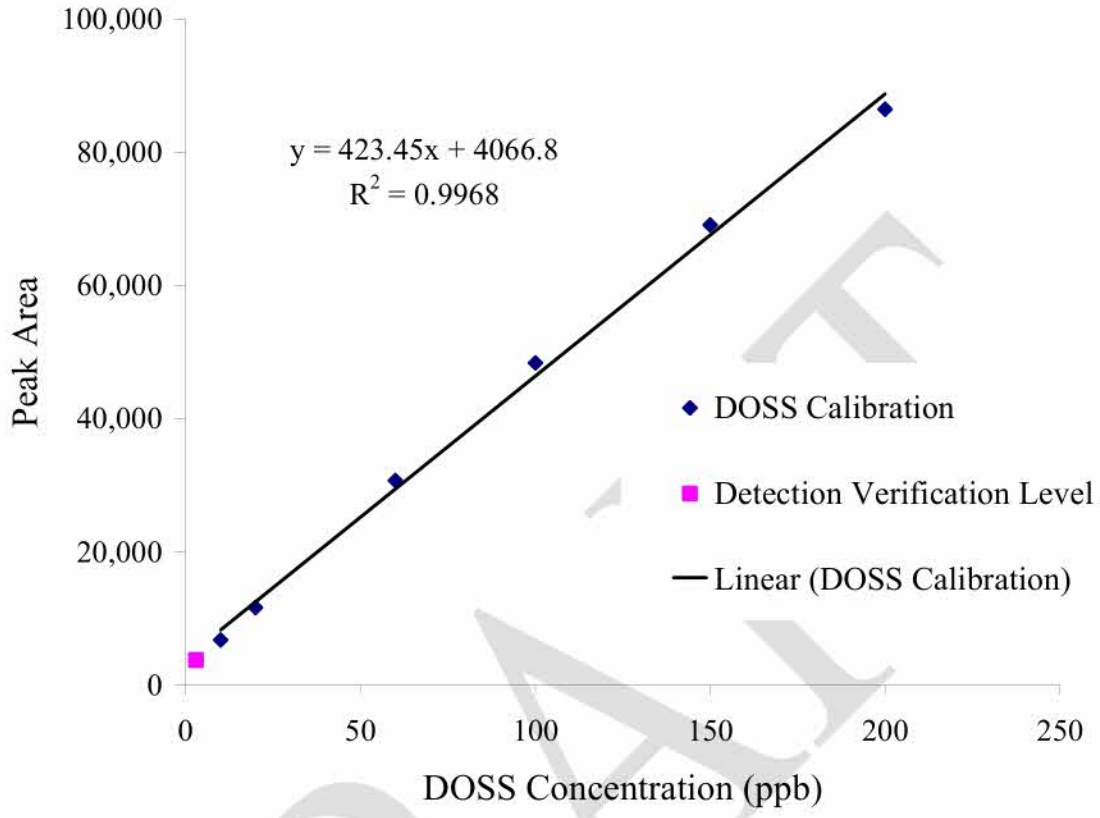

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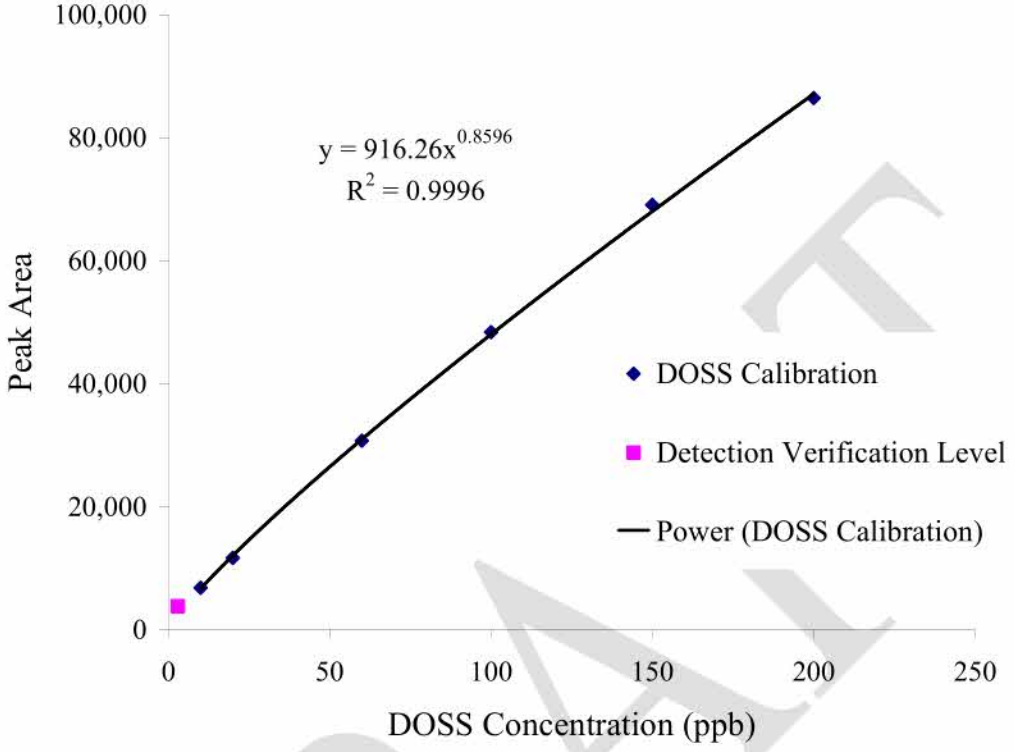
Figure 4. Ten to 200 ppb DOSS quadratic calibration curve (5 millimolar ammonium formate in 50% acetonitrile/50% water).



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APPENDIX B. RAM-DOSS BY AGILENT LC-MS/MS

LIQUID CHROMATOGRAPHY / MASS SPECTRAL IDENTIFICAITON (using AGILENT instrument)

Instrumentation

i. Agilent 6410 Triple Quad Mass Spectrometer with Mass Hunter Version v3.0 for system control and Data acquisition and processing
ii. Agilent 1200 Binary HPLC pump, Auto sampler, Column compartment and online Degasser.

b. LC conditions
   i. Zorbax Eclipse XBD C18 analytical column 2.1 x 50 mm, 3.5 micron particle size(part no.971700-902)
   ii. Eluent
      1. A: 99% water +1% ACN with 0.1% formic acid
      2. B: 99% ACN +1% water with 0.1% formic acid
   iii. Injection volume: 15uL
   iv. LC conditions (see table below)

Time (min)	Flow (mL/Min)	%A (water with 0.1% Formic)	%B (ACN with 0.1% Formic)
0-1	0.4	98	2
4	0.4	60	38
7	0.4	20	80
12	0.4	2	98
12.1	0.4	98	2

c. Mass Spectrometer Conditions
   i. Data Acquisition parameters(see table below)

Parameter	Settings
Scan Mode	negative
Capillary Voltage	4.0 kV
To Waste	Until 2.6 min
Segments	3
Gas Temperature	350 °C
Gas Flow	6 L/min
Nebulizer	15 psi

Compound	Ion	Segment	Prec. ion	Prod. ion	Frag (V)	CE (V)	Multiplier (V)
DOSS	M-Na	1	421	81	188	30	600
DOSS-D34	M-Na	1	445.4	81	188	30	600

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