# Simultaneous Determination of Oil Dispersants in Seawater and Crude Oil by LC and Tandem MS

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Application Note 586

## Key Words
Environmental analysis, oil spill, Corexit®, Deepwater Horizon, Gulf of Mexico, seawater

## Goal
To develop a liquid chromatography with tandem mass spectrometry (LC-MS/MS) method capable of simultaneously detecting and quantifying DOSS and 2-butoxyethanol in a single chromatographic run without preconcentration or cleanup steps. This method can serve as a tool to track Corexit® after its usage in oil spills and determine if Corexit EC9527A was employed.

## Introduction
On April 20, 2010 the *Deepwater Horizon* (MC-252) oil platform caught fire and sank in the Gulf of Mexico, creating a large release of oil and gas from the riser pipe and uncapped well head. Efforts to contain and clean up the spill included heavy use of oil dispersants both above and below the surface. The dispersants Corexit EC9500A and Corexit EC9527A (formerly Corexit 9500 and Corexit 9527, produced by Nalco, Naperville, IL) were approved for use in the Gulf of Mexico oil spill by the U.S. Environmental Protection Agency (EPA).[1] At least 1.8 million gallons of dispersants were applied during the response and recovery process.[2] Corexit EC9500A was the main product used in that effort.

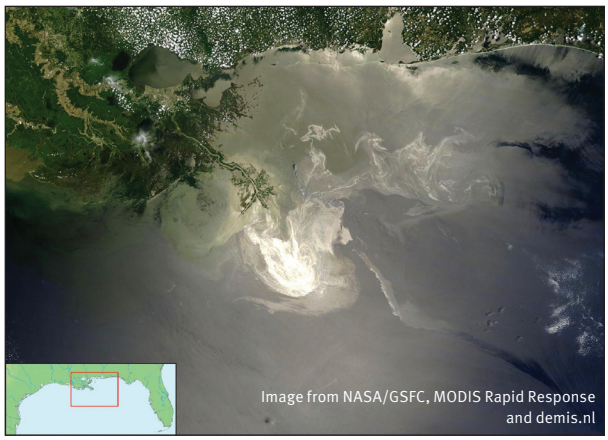


Figure 1. Satellite view of oil slick in the Gulf of Mexico on May 24, 2010

According to available material safety data sheets, the components of Corexit EC9500A are dioctyl sulfosuccinate sodium salt (DOSS) (10–30% w/w), hydrotreated light petroleum distillates (10–30% w/w), and propylene glycol (1–5% w/w).[3] Corexit EC9527A contains mainly 2-butoxyethanol (30–60% w/w) and DOSS (10–30% w/w).[5] These mixtures of solvents and surfactants reduce the interfacial tension between water and oil, facilitating the breakup of the oil into tiny droplets that are easily dispersed by wind and wave action.[4] The structures of 2-butoxyethanol and DOSS are shown in Figure 2.

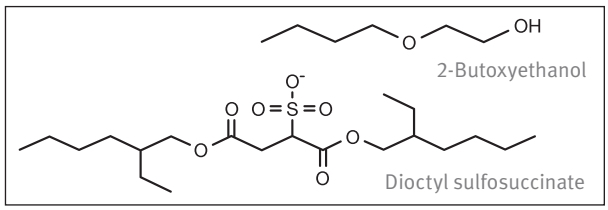


Figure 2. 2-butoxyethanol and dioctyl sulfosuccinate (DOSS), the main components of Corexit formulations

Although Corexit formulations have been found to have only low-to-moderate toxicity to most aquatic species, tracking these formulations in the environment is still a priority because much of their fate is still not well understood.[6] The large amounts of Corexit used in the Gulf of Mexico gave rise to the need for an analytical method capable of detecting its presence in seawater even when large dilution factors are expected.

2-butoxyethanol is of interest because it is found only in Corexit EC9527A. Despite the fact that a variety of other sources can contribute to its presence in coastal areas, chronic background environmental concentrations of 2-butoxyethanol are expected to be low because of its high miscibility in water and its fast biodegradation (half-life of 1–4 weeks) in environmental waters.[7]

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However, applications of Corexit EC9527A in an oil spill response could potentially yield localized high concentrations of 2-butoxyethanol in surface waters. Therefore, an LC-MS/MS method capable of simultaneously detecting and quantifying the two main components of Corexit EC9527A could be useful to assess if this formulation was used.

## Experimental

### Reagents and Solvents

2-butoxyethanol was from the Acros Organics brand, part of Thermo Fisher Scientific. The surrogate standards sodium dodecyl-$d_{25}$ sulfate (DDS-$^{2}H_{25}$) and 2-butoxyethanol-$^{2}H_{4}$ were purchased from CDN Isotope Laboratories (Quebec, Canada). Certified DOSS and DOSS-$^{13}C_{4}$ standards were purchased from Cambridge Isotopes Laboratories (Andover, MA). Stock and working solutions of all compounds were prepared in acetonitrile. The concentrations of the stock solutions were as follows: DOSS and DOSS-$^{13}C_{4}$ were 100 mg/L (certified standards); 2-butoxyethanol was 8000 mg/L; 2-butoxyethanol-$^{2}H_{4}$ was 20000 mg/L; and DDS-$^{2}H_{25}$ was 72 mg/L. Working solutions concentrations are presented in the Sample Preparation section. Artificial seawater was prepared to 3.5% w/v using the commercially available Instant Ocean® sea salt. Chromatographic studies were performed using Fisher Chemical™ Optima™ LC/MS-grade formic acid, acetonitrile, and water.

### Sample Preparation

#### Seawater

Seawater samples were collected from Biscayne Bay in Florida and filtered through 0.45 µm fiberglass filters. A 5 mL seawater subsample was placed in a glass vial containing 2.5 mL of Optima-grade acetonitrile and stored until analysis. Then, 1200 µL of the acetonitrile-diluted seawater was transferred to a 2 mL amber LC vial already containing 47 µL of acetonitrile and 200 µL of artificial seawater. To that was added 18.9 µL of DDS-$^{2}H_{25}$ surrogate (7.9 mg/L in acetonitrile), 18.8 µL of 2-butoxyethanol-2H4 surrogate (8.0 mg/L in acetonitrile), and 15.8 µL of DOSS-$^{13}C_{4}$ surrogate (1.9 mg/L in acetonitrile) for a final volume of 1500 µL that maintained the 33.3% v/v of acetonitrile. The samples were thoroughly mixed using a vortex and analyzed directly by LC-MS/MS.

#### Crude oil

A sample of sweet-light crude oil from the MC-252 riser, known to contain DOSS, and a sweet-light crude oil from the Wilcox formation in Texas, were used to test the method. The crude oil samples (5.0 µL) were added to 2 mL amber LC vials and spiked with 37.5 µL of 2-butoxyethanol-$^{2}H_{4}$ surrogate and 40.0 µL of DOSS-$^{13}C_{4}$ surrogate. The surrogate-fortified oil was suspended in 1260 µL of acetonitrile, capped, and mixed using a vortex for 2 min. This resulted in a two-phase system with undissolved oil on the vial walls. For instrumental analysis, an aliquot from the acetonitrile phase of each sample was added to a new 2 mL amber LC vial containing 1000 µL of artificial seawater and 18.9 µL DDS-$^{2}H_{25}$ surrogate. Acetonitrile was added to make a final volume of 1500 µL. To minimize analysis time and ensure method uniformity, the injected sample was prepared to match the 66% seawater and 33% acetonitrile matrix of the calibration solutions and seawater samples.

#### Calibration solutions

Calibration solutions were prepared in artificial seawater with the same salt and acetonitrile ratio of the analysis-ready seawater and oil samples. Then, 1000 µL of artificial seawater was transferred to a 2 mL LC amber vial, and 18.9 µL of DDS-$^{2}H_{25}$ surrogate, 18.8 µL of 2-butoxyethanol-$^{2}H_{4}$ surrogate, and 15.8 µL of DOSS-$^{13}C_{4}$ surrogate were added. Increasing amounts of DOSS and 2-butoxyethanol were added to the solutions, and acetonitrile was added to make a final volume of 1500 µL. A seven-point calibration set was freshly prepared for each analysis day.

### Liquid Chromatography

HPLC analysis was performed using a Thermo Scientific™ Accela™ quaternary pump equipped with an HTC-PAL™ autosampler system (CTC Analytics, Zwingen, Switzerland).

**LC Parameters**

| | | | |
|---|---|---|---|
| Column: | Thermo Scientific™ Hypersil GOLD™ aQ column (50 mm × 2.1 mm, 3 µm particle size) | | |
| Pre-column | Hypersil GOLD aQ (10 mm x 2.1 mm, 3 µm particle size) | | |
| Injection volume | 20 µL | | |
| Run time | 10 min | | |
| Flow rate | 325 µL/min | | |
| Mobile phase A | 0.1% formic acid and 1% water in acetonitrile | | |
| Mobile phase B | 0.1% formic acid in water | | |
| Gradient | Time | %A | %B |
| | 0.0 | 2 | 98 |
| | 0.9 | 2 | 98 |
| | 3.7 | 98 | 2 |
| | 5.6 | 98 | 2 |
| | 5.9 | 2 | 98 |
| | 10.0 | 2 | 98 |

Instrument control and data acquisition was performed using Thermo Scientific™ Xcalibur™ software version 2.1.

## Mass Spectrometry

Detection of analytes was performed on a Thermo Scientific™ TSQ Quantum Access™ triple-stage quadrupole mass spectrometer equipped with a Thermo Scientific™ Ion Max™ API source with an electrospray ionization (ESI) probe. The source was operated in positive ion mode for the first 4 min of the chromatographic separation for the detection of 2-butoxyethanol and 2-butoxyethanol-$^{2}H_{4}$ and then switched to negative ion mode to enable detection of DOSS, DOSS-$^{13}C_{4}$, and DDS-$^{2}H_{25}$. Optimized MS parameters were as follows:

| MS Parameters | |
|---|---|
| **Positive ion mode segment** | |
| Capillary voltage | 4.5 kV |
| Tube lens | 50 V |
| Auxiliary gas ($N_2$) | 15 arbitrary units |
| **Negative ion mode segment** | |
| Capillary voltage | 4 kV |
| Tube lens | -80 V |
| Auxiliary gas ($N_2$) | Not used |
| **Both segments** | |
| Capillary temperature | 325 °C |
| Sheath gas ($N_2$) | 60 arbitrary units |

Data were acquired in selected-reaction monitoring (SRM) mode. Identities of the precursor and product ions and the optimized collision parameters are provided in Table 1. The flow from the LC was diverted to waste for the first 1.5 min to prevent the accumulation of salts into the mass spectrometer source. A typical chromatogram for a spiked seawater sample is shown in Figure 3.

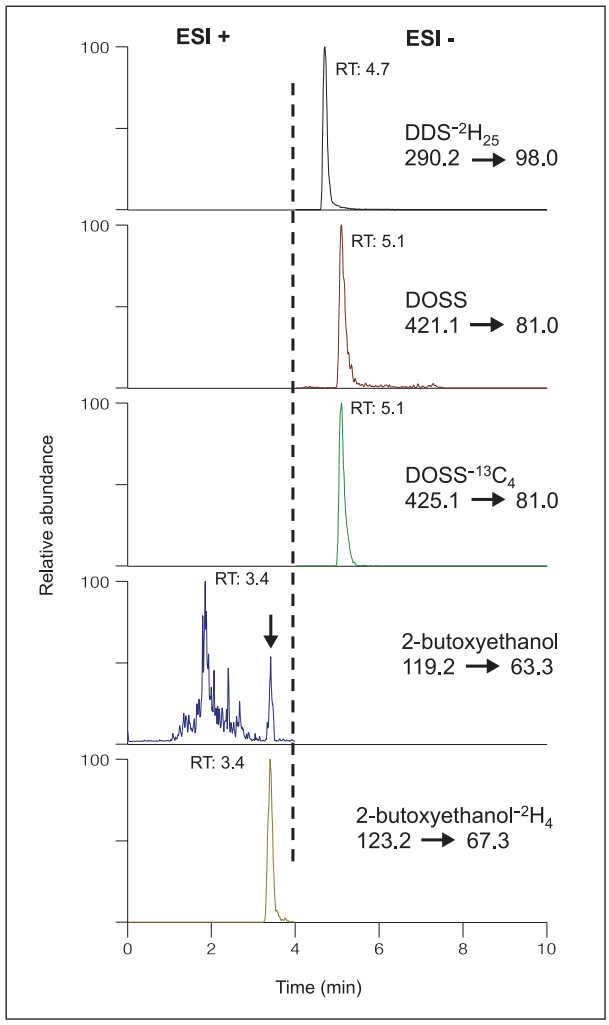


Figure 3. LC-ESI-MS/MS chromatograms of DOSS and 2-butoxyethanol in seawater at spike levels of 0.778 µg/L and 2.56 µg/L, respectively, and their surrogates

Table 1. Summary of the retention times, masses, and optimized SRM parameters

| Compound | RT (min) | Parent ion *(m/z)* | Collision Pressure (mTorr) | Quantifying ion *(m/z)* | Collision energy (eV) | Qualifying ion *(m/z)* | Collision energy (eV) |
|---|---|---|---|---|---|---|---|
| 2-butoxyethanol | 3.4 | 119.2 | 0.8 | 63.3 | 5 | 45.4 | 9 |
| 2-butoxyethanol-$^{2}H_{4}$ | 3.4 | 123.2 | 0.8 | 67.3 | 6 | - | - |
| DDS-$^{2}H_{25}$ | 4.7 | 290.1 | 1.5 | 98.0 | 42 | - | - |
| DOSS | 5.1 | 421.1 | 1.5 | 81.0 | 25 | 227.1 | 21 |
| DOSS-$^{13}C_{4}$ | 5.1 | 425.1 | 1.5 | 81.0 | 25 | - | - |

## Results and Discussion

### Chromatographic Method Development

Preliminary work indicated that 2-butoxyethanol needs to be ionized in a very narrowly defined pH range in the electrospray ionization source. Therefore, the pH was kept constant throughout the run by adding the same concentration of formic acid to both the aqueous and the organic mobile phases. A solution of 0.1% formic acid in water (pH 2.8) was used in combination with 0.1% formic acid and 1% water in acetonitrile. This approach provided acceptable peak shape and intensity for the negative mode signals and allowed good ionization of 2-butoxyethanol (Figure 3).

### Seawater Sample-Preparation Development

Signal suppression was observed for all analytes when fortified, undiluted seawater was injected relative to solutions of the same concentration in deionized water. Two experiments were conducted to determine the optimum dilution conditions that would provide adequate signals for quantification. In a first experiment, acetonitrile was compared to deionized water as a dilution solvent. A fortified seawater sample was diluted from 100% to 50%, with the dilution solvent being progressively changed from deionized water to acetonitrile, while keeping the dilution factor constant. As observed in Figure 4, the DOSS peak area increased to a maximum as the percentage of acetonitrile increased, indicating that acetonitrile was a better dilution solvent than water.

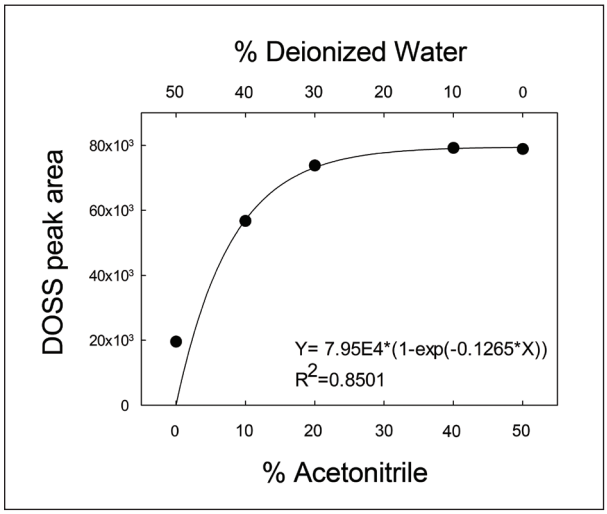


Figure 4. Comparison between acetonitrile and deionized water as solvents

In a second experiment, the optimal seawater-to-acetonitrile ratio was established by progressively diluting a fortified seawater solution. Figure 5 shows that DOSS peak area increases to a maximum between 20% and 30% v/v of acetonitrile, before following the expected dilution trend.

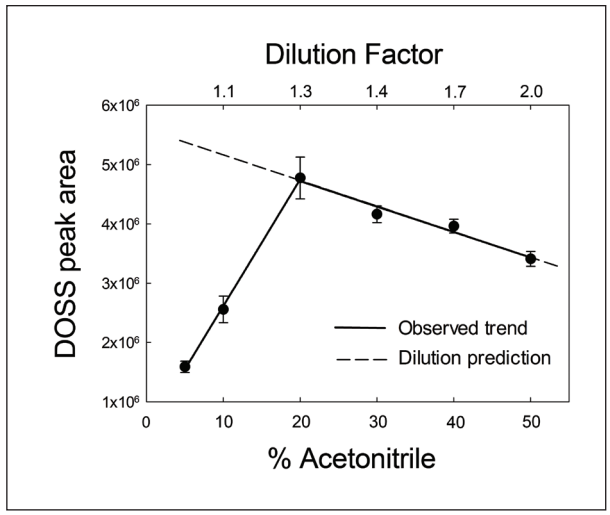


Figure 5. Dilution experiment of a 10 µg/L DOSS-fortified seawater sample

These results suggested that acetonitrile may reduce the interaction between DOSS and the glass vial surface. To investigate the storage effect of sample containers, 5 µg/L DOSS-fortified seawater samples were stored in three common types of sampling bottles (glass, PTFE, and PE) at or below 4 °C. Subsamples were taken at 0, 1, 3, and 25 hours and analyzed. Based on the dilution experiment results, a second set of fortified seawater samples were stored in the same bottle types and acetonitrile was added to 33% v/v (5:1 seawater/acetonitrile ratio). The results are shown in Figure 6. In the absence of acetonitrile, the recoveries of DOSS were severely reduced from the start of the experiment in all three types of sampling bottles. However, the samples preserved with 33% v/v acetonitrile produced stable DOSS signals up to 25 h. Therefore, dispensing 10.0 mL seawater + 5.0 mL acetonitrile into a 20 mL glass vial (33% acetonitrile) at the moment of sample collection allows for sample storage and transport to the laboratory with minimal losses.

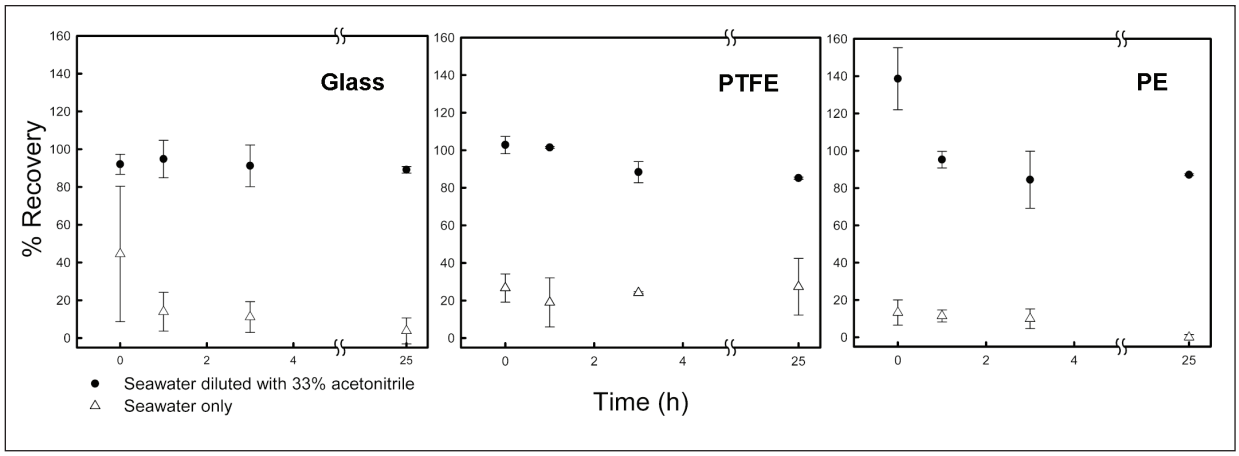


Figure 6. Glass, PTFE, and PE bottles effect on the recovery of 5 µg/L DOSS from seawater samples and acetonitrile-diluted seawater samples

### Method Performance on Seawater Samples

Calibration curves were produced by plotting the peak area ratio (analyte/isotopically labeled surrogate) against the concentration of each analyte, from the injection of seven standard solutions run in triplicates. The concentration ranges in artificial seawater varied from 0.5 to 20 µg/L and 2.5 to 30 µg/L for DOSS and 2-butoxyethanol, respectively. Linearity was observed for both analytes in the range used ($R^2 > 0.995$). Since there was no extraction or clean-up step in the analysis of seawater, the quantitation of DOSS was performed directly from the DOSS/DOSS-$^{13}C_4$ peak area ratio. DDS-$^{2}H_{25}$ was added to match the matrix to that of the calibration curves, as this compound is necessary for quantitation in crude oil. However, the use of the DOSS/DDS-$^{2}H_{25}$ peak area ratio for quantitative purposes in seawater yielded very similar results, suggesting that DDS-$^{2}H_{25}$ could also be used as a suitable surrogate if the isotopically labeled DOSS is unavailable or is prohibitely expensive.

To calculate the method detection limits (MDL) for the target analytes, seven replicates of seawater samples were spiked at concentrations of 4.53 µg/L for DOSS and 23.3 µg/L for 2-butoxyethanol. The MDLs were calculated according to procedures outlined by the EPA.[8] The results are shown in Table 2. Excellent recoveries were obtained from fortified seawater samples, and the method is adaptable to other matrices like crude oil.

The EPA has listed aquatic life benchmarks of 165 µg/L for 2-butoxyethanol and 40 µg/L for DOSS and has suggested reporting limits for environmental analysis of 125 µg/L and 20 µg/L, respectively.[9] The detection limits reported in this work for 2-butoxyethanol (2.36 µg/L) and DOSS (1.34 µg/L) are well below the required reporting limits and are suited for environmental monitoring.

Table 2. Method detection limits and recovery in fortified seawater and light-sweet crude oil from the Wilcox formation in Texas. Water fortification levels were 23.7 and 4.53 µg/L for 2-butoxyethanol and DOSS, respectively. For the Wilcox formation crude oil, fortification levels were 16.8 and 2.45 mg/kg.

| Matrix Type | Unit | 2-Butoxyethanol MDL* | Mean | Average % Recovery | DOSS MDL | Mean | Average % Recovery |
|---|---|---|---|---|---|---|---|
| Seawater | µg/L | 2.36 | 22.4 | 96 ± 3 | 1.34 | 4.44 | 98 ± 9 |
| Crude Oil | mg/kg | 4.46 | 17.5 | 104 ± 8 | 0.723 | 2.26 | 92 ± 9 |

* Method detection limit (MDL = 3.143 x SD), n=7

### Crude Oil Analysis

Calibration curves for the crude oil analysis were produced by plotting the peak-area ratio (analyte/DDS-$^{2}H_{25}$) against the analyte concentration in the injected solution and then calculating the concentration in the original weight of crude oil used. To correct for the extraction step, the average relative response factor (RRF) of each isotopically labeled surrogate was used.

Method detection limits for the crude oil analysis are shown in Table 2. Excellent recoveries were obtained for both analytes, suggesting that the single-step extraction procedure with acetonitrile is enough to quantify both tracers in the crude oil matrix. As expected, none of the analytes were detected in the sweet-light crude oil from the Wilcox formation. The oil that originated at the MC-252 riser contained 4.0 ± 0.2 mg/kg of DOSS. However, 2-butoxyethanol was not detected in the MC-252 oil sample.

## Conclusion

The method provides a simple yet robust tool for the quantification of two key indicator components of commercial Corexit formulations in seawater and crude oil. It could be used to monitor the fate and transport of dispersant in the months following an unintended oil release. This direct-injection LC-MS/MS method with simultaneous detection of both tracer compounds in two different matrices could be quickly adopted by many laboratories with LC-MS/MS capabilities.

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