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DETERMINATION OF DIOCTYLSULFOSUCCINATE IN SELECT SEAFOODS USING A QuEChERS EXTRACTION WITH LIQUID CHROMATOGRAPHY- TRIPLE QUADRUPOLE MASS SPECTROMETRY

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Abstract

A rapid extraction procedure based upon QuEChERS (Quick Easy Cheap Effective Rugged Safe) methodology combined with analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS) has been developed to test for the presence of the surfactant, sodium dioctylsulfosuccinate in seafood. This procedure draws upon and complements an earlier protocol that was designed to screen for polycyclic aromatic hydrocarbons (PAHs) in seafood. Generally, a 5-g portion of homogenized seafood was fortified with a deuterated standard of DOSS and mixed with 5 mL of water and 15 mL of acetonitrile. Then, magnesium sulfate (6 g) and sodium acetate (1.5 g) were added and the mixture was shaken vigorously. The preparation was centrifuged at 3000 x g and a portion of the acetonitrile layer was filtered through a 0.2 micron filter. After further dilution with acetonitrile, the extract was analyzed by LC-MS/MS. The method was validated in four matrices that were fortified with DOSS at levels ranging between 0.1 μg/g and 1.0 μg/g. Performance characteristics were determined for the method including linearity, precision, and an estimate of the detection limit. Additionally, samples of finfish, oysters and crab that were exposed to Corexit 9500 were analyzed to demonstrate the extraction of incurred residues of DOSS. The methodology was developed and validated simultaneously at the U.S. Food and Drug Administration's Forensic Chemistry Center (FDA/FCC) and the National Oceanic and Atmospheric Administration's Northwest Fisheries Science Center (NOAA/NWFSC).

Date: 10/27/2010

Introduction

The Deepwater Horizon disaster released in excess of 4.9 million barrels of oil into the Gulf of Mexico (1). Efforts to manage this oil spill included collection, burning and the use of chemical dispersants. Dispersants break up the oil into small droplets within a shell of a surface active agent (2). These droplets diffuse throughout the total volume of water. This process reduces the local concentration of the oil and, thereby, its imminent toxicity and purportedly enhances the access of microorganisms that are capable of degrading the oil. One of the principal dispersants used was Corexit 9500 while small amounts of Corexit 9527 from existing stockpiles may have been used (3). A significant component of Corexit 9500 and Corexit 9527 is the surface active agent, sodium dioctylsulfosuccinate (DOSS, CAS 577-11-7) (3) which is also known as sodium bis(2-ethylhexyl)sulfosuccinate. The method described in this paper was developed to detect the presence of DOSS in seafood. Due to its low volatility and potential to persist longer in the environment than the other ingredients of Corexit, DOSS is considered the most appropriate marker for possible exposure to Corexit 9500 and 9527. To keep things in perspective, it is important to note that the FDA has no objection to the use of DOSS as a food additive in beverages as a wetting agent or solubilizer for flavor emulsion stabilizers at levels up to 10 ppm (GRAS Notice, GRN No. 000006) (4). DOSS is also utilized in the formulation of some pharmaceuticals and has therapeutic value as a stool softener (5) which is available over-the-counter in dosages as high as 250 mg/capsule (6).

A rapid method to determine polycyclic aromatic hydrocarbons (PAHs) in seafood had previously been developed at the FDA/FCC laboratory (7) and elsewhere (8, 9). The PAH method employed an extraction protocol based upon QuEChERS methodology (10, 11) with subsequent analysis by high performance liquid chromatography with fluorescence detection (HPLC-FLD). The extraction generally proceeded in two steps with an initial partitioning of PAHs into acetonitrile driven by the addition of sodium acetate for pH control and magnesium sulfate for the entrainment of water. There was provision for an additional clean-up of the acetonitrile extract if necessary by the addition of a strong anion exchange resin and a C-18 solid phase extraction medium. The PAH method was found to produce satisfactory results for fortified samples of seafood and for a standard reference material (National Institute of Standards and Technology SRM 1974b) with only the initial extraction into acetonitrile followed by filtration through a 0.2 μm PTFE filter (7).

This method for determining DOSS utilized the same one step extraction into acetonitrile which was followed by filtration and analysis using liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS). For logistical reasons, some early work at the FDA was conducted using an ion trap mass spectrometer, but substantial improvements in sensitivity and precision were realized by migrating to a triple quadrupole instrument. A deuterated internal standard (sodium bis(2-ethylhexyl-d_{17})sulfosuccinate, d-$_{34}$ DOSS) was introduced to compensate for potential matrix effects and/or loss of the analyte during the extraction. In addition, the NOAA/NWFSC laboratory also introduced a deuterated standard of sodium

dodecylsulfate (d_{25}-SDS) after the extraction procedure so that the recovery of d_{34}-DOSS could be monitored with every sample. The procedure has been applied to a variety of edible seafood which included: oysters, shrimp, finfish and crab. All results below are reported in terms of the sodium salt of dioctylsulfosuccinate which is referred to as DOSS. The instrumentation is blind to the counterion and only monitors the dioctylsulfosuccinate anion and its fragments.

This methodology was developed and validated simultaneously at FDA/FCC and NOAA/NWFSC.

Experimental

Equipment FDA/FCC

- Model 1200 liquid chromatograph with binary pump, microdegasser, autosampler and thermostatted column compartment (Agilent Technologies).
- API 5500 LC/MS/MS mass spectrometer running under Analyst 1.5 (Applied Biosystems/MD Sciex)
- ACE 3 C18 column, 50 mm X 3.0 mm id X 3 μm d_p (Advanced Chromatography Technologies p/n ACE-111-0503)
- Robot Coupe processor with stainless steel bowl (Robot Coupe p/n R301UB)
- Magic Bullet® Blender (used for tissue portions < 125 g, Homeland Houswares, LLC)

Equipment NOAA/NWFSC

- Aquity ultra performance liquid chromatograph (Waters)
- Quattro Micro API triple quadrupole mass spectrometer system (Micromass, Waters)
- Zorbax RRHD SB-C18 column, 50 mm X 2.1 mm id X 1.8 μm d_p (Agilent Technologies).
- Handheld mono blender (Williams & Sonoma, p/n 1555317)
- Vortex Mixer (Fisher Scientific, p/n 128145Q)

Supplies

- Centrifuge capable of 3000 x g for 50 mL centrifuge tubes
- Buffered QuEChERS extraction tubes with foil packet containing 6 g of magnesium sulfate and 1.5 g of sodium acetate (AOAC Method 2007.01, for use with 15 g samples, Agilent Technologies p/n 5982-5755)
- Ceramic homogenizers for 50 mL tubes, (Agilent Technologies p/n 5982-9313)
- Syringes, without needles, nonsterile, BD Luer-Lok Tip, 5 mL capacity (Fisher Scientific p/n 14-823-16D)
- PTFE syringe filters, 0.20 μm pore size, 25 mm dia., (Fisher Scientific p/n SLFG 025NK)
- 4 mL amber glass vials with PTFE lined caps, (Fisher Scientific p/n B7800-2A)

Date: 10/27/2010

Reagents and Standards

- Acetonitrile, HPLC grade / LC-MS grade (Fisher, p/n A998 / A995-4 or equivalent)
- Water, 18.2 MΩ water from a Millipore Milli-Q Gradient A-10 water source (or equivalent) or LC/MS Grade (J.T. Baker, p/n 9831-03) or equivalent.
- Formic Acid, 99.5% LC-MS grade (Fisher, p/n A117-10X1AMP)
- Water with 0.1% Formic Acid, HPLC grade (Fisher, p/n HB523-4)
- Acetonitrile with 0.1% Formic Acid, HPLC grade (Fisher, p/n 9823-4)
- Docusate sodium [viz. DOSS] (Sigma-Aldrich, p/n D1685).
- Sodium bis(2-ethylhexyl-d_{17})sulfosuccinate (Sigma-Aldrich/Isotec, p/n 710652/SPEC)
- Sodium d_{25}-dodecyl sulfate (Sigma-Aldrich, p/n 451851)

Preparation of Standards

FDA/FCC

Stock Solutions

A stock solution of DOSS (viz. Docusate sodium) was prepared at a concentration of 1500 µg/mL in acetonitrile with the addition of a small amount of water (about 5% of the total volume) to aid in dissolution. Lower concentrations were prepared by dilution with acetonitrile.

A stock solution of the internal standard, d_{34}-DOSS, was prepared at a concentration of 600 µg/mL in acetonitrile. A small amount of water (about 5% of the total final volume) was added to aid in dissolution.

Working Standards

Calibration standards were prepared at 0, 1, 2, 5, 10, 25 and 50 ng/mL of DOSS in acetonitrile. Deuterated internal standard (d_{34}-DOSS) was incorporated into each working standard at a concentration of 20 ng/mL.

Standard Solution for Fortification Experiments

A solution for fortification experiments was prepared at a level of 10 µg/mL, so that an aliquot of 50 µL would correspond to 0.5 µg of DOSS. When a 50 µL aliquot was added to a 5 g sample of seafood, the nominal concentration was 0.1 µg/g. When taken through the method (assuming 100% recovery) the concentration of DOSS in the final extract was 0.5 µg / 15 mL X 1/5 (dilution factor, see below) X 1000 (ng/µg) = 6.7 ng/mL. When fortification was conducted at a higher level, 250 µL of the fortification solution was added for a final concentration of 5 X 6.7 = 34 ng/mL.

Internal Standard

The stock solution of d_{34}-DOSS (600 µg/mL) was diluted (1 + 19) (v/v) with acetonitrile for an intermediate stock of 30 µg/mL. For the analysis of samples, a 50 µL aliquot of the intermediate stock solution of the internal standard was added at the beginning of the extraction procedure. After extraction and a (1+4) (v/v) dilution with acetonitrile, the

nominal final concentration of the internal standard in the solution which was presented to the LC-MS/MS system was: 50 µL X 30 ng/µL X 1/15 mL X 1/5 (dilution) = 20 ng/mL.

NOAA/NWFSC

Stock Solutions

Stock solutions of DOSS, d_{34}-DOSS and d_{25}-SDS were prepared at concentrations of 550, 510 and 500 µg/mL in methanol. A 10-µL volume of these deuterated solutions was used to spike all samples analyzed. The DOSS stock solution was also used for the spiked matrix validation. Further dilutions of these stock solutions were prepared in acetonitrile.

Working Standards

Calibration standards were prepared at 1, 5, 25, 100, 500, 2500 and 10,000 ng/mL of DOSS in acetonitrile. Deuterated d_{34}-DOSS and d_{25}-SDS were added in each calibration level at a fixed concentration of 100 ng/mL. The LC-MS/MS quality control standard (LC-QC), used for checking the stability and continuous calibration of the LC-MS/MS, contained DOSS, d_{34}-DOSS and d_{25}-SDS all at concentrations of 50ng/mL in acetonitrile.

Standard Solution for Fortification Experiments

To spike approximately 5 g of different tissues at a final concentration of 0.1, 0.25 and 1 ppm (µg of DOSS per gram of tissue), respectively, the following DOSS solutions were used (volume added): 550 µg/mL (10 µL), 55 µg/mL (22.7 µL) and 55 µg/mL (10 µL).

Samples

Seafood samples (finfish, shrimp, crabs, and oysters) used in the fortification experiments in this study had been previously composited and stored frozen.

In addition, samples of finfish, crabs and oysters were exposed to Corexit 9500 at a concentration of 100 mg/L in tanks which contained seawater. This relatively high concentration was used to maximize the likelihood that incurred residues would be present. Homogenates of each species were split and half was sent to the FDA/FCC laboratory while the other half was sent to NOAA/NWFSC. These samples were prepared at FDA's Gulf Coast Seafood Laboratory (GCSL).

Sample Composite Preparation

Samples were composited and homogenized by blending in a food processor or blender for 2 to 3 minutes. Seafood samples were received and stored frozen, but were partially thawed prior to homogenization.

Date: 10/27/2010

Extraction Procedure

Finfish, Shrimp and Crab

For analysis of finfish, shrimp and crab, approximately 5 grams of homogenized sample composite and a ceramic homogenizer were placed in a QuEChERS extraction tube. Then an aliquot of the deuterated internal standard (d_{34}-DOSS) was added (FDA/FCC: 50-µL aliquot of a 30 µg/mL solution; NOAA/NWFSC: 10-µL of a 510 µg/mL solution). Five grams of water was added and the tube was vigorously shaken for 1 minute. After the addition of 15 mL of acetonitrile the tube was again shaken for 1 minute. Six grams of magnesium sulfate and 1.5 g of sodium acetate (viz. the contents of a QuEChERS extraction packet) were added, the tube was again shaken for 1 minute after which the tube was centrifuged at 3000 x g for 10 minutes. Finally, a 4-mL portion of the upper (acetonitrile) layer was filtered through a 0.2 µm PTFE cartridge filter to complete the sample preparation. After the extraction step and right before the centrifuge, the NOAA/NWFSC laboratory spiked the samples with 10µL of a 500 ng/µL solution of d_{25}-SDS. This deuterated standard was later used to calculate the recovery of the extraction process

Oysters

Oysters had been previously found to contain sufficient water in them so that the addition of 5 g of RODI water at the beginning of the preparation was unnecessary for the extraction. That step was eliminated at FDA/FCC but the rest of the extraction proceeded as described above. NOAA/NWFSC treated the oysters like the other samples.

Fortification of Samples to Demonstrate Method Efficiency

At the FDA/FCC laboratory, a 5 g portion of homogenized composite was fortified with 50 µL of the fortification solution that contained DOSS at 10 µg/mL. This provided a fortification level of 0.1 µg/g. Alternatively, 250 µL of the 10 µg/mL solution was added to provide a high level spike at 0.5 µg/g.

At the NOAA/NWFSC laboratory, three levels of fortification of 5-g portions were used: 0.1 µg/g, 0.25 µg/g or 1.0 µg/g.

Method Blanks

Method blanks were prepared by substituting 5 g of water in place of sample composite and performing the extraction procedure as described above.

Liquid Chromatography with Mass Spectral Analysis

Samples, standards and blanks were analyzed using the FDA/FCC and NOAA/NWFSC LC-MS/MS systems described above. There were modest differences between the two protocols.

FDA/FCC

Prior to analysis, the prepared extract was diluted (1+4) (v/v) with acetonitrile. When large levels of DOSS were encountered and additional dilution was required, the additional dilution was made using acetonitrile that contained the internal standard at a level that matched that of the diluted prepared extract (viz. 20 ng/mL).

At the FDA/FCC laboratory, a 5 µL portion of the diluted extract was injected onto an ACE 3 C18 column (50 mm X 3.0 mm id X 3 µm d_f) that was operated at 40°C with a flow rate of 0.50 mL/min. The gradient was composed of a mixture of acetonitrile and water, each of which contain 0.1% (v/v) formic acid. See Table 1.

Table 1. FDA/FCC Gradient Program

Time (minutes)	Acetonitrile (% by volume)
0	45
2.0	45
5.0	95
8.0	95
8.01	45
11.0	45

Under these conditions, DOSS and the deuterated internal standard both eluted at about 4.4 minutes with DOSS slightly later than d_{34}-DOSS.

Prior to analysis, the mass spectrometer was tuned in negative ion mode using infusions of DOSS and d_{34}-DOSS solutions, each at 3 µg/mL in 50% aqueous acetonitrile that contained 0.1% (v/v) formic acid. The operating conditions described in Table 2 were selected.

Table 2. FDA/FCC Operating Parameters

Operating Parameter	Setting
Drying Gas Temperature	600°C
Gas 1 Flow Rate	50 units
Gas 2 Flow Rate	50 units
Ion Spray Voltage	- 3.5 kV
CAD Gas Flow Rate	7 units
Curtain Gas Flow Rate	35 units

The mass spectrometer was configured to monitor the transitions as described in Table 3 and Table 4, below.

Table 3. FDA/FCC Mass Fragmentation Parameters for DOSS

Precursor Ion (m/z)	Product Ion (m/z)	Dwell Time (ms)	Collision Energy (V)
421.2	80.9	100	-18
421.2	227.2	100	-30
421.2	291.2	100	-30

Table 4 FDA/FCC Mass Fragmentation Parameters for d34-DOSS (Internal Standard)

Precursor Ion (m/z)	Product Ion (m/z)	Dwell Time (ms)	Collision Energy (V)
455.3	80.9	100	-24

NOAA/NWFSC
A small portion of the filtered extract is diluted (1+14) (v/v) with acetonitrile prior to analysis by LC-MS/MS.

At the NOAA/NWFSC laboratory, a 10 µL portion of the diluted extract was injected onto a Zorbax RRHD SB-C18 column, 50 mm X 2.1 mm id X 1.8 µm d_p that was operated at 45^{o}C with a flow rate of 0.30 mL/min. The gradient was composed of a mixture of acetonitrile and water, each of which contained 0.1% (v/v) formic acid. See Table 5.

Table 5. NOAA/NWFSC Gradient Program

Time (minutes)	Acetonitrile (% by volume)
0	40
7.0	100
10.0	100
11.0	40
15.0	40

Under these conditions, d_{-25} SDS eluted at 3.46 minutes while d_{34}-DOSS eluted at 4.30 minutes and DOSS eluted at 4.40 minutes.

Prior to analysis, the mass spectrometer was tuned in negative ion mode using infusions of DOSS, d_{34}-DOSS and d_{25}-SDS, each at about 5 µg/mL. The standards were introduced in

mobile phase at a composition that was similar to that at which the component would elute during analysis. The operating conditions described in Table 6 were selected.

Table 6. NOAA/NWFSC Operating Parameters

Operating Parameter	Setting
Desolvation Temperature	350°C
Desolvation Gas Flow Rate	600 L/hr.
Cone Gas Flow	OFF
Capillary Voltage	- 3.0 kV
Gas Cell Pirani Pressure	3.60 X 10^{-3} mbar
Source Temperature	125°C

The mass spectrometer was configured to monitor the transitions as described in Table 7, Table 8 and Table 9, below.

Table 7. NWFSC Mass Fragmentation Parameters for DOSS

Precursor Ion (m/z)	Product Ion (m/z)	Dwell Time (ms)	Cone Voltage (V)	Collision Energy (V)
421.04	80.72	100	40	-35

Table 8. NOAA/NWFSC Mass Fragmentation Parameters for d_{34}-DOSS

Precursor Ion (m/z)	Product Ion (m/z)	Dwell Time (ms)	Cone Voltage (V)	Collision Energy (V)
455.15	80.82	100	40	-35

Table 9. NOAA/NWFSC Mass Fragmentation Parameters for d_{25}-SDS

Precursor Ion (m/z)	Product Ion (m/z)	Dwell Time (ms)	Cone Voltage (V)	Collision Energy (V)
290.37	97.80	160	45	-30

Date: 10/27/2010

Data Analysis
For quantitative purposes, the integrated signal from the transition, 421.2 to 80.9, which is associated with DOSS was divided by the integrated signal from the transition, 455.3 to 80.9, which is traceable to d_{34}-DOSS. This ratio was plotted against the ratio of the standard concentration to that of the internal standard concentration to generate the calibration curve.

A calibration curve was fitted to the data using a linear regression of the [Area Ratio] and the [Concentration Ratio] using a weighting of "1/x" (FDA/FCC) or a "fit weighting null" (NOAA/NWFSC).

Samples were placed on the curve as the [Area Ratio] and the corresponding [Concentration Ratio] was converted to the concentration of DOSS by multiplying by the concentration of the internal standard, d_{34}-DOSS.

The concentration of DOSS from the calibration curve was converted into the amount of DOSS in the samples as follows:

FDA/FCC:
Amount DOSS (as sodium salt) = DOSS (curve, ng/mL) X 5 (dilution factor) X 15 (mL)

NOAA/NWFSC:
The process was similar to that described above but takes place within the Aquity software package. The amount of d_{34}-DOSS that was added to the sample was provided by the operator and the software returned the amount of DOSS in the sample. In addition, the recovery of d_{34}-DOSS through the extraction was determined based upon the response for d_{34}-DOSS in relation to that observed for d_{25}-SDS. This assessment of the recovery of d_{34}-DOSS through the extraction was provided for each individual sample.

The amount of DOSS in the sample was converted into a concentration in the seafood by dividing by the sample weight.

The percent recovery from fortified samples is given by:

$$\frac{[\text{Determined DOSS (ng)} - \text{Incurred DOSS (ng/g) X Sample Wt. (g)}]}{[\text{Amount of DOSS Added to Sample (ng)}]} \times 100$$

where the incurred DOSS is determined through the analysis of the sample prior to fortification.

Quality Assurance Plan and Strategy for Screening Samples for DOSS
The following discussion presents examples of protocols for screening samples for the presence of DOSS using the methodology described above.

Date: 10/27/2010

The parameters described below emulate those which were provided in the method for PAHs that was provided earlier (7).

Prior to beginning the analysis of samples, a method limit of detection (LOD) is determined. To this end, a calibration curve is established over the working range (*eg.* 1 ng/mL to 50 ng/mL) although this will vary from instrument to instrument. Then, at least 5 replicates of a seafood which has been fortified with DOSS at a level sufficient to show a relative standard deviation of about 10% are taken through the procedure. The standard deviation of the ratio between the area of the transition that is traceable to DOSS (421.2 to 80.9) to that traceable to d_{34}-DOSS (internal standard) (455.3 to 80.9) is defined. This standard deviation is tripled and placed on the calibration curve and converted into a concentration of DOSS to define the method LOD. A method limit of quantitation (LOQ) can be derived in a similar manner with the standard deviation being multiplied by 10 rather than 3.

This protocol is configured for the analysis of up to 30 samples per batch (although 20 is preferred). A calibration curve is analyzed at the beginning and at the end of each batch. A check standard is evaluated every 8-10 samples. One method blank is examined to ensure that there are no obvious contamination issues with the reagents/supplies. One example of seafood that is known to be uncontaminated is fortified at a level of 0.10 μg/g and taken through the method to show recoverability. Alternatively, a sample with a known incurred residue of DOSS may be analyzed with each batch (a positive control).

QC Elements and Acceptance Criteria

- A minimum of three calibration standards are analyzed across the working range of the method to demonstrate linearity with $r^2 \geq 0.99$.

- Calibration standards are analyzed at the beginning and end of each batch of 20 or fewer samples. A check standard (in the middle of the working range) can be analyzed periodically to confirm that the curve is stable. For successive runs of the same standard, the ratio of the Area of DOSS to that of d_{34}-DOSS must be within +/- 20% of the prior value. If this is not the case, then the calibration curve should be re-run and the affected samples should be re-analyzed.

- A minimum of one fortified sample should be analyzed with each batch of samples. The recovery of a spike of 0.10 μg/g DOSS must be in the range 75% - 120%. Or, the value for the incurred residue must be within +/- 20% of the average historic value for that sample.

- One method blank made with 5 g water in place of sample matrix must be analyzed with each batch of 20 or fewer samples. Evidence of significant contamination with DOSS would call into question any positive results for DOSS and fresh portions from these would need to be taken through the entire procedure again.

- In the event that DOSS is detected in the sample at levels above the method LOD, then the identity needs to be confirmed by retention time match and an examination of the transitions at (421.2 to 227.2) and (421.2 to 291.2). For each of these, the ratio of the integrated area associated with the confirmatory transition to that of the primary transition (421.2 to 80.9) needs to be within 20% of that of a standard at about the same concentration. For low-level samples, it may be advisable to analyze the extract prior to the five-fold dilution for analysis. There is no need to be concerned about the internal standard in this case since the purpose of the re-analysis is simply to confirm the identity of DOSS.

- Any positive results in a batch of samples, should be analyzed again (for example, on the following day) and the two quantitative results need to agree within 25% or the sample should be analyzed a third time and the average of all three determinations should be reported with an estimate of the standard deviation unless one can be rejected using an outlier test such as the Dixon Test (12)

So, for example, a typical batch of samples might resemble the following:

Calibration Standards
Sample 1
Fortified Sample 1
Acetonitrile Blank (no Internal Standard)
Method Blank
Samples 2 through 6
Check Standard
Samples 7 through 14
Check Standard
Samples 15 through 22
Check Standard
Samples 13 through 30
Calibration Standards

Date: 10/27/2010

Results and Discussion

Calibration of the LC-MS/MS Systems and Estimates of Performance

FDA/FCC

Calibration curves were generated using standards of 0, 1, 2, 5, 10, 25 and 50 ng/mL. The standards were run at the beginning and the end of each batch of samples. For each point on the calibration curve, the integrated signal due to DOSS was divided by that due to d_{34}-DOSS and this ratio was linearly regressed against the ratio of the concentration of the standard divided by that of the internal standard (viz. 20 ng/mL) using a weighting of 1/x. A typical value for the area due to d_{34}-DOSS was 4 X 10^5 counts. Precision in the middle of the working range was determined from 7 replicates of a standard of 10 ng/mL. The solution LOD based upon the standard curve was estimated from the standard deviation of 7 replicates of a standard of 0.5 ng/mL on Day 1 and from 7 replicates of a standard of 0.2 ng/mL on Day 2. The standard deviation of the Area Ratio (DOSS/d_{34}-DOSS) was tripled and placed on the calibration curve to define a Concentration Ratio which was multiplied by the concentration of d_{34}-DOSS to yield the solution LOD. The LOD in seafood was extrapolated from the solution LOD assuming that 5 g of sample was extracted into 15 mL of acetonitrile and diluted 1/5 prior to analysis.

Performance characteristics that are derived from these calibration curves are presented in Table 10.

Table 10. Figures of Merit from FDA/FCC Calibration Curves

	Slope	Intercept	Correlation Coefficient	Precision (% RSD)	Solution LOD (ng/mL)	Seafood LOD (ng/g)
Day 1	0.681	4.4 X 10^{-7}	0.9998	1.4	0.10	1.5
Day 2	0.701	-2.4 X 10^{-7}	0.9998	1.8	0.06	0.9
Day 3	0.677	3.2 X 10^{-7}	0.9999	ND~	ND	ND
Day 4	0.664	1.4 X 10^{-7}	0.9999	ND	ND	ND
Day 5	0.672	4.3 X 10^{-7}	0.9998	ND	ND	ND
Day 6	0.697	5.7 X 10^{-7}	0.9998	ND	ND	ND
Day 7	0.707	4.1 X 10^{-7}	0.9999	ND	ND	ND
Day 8	0.690	6.3 X 10^{-8}	0.9998	ND	ND	ND
Day 9	0.711	4.9 X 10^{-7}	0.9999	ND	ND	ND
Day 10	0.702	-9.1 X 10^{-8}	0.9999	ND	ND	ND
Day 11	0.693	8.9 X 10^{-8}	0.9999	ND	ND	ND
Day 12	0.685	1.6 X 10^{-7}	0.9998	ND	ND	ND

~ ND = not determined

The calibration curves are stable across time and linear across at least 1.5 orders of magnitude. FDA/FCC did not aggressively pursue establishing the upper limit of the linear range. The solution LOD appears to be on the order of 0.1 ng/mL in solution which suggests a LOD in seafood of approximately 2 ng/g.

To estimate the LOD of the method as a whole, 5-g portions of seafood were fortified with DOSS at a level of 5.5 ng/g and taken through the procedure. Seven fortified replicates from each species were analyzed along with two controls. No signal was observed from the controls. The standard deviation of the Area ratio (DOSS/d_{34}-DOSS) was tripled and treated as above to yield the estimate of the method limit of detection (LOD). The method LOQ was estimated in the same manner using a factor of 10 rather than 3. The results of these experiments are presented in Table 11.

Table 11. FDA/FCC Estimated Method LOD and LOQ from Fortified Samples

	Crab	**Finfish**	**Oysters**	**Shrimp**
RSD (%) [@]	13.6	22.2	19.1	12.0
Method LOD (ng/g)	2.2	3.6	2.7	2.0
Method LOQ (ng/g)	7.4	12	9.1	6.7
[@] Percent Relative Standard Deviation of the Area Ratio (DOSS/d-34 DOSS) for 7 replicates of the seafood spiked at 5.5 ng/g				

The estimates of the LOD derived from fortified samples do not differ markedly from those which were obtained from the analysis of low level standards, see Table 10, and, taken together, these suggest a method LOD of 3 ng/g in seafood with a corresponding method LOQ of 10 ng/g in seafood. These experiments were conducted on Days 9, 10 and 11.

NOAA/NWFSC

A calibration curve was determined using seven concentrations of DOSS: 1.0, 5.0, 25, 100, 500, 2500 and 10,000 ng/mL. Each solution was injected twice. The correlation coefficient of the curve was > 0.999. The percent deviation of DOSS concentration predicted by the calibration curve for the calibration curve solutions which were analyzed is presented in Table 12.

Table 12. Percent Deviation for Calibration Solutions of DOSS

Actual Concentration (ng/mL)	Calculated Concentration (ng/mL)	Percent Deviation (%)
1	1.3	30
5	4.6	8
25	22.	12
100	94.	6
500	463.	7
2500	2559.	2
10000	9987	0.1

The LOD and LOQ of the method for 5 g of sample extracted were estimated to be 15 ng/g and 45 ng/g, respectively based upon the lowest standard of the calibration curve. The signal to noise ratio of this standard of 1 ng/mL was 10:1. The extract was diluted 1/15 so the method LOQ in seafood would be 1 ng/mL X 15 (dilution factor) X (15 mL extract) X 1 / 5 (g seafood) = 45 ng/g.

Fortified Samples

To examine recoverability, samples of seafood of each of four types (viz. crab, finfish, oysters and shrimp) were analyzed. No measurable levels of DOSS were observed in any of these samples. DOSS was added to portions of these samples at several different levels from 0.1 μg/g to 1.0 μg/g. Each analysis was conducted on a 5-g portion of the sample (or 5 mL of water). Recoveries are presented in Table 13.

Table 13. Recovery of DOSS from Fortified Samples Conducted at Both Laboratories

Species	Fortification Level (μg/g seafood)	Recovery (%)	
		FDA/FCC	NOAA/NWFSC
Method Blank	0.11 / 0.10 [@]	98.7	86.9
	0.55	94.4	
	1.0		85.1
Crab	0.11 / 0.10	92.2 (4.0) [#]	78.5 (2.6)
	0.25		98.9
	0.55	95.7 (4.8)	
	1.0		112 (18)
Finfish	0.11 / 0.10	95.7 (0.3)	79.0 (11)
	0.25		95.7 (4.4)
	0.55	99.2 (1.3)	
	1.0		97.7 (0.6)
Oysters	0.11 / 0.10	95.6 (2.5)	77.7 (3.7)
	0.25		94.9
	0.55	98.0 (2.3)	
	1.0		107.7 (2.8)
Shrimp	0.11 / 0.10	93.8 (1.5)	79.3 (9.3)
	0.25		81.8 (6.3)
	0.55	92.0 (4.6)	
	1.0		88.6 (7.5)

[@] FCC spiked at 0.11 μg/g while NWFSC spiked at 0.10 μg/g.
[#] The number in parentheses is the range for two determinations.

Incurred Residues

Seafood species were exposed to Corexit 9500 as described in the section, "Samples", above. Representative samples were sent to both the NOAA/NWFSC laboratory and the FDA/FCC laboratory for analysis. The results of these studies will be published elsewhere but a selection of the analytical results for incurred DOSS residues are shown in Table 14. The purpose of presenting these data is to demonstrate the analysis of incurred residues of DOSS by two independent laboratories.

Date: 10/27/2010

Table 14. DOSS Concentration as an Incurred Residue in Selected Samples

Species	DOSS Concentration in Seafood (μg/g)	
	FDA/FCC	**NOAA/NWFSC**
Crab	0.92 0.89 *0.84* [#] *0.87* [#] *0.92* [#]	0.87 0.95
	0.50 0.49	0.55 0.60
	0.14 0.14	0.16 0.22
	0.022 0.024	0.05 < 0.05
Finfish	< LOD	< 0.05
	< LOD	< 0.05
	0.019 0.015	< 0.05 < 0.05
	0.013 0.013	< 0.05 < 0.05
Oysters	19 18	16 18
	12 12	11 13
	0.20 0.20	0.21 0.21

[#] These data were generated using an ion trap instrument using external calibration with no internal standard added.

In conclusion, this extraction protocol (that was shown to be effective for PAHs (7)) has been shown to be quantitative for a component of Corexit 9500, DOSS, in two independent laboratories. Triple quadrupole mass spectrometry provided limits of detection for DOSS to levels as low as a few parts per billion (ng/g) in seafood, although this was dependent upon the specific instrument used. The methodology has been demonstrated to be capable of reliably measuring both spiked as well as incurred residues of DOSS. The extent of agreement between results obtained at two independent laboratories with slight variations in procedure was excellent and demonstrated the robustness of the method.

Date: 10/27/2010

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