

Determination of Dioctylsulfosuccinate (DOSS) in Oysters Using Single Quadrupole Mass Spectrometry

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Overview

A simplified method for extraction of sodium dioctylsulfosuccinate (DOSS) from oysters is demonstrated in this work. Previous methods employed extraction protocols based on QuEChERS methods.¹ Here, a Thermo Scientific Dionex ASE™ 350 Accelerated Solvent Extractor allowed single-step extraction using a mixture of water and acetonitrile to achieve high extraction efficiencies. The extract was filtered and then diluted to a final concentration within the valid calibration range established. Use of standards in deionized water demonstrated linear response across three orders of magnitude and provided the basis for determination of DOSS concentrations in oyster extracts and salt water samples. In addition to linear response, high recoveries and low carryover were demonstrated. The Thermo Scientific MSQ Plus™ Mass Detector (single quadrupole) provided the sensitivity and selectivity when coupled with HPLC chromatography. The complete extraction and analysis proved to be rugged, and it was possible to identify the target analyte even at very low concentrations and in complex biological matrices.

Introduction

During the Deepwater Horizon disaster, over 1.8 million gallons of dispersants were used in the Gulf of Mexico.² The primary dispersant used was COREXIT® 9500 (Nalco Co., Naperville, IL) along with small amounts of COREXIT 9527. Both these proprietary mixtures contain DOSS as the major component.³ DOSS has low volatility and a potential to persist in the environment, and there is concern that COREXIT component substances have a potential to bioconcentrate.⁴ Analysis for DOSS serves as a useful marker to assess the presence and persistence of these dispersants. This study was undertaken to identify a rapid extraction technique and subsequent analysis that would allow for the identification and quantitation of DOSS.

Methods

Instrumentation

Extraction

Dionex ASE 350

Chromatography

Thermo Scientific Dionex UltiMate™ 3000 LC

LPG-3000 Series Low-Gradient Pump

FLM-3100 Flow Manager

WPS-3000 Autosampler

MSQ Plus Mass Detector (single quadrupole)

AXP-MS Auxiliary Pump

Thermo Scientific Dionex Chromeleon™ 6.8 SR10 Chromatography Data System software

Extraction Conditions

The oyster emulsion (prepared as described in Chemicals and Reagents section) was maintained at 4 °C until just prior to extraction. To prepare the extraction vessels, a clean glass fiber filter (P/N: 068092) was placed on the bottom of the 10 mL stainless steel extraction vessel and the bottom cap was screwed in place. The extraction vessel was then loaded with 1g of diatomaceous earth (ASE prep DE-CAS 68855-54-9, P/N: 062819) that was ground with a mortar and pestle. A 1 mL aliquot of oyster emulsion was loaded on top of the DE. The remaining space in the extraction vessel was filled with DE, a second glass fiber filter was placed on the top of the vessel, and the top cap was screwed in place. For spiked samples, an aliquot of DOSS was added directly to the oyster emulsion prior to filling the cell with DE.

The extraction vessels were loaded in the top carousel of the ASE 350 instrument, and 60 mL glass collection vials were weighed then placed in the bottom carousel. The extraction mode used was standard, with an oven temperature of 100 °C, extraction pressure of 1000 psi, and a static cycle time of 5 minutes for a single cycle followed by a rinse volume of 60% and a gas purge of the vessel for 90 seconds. The extraction solvent used was 1:1 acetonitrile/water. Between samples, a 5 mL rinse of solvent was used to minimize carry-over.

Following extraction, the collection vessels were weighed and the difference from tared weight noted for use in calculating the concentration factor for each sample analyzed.

Chromatographic Conditions

HPLC System

Column: Thermo Scientific Acclaim™ 120 C18 column
(3 μm, 120 Å, 2.1 mm × 150 mm)

Mobile Phase: Solvent A = Water + 0.1% Formic Acid
Solvent B = Acetonitrile + 0.1% Formic Acid

Gradient	Time (min)	% B
	0	45
	3	45
	6	95
	9	95
	9.01	45
	12	45

Flow Rate: 0.45 mL/min

Inj. Volume: 25 μL (full loop)

Temperature: 40 °C

Mass Spectrometer Parameters for DOSS:

ESI, negative ion

SIM scan, 421.1 *m/z* with 0.7 *m/z* span, dwell time: 0.8 second

N₂ pressure: 80 psi

Probe Temperature: 400 °C

Needle Voltage: 2 kV

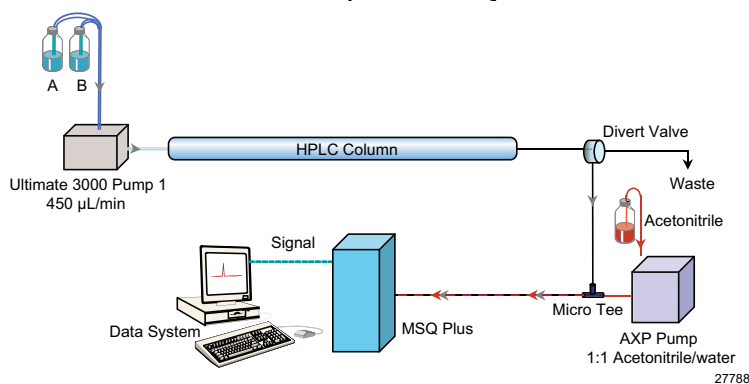
Cone Voltage: 70 V

A divert valve was plumbed after the column to allow the eluent and other background compounds to be directed away from the mass spectrometer before and after the elution of the analyte of interest. A flow rate of 0.1 mL/min of 1:1 water and acetonitrile was added using a low-volume mixing tee prior to the mass spectrometer. This provided a steady stream of fluid to the mass spectrometer during eluent diversion to ensure the needle and spray interface did not dry out.

Divert Valve:

Time (min)	Position
0	To Waste
3.0	To Mass Spectrometer
6.5	To Waste

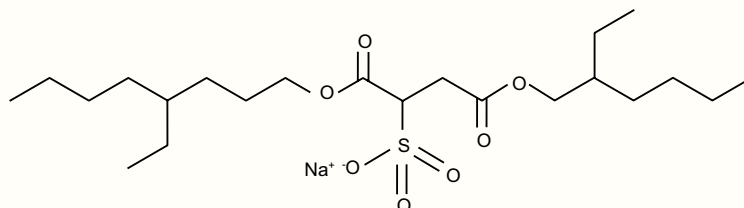
FIGURE 1. Schematic of the HPLC-mass spectrometer system used here.



Chemicals and Reagents:

DOSS was purchased from Sigma-Aldrich (CAS 577-11-7, Aldrich: 323586). The chemical structure is shown below.

FIGURE 2. Chemical structure for DOSS.



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Acetonitrile was obtained from Burdick & Jackson (HPLC grade, AH015-4). Deionized (DI) water was produced by a Millipore water station with 18.2 MΩ.cm resistance. Formic acid was obtained from Fluka (CAS 64-18-6, Fluka: 06440). Commercially available synthetic sea salt (Instant Ocean Sea Salt, Spectrum Brands, Inc.) was prepared following manufacturer's recommendations. A solution of approximately 3.5% salinity of synthetic sea water (SW) was prepared by dissolving 30 g aquarium salt into 1 L DI water.

A primary stock solution of DOSS was prepared at 5000 ug/ml (ppm) in DI water. Working stock solutions were prepared by diluting the primary stock solutions with DI water individually into 50 ppm and 500 ppb concentrations. These were used to subsequently prepare calibration standards.

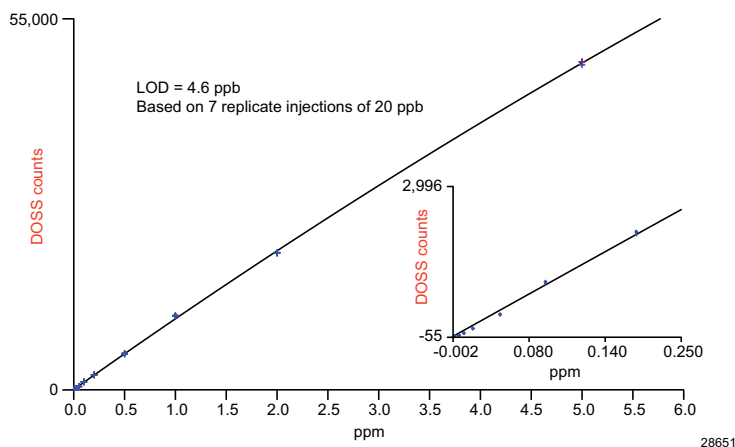
Oysters were obtained from a local market (Fresh Pacific Oysters, Goose Point Oysters brand, Willapa Bay, Washington). A food blender (Oster® 10-speed) was run at high setting for 1 min using one 8 oz package of oysters and their liquor to create a homogeneous emulsion. The emulsion was stored at 4 °C and the bottle was shaken to resuspend the emulsion before sampling. Syringe filters (IC tAcrodisc® 25 mm syringe filter with 0.2 μm Supor [PES] membrane, PALL Scientific P/N 4583T) were used to remove particulate material from the postextraction fluid.

Results

Calibration

DOSS standards were prepared in DI water at ten concentrations: 5, 10, 20, 50, 100, 200, and 500 ppb and 1, 2, and 5 ppm. Full loop injections of 25 μL yielded a total amount of 125 pg to 125 ng loaded on column (Figure 3). Samples were prepared in synthetic SW and spiked at 50, 100, 200, 500 and 1000 ppb. These samples were diluted 10x with acidified acetonitrile (CH₃CN + 0.1% formic acid) to improve linearity of recovery.

FIGURE 3. Calibration for DOSS in DI water.



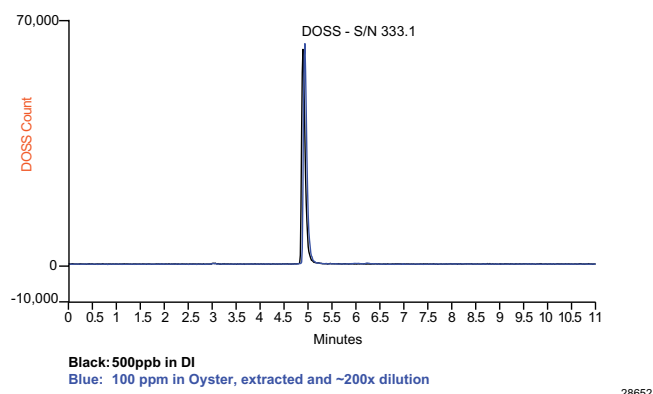
Oyster Samples

Oyster emulsion was spiked with DOSS at 2.5, 10, 25, 100, and 250 ppm levels and also analyzed as unspiked blanks. Three samples at each level were prepared and extracted. Collection vials were weighed before and after the extraction to calculate the dilution ratio. All extractions were filtered using a syringe filter and a 0.1 mL aliquot diluted to 1 mL with 1:1 acetonitrile and water. This prepared sample was then placed in the thermostatted autosampler at 5 °C to await analysis.

Chromatography

As shown in Figure 4, DOSS was well retained on the C18 column. It was necessary to use acidified solvents to keep the analyte in its nonionized neutral form during separation. The low organic solvent concentration (45%) at the beginning of the run allowed the inorganic salts to be washed off the column and diverted away from the MS. A gradient of increasing organic concentration followed that reduced the total elution time necessary to recover the analyte, and ended at a high organic concentration (95%) to wash any strongly retained compounds off the column. This improved method ruggedness when using SW samples with high inorganic content, or oyster extractions which are high in both inorganic and organic content.

FIGURE 4. Chromatogram comparison



Mass Spectrometry (MS)

The aim of the study was to develop a selective, sensitive method for direct analysis of DOSS in oysters and SW samples. The mass spectrometer provides inherent selectivity based on mass-to-charge (m/z) ratio and a selected ion monitoring (SIM) was chosen for sensitivity. The ionization parameters for the analyte were optimized starting with varying cone voltage, followed by needle voltage, and finally probe temperature. Optimal parameters are recorded in the chromatographic conditions section. The scan dwell time was optimized to give good peak shape given the chromatographic width of the peak. Longer dwell times result in greater signal accumulation but reduce the number of points across the chromatographic peak. Thus, narrow chromatographic peaks require shorter dwell times to maintain good peak shape. It should be noted that optimal parameters for MS analyses are instrument- and compound-dependent. Therefore, analysts wishing to repeat these experiments are advised to evaluate the parameters described above to determine optimal values for different systems.

Method Performance

Calibration over three orders of magnitude (5 ppb to 5 ppm) with triplicate injections at each level showed excellent linear response with a correlation coefficient of 99.96%. A method detection limit (MDL) was estimated using the standard deviation obtained from seven replicate injections of a 20 ppb standard, then calculated using the following equation:

$$MDL = s_{(n-1)} \times t_{(99\%)}$$

where s is the standard deviation and t is the student's t at 99% confidence interval. The calculated MDL was 4.6 ppb. Carryover was evaluated by analyzing DI water after the analysis of a 100 ppb standard. The resulting average of three sets of analyses gave an average value below the MDL. Accuracy was calculated as observed amount/specified amount \times 100%.

Table 1 summarizes the recovery at various concentrations in SW, corrected for a ten-fold dilution with acidified acetonitrile. This demonstrated that DOSS can be measured in both clean water and SW.

Actual (ppb)	Measured	Recovery (%)
50	67	134
100	100	100
200	232	116
500	464	92.8
1000	1010	101

The performance of the extraction efficiency was evaluated over two orders of magnitude. The extract process resulted in a ~20-fold dilution. The extract was diluted ten-fold to bring the analyte concentration within the range of calibration for the system. Table 2 shows the recovered concentrations. The measured concentrations are for the diluted extract, while the final calculation was made by multiplying the measured concentration by the extraction volume and the factor of 10x dilution. Thus, final concentration = measured concentration \times extraction volume \times 10. The highest measured concentration at 250 ppm represents half the current U.S. FDA limit for DOSS in oysters.⁵ The data show that concentrations 100 times below the current limit are easily detected. Should future requirements specify lower detection limits than currently shown, the method can be adjusted by reducing or eliminating the extract dilution step and still achieve an additional order of magnitude in sensitivity.

Actual (ppm)	Measured* (ppb)	Final Conc. Calculated (ppm)	% Recovery	Std. Dev.	RSD
0	0	0	†	†	†
2.5	12.8	2.6	104.4	0.4	15
10	61.3	12.6	125.9	0.5	4
25	123.4	25.4	101.8	6.6	26
100	440.8	91.1	91.1	15.1	17
250	1329.6	243.6	97.4	25.4	10

* Three extractions at each level, ~200x dilution from original concentration

† No peak observed

DOSS stability was evaluated to determine if degradation occurred during sample storage. Oyster emulsion was spiked with 50 ppm DOSS (Table 3). The first group of samples was immediately extracted, filtered, and diluted. The extract was separated into three aliquots: one aliquot was immediately analyzed; the second was stored at room temperature for three days; and the third was stored in a refrigerator for three days. At the same time the first oyster emulsion was prepared, a second and third batch were prepared and loaded into extraction vessels. In this case extraction was delayed for three days, with half the vessels stored at room temperature and half under refrigeration. After this time, the contents of the vessels were extracted and prepared using the same extraction method. The extracts were analyzed the same day they were extracted, along with the extracts from day one. The results suggest that the worst case scenario is allowing the DOSS and oyster emulsion to remain at room temperature, as this showed the largest loss in recovery. Immediate extraction or maintaining the oyster emulsion at cold temperatures produced higher recoveries. It is therefore recommended to keep samples cold and minimize time to analysis.

Extract Day 1	% Recovery
Measured on Day 1	100.6
Maintained cold, measured on Day 4	83.2
Room temperature, measured on Day 4	89.8
Extract Day 4	
Maintained cold until extracted	83
Maintained at room temperature until extracted	63.4

Conclusion

A robust method for analysis of DOSS has been demonstrated here. Extraction from an oyster matrix was accomplished using the ASE 350 system, allowing for quick, simple, single-step extraction. The Ultimate 3000 HPLC system with the Acclaim C18 column provided chromatographic separation. The MSQ Plus Mass Detector provided molecular ion analyte selectivity, and the use of the SIM function provided good low-level quantitation. The combination of efficient extraction and sensitive detection resulted in a method that easily exceeds the limits of detection set by the U.S. FDA for allowable limits of DOSS in oysters.

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