AN ABSTRACT OF THE DISSERTATION OF

Benjamin J. Place for the degree of Doctor of Philosophy in Chemistry presented on July 23, 2012.
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The development of analytical methods for emerging contaminants creates many unique challenges for analytical chemists. By their nature, emerging contaminants have inherent data gaps related to their environmental occurrence, fate, and impact. This dissertation is a compilation of three studies related to method development for the structural identification of emerging contaminants, the detection and quantification of chemicals used in unprecedented quantities and applications, and the extraction of compounds from complex matrices where the solvent-solute-matrix interactions are not completely understood. The three studies present analytical methods developed for emerging contaminants in complex matrices, including: fluorochemical surfactants in aqueous film-forming foams, oil dispersant surfactants in seawater, and fullerene nanomaterials in carbonaceous solids.

Aqueous film-forming foams, used in military and commercial firefighting, represent environmentally-relevant commercial mixtures that contain a variety of fluorochemical surfactants. Combining the surfactant-selective ionization of fast

atom bombardment mass spectrometry with high resolution mass spectrometry, chemical formulas for 11 different fluorochemical classes were identified. Then AFFF-related patents were used to determine the structures. Of the eleven classes of fluorochemicals, ten have little, if any, data on their environmental occurrence, fate, and potential impacts in the peer-reviewed literature. In addition, nine of the identified classes had either cationic or zwitterionic functionalities and are likely to have different transport properties compared to the well-studied anionic fluorochemicals, such as perfluorooctanoate.

After the Deepwater Horizon oil spill in the summer of 2010, one of the emergency response methods for the mitigation of the oil's environmental impact was the use of unprecedented amounts of oil dispersant to break down the oil slick and encourage biodegradation. This event illustrated the need for rapid analytical method development in order to respond to the potential environmental disaster in a timely manner. Using large volume injection liquid chromatography with tandem mass spectrometry, an analytical method was developed for the trace analysis of the multiple dispersant surfactant classes and the potential degradation products of the primary surfactant. Limits of detection ranged from 49 – 3,000 ng/L. The method provided excellent recovery (86 – 119%) and precision (10 – 23% RSD), while also accommodating for the high salinity of seawater samples and analyte contamination.

Despite the fact that fullerene nanomaterials have been studied for almost three decades, research is still being conducted to fully understand the environmental properties of these materials. Previous studies to extract fullerenes from environmental matrices have resulted in low efficiency, high variability, or the extraction efficiencies have gone unreported. Extraction by ultrasonication with toluene and 1-methylnaphthalene increased the recovery 5-fold of a spiked, isotopically-labeled C_{60} surrogate from carbon lampblack as compared to that of the conventional approach of extracting with 100% toluene. The study revealed the importance of evaluating experimental variables such as extraction solvent composition and volume, and sample mass, as they have a significant impact on the quantitative extraction of fullerenes from environmental matrices.

Analytical Method Development for the Identification, Detection, and Quantification of Emerging Environmental Contaminants in Complex Matrices

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Benjamin J. Place, Author

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CONTRIBUTION OF AUTHORS

For all of the following studies, Dr. Jennifer Field provided insight and assistance in the experimental design and manuscript writing process for all of the research presented. For the development of the oil dispersant analytical method, Dr. Ewan Sinclair, of ALS Analytical Laboratories, provided technical assistance in the analysis of the Corexit formulations and in the manuscript review process. Markus Kleber provided early technical discussions on the development of the method and contributed during the manuscript writing process.

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CHAPTER 1: INTRODUCTION

A primary goal of environmental chemists is the identification and mitigation of chemical contaminant risk to humans and biota in the environment. Some of these chemical contaminants have been identified and rigorously studied for decades, and are often referred to as ‘legacy chemicals’ (such as the heavy metals lead and arsenic). Although there is still emerging research on some aspects of these legacy chemicals (e.g., biological mechanisms of toxicity), the identity and environmental behavior of these compounds are well understood.

Along with exposure to these well defined legacy compounds, environmental chemists, engineers, and toxicologists continuously strive to identify new compounds of concern and discover new routes of exposure and/or mechanisms of toxicity. It is from this perspective that the topic of ‘emerging contaminants’ becomes defined. Although there are multiple definitions for ‘emerging contaminants’ in the peer-reviewed literature,(1-3) the definition for the purposes of the following study is: *chemical compounds in which recent studies have reported new identities, uses, or properties that are deemed environmentally important*. Environmental information on these chemical compounds is limited or nonexistent, for occurrence, fate, and effects (e.g. toxicity). This lack of information makes comprehensive risk assessment for emerging contaminants difficult and further research is required to fill the data gaps.

Analytical chemists play an important role in the process of determining the risk posed to humans and other biota by emerging contaminants. The chemicals in the following research represent emerging contaminants in different stages of their ‘emergence’ status: unknown identities, environmental fate, and presence. The objective of this research was to develop analytical methods for the (1) identification, (2) detection and quantification, and (3) extraction of emerging contaminants from environmentally-relevant matrices. The nature of the emerging contaminants necessitates that innovative tools be developed. This dissertation is a compilation of studies regarding three emerging contaminants with differing chemical properties: fluorochemical surfactants, hydrocarbon surfactants, and fullerene nanomaterials.

Fluorochemicals and Aqueous Film-Forming Foams

Fluorochemical Properties and Use

Fluorochemicals are named for the alkyl chain where the covalently bonded hydrogen have been replaced by fluorine. Non-fluorinated functionalities, such as carboxylic acid and/or amine functional groups, are then added to vary the compound’s properties.(4) The fully fluorinated carbon backbone results in hydrophobic and oleophobic characteristics that cause fluorochemicals to have unique properties for many industrial and commercial uses, ranging from lubricants and electroplating to cosmetics.(4) Fluorochemical surfactants, due to their hydro- and oleophobic fluorinated chain and their polar head group, are recognized worldwide for their superior surface activity and surface tension-lowering capabilities.(4)

There are two major manufacturing processes for the synthesis of fluorinated compounds: electrofluorination and fluorotelomerization. The different processes produce structurally-distinct fluorochemicals with characteristic fluoroalkyl chain lengths.(4, 5) These differences have been previously exploited for the identification of fluorochemicals by manufacturing source.(6) Electrofluorination is a technique dominated by the 3M Company in the US, while the fluorotelomerization synthetic route is the primary technique for other fluorochemical manufacturers.(5)

Another use of fluorinated surfactants is as components in aqueous film-forming foams (AFFFs), which are chemical mixtures used for fighting hydrocarbon-based fires (e.g., fuel fires, plane crashes),(7) Developed in 1963 by the US Naval Research Laboratory,(8) fluorochemical-based AFFF continue to be used by the US military and other public and private sector organizations (such as commercial airports and refineries) where the exceptional firefighting capabilities of AFFF are required for safety reasons. Previous studies report increased concentrations of fluorochemicals in environmental systems directly related to the release of AFFF into the environment, from both accidental and intentional releases.(9-12)

Fluorochemicals as Emerging Contaminants

As a class of compounds with unique properties, fluorochemicals have been studied for their impact on the environment for decades. In addition to their commercially-useful properties, multiple studies reported toxic properties of these fluorochemicals.(13-18) The results of these studies, and studies on the occurrence of fluorochemicals, classify some of these compounds as persistent, bioaccumulative, and/or toxic.(13) The focus of most prior research is on a large list of known fluorochemicals, including perfluoroalkyl carboxylic acids, perfluoroalkyl sulfonates, and fluorotelomer sulfonates. Examples of these are perfluorooctanic acid(PFOA), perfluorooctane sulfonate (PFOS), and 6:2 fluorotelomer sulfonate (6:2 FTS).(9-12, 19) While the primary focus and energy of environmental chemists is on these well-known compounds, the list of environmentally-relevant fluorochemicals is still incomplete. While some components of AFFF are reported previously,(12) many proprietary AFFF formulations contain fluorochemicals that have are not identified. These potentially novel fluorochemicals can be classified as ‘emerging contaminants’ under the above definition. The structures of these fluorochemical compounds must be determined before quantitative analytical methods can be developed, which are needed to better understand the fate and presence of AFFF fluorochemicals in the environment.

Current Analytical Methods

Existing quantitative methods use liquid chromatography paired with tandem mass spectrometry for trace level detection of known fluorochemicals in environmental matrices, including water, sediments, sludges, and landfill leachates.(9-12, 19-24) These methods cannot be directly applied to AFFF formulations, as tandem mass spectrometry does not have capability to determine the structural identities of unknown compounds nor to detect compounds of unknown identities. A compound identification technique referred to as 'non-target screening' has emerged over the last few years with the increased capabilities and accessibility of mass spectrometers to perform high resolution analyses.(25, 26) This technique allows for analysis of samples without *a priori* knowledge, although the methods currently used are not capable of fully identifying compounds without additional analyses.

To improve non-targeted screening, a first step screening tool can be applied to prioritize compounds by specific properties unique to the compounds of interest. For example, in the case of AFFF-based fluorochemicals, their superior surface activity compared to other organic compounds is a property that can be effectively exploited through the use of surface active-sensitive techniques such as fast atom bombardment mass spectrometry (FAB-MS).(6, 27-29) The study discussed in Chapter 2 illustrates the use of a targeted screening method to accurately determine the structural identities of multiple fluorochemicals contained in US military-use AFFF.

Oil Dispersants

Deepwater Horizon Oil Spill

The explosion aboard the Deepwater Horizon oil rig in April 2010 resulted in the eventual release of millions of liters of oil into the Gulf of Mexico. Immediate response was taken to mitigate the environmental impact of the oil spill, the responses included controlled oil burns, physical removal of oil on the surface, and dispersion of the oil with the use of oil dispersants, which break up oil plume and encourage biodegradation of the individual oil constituents.(30, 31) While dispersants do decrease the appearance and impact of oil at the surface, there are toxic effects associated with biota exposure to dispersants and dispersed oil.(32-35)

During the first few months of the oil release and dispersant application, the identities of the chemical constituents of the Corexit formulations were not publicly released and therefore no quantitative analytical methods could be developed to track the dispersant through the marine environment. Because the function of dispersant includes creating water-soluble micelles around the hydrophobic hydrocarbons contained in crude oil, it can be safely assumed that surfactants are a major component in the dispersant formulations. In order to identify the surfactant components of the oil dispersant, initial research was performed using FAB-MS. After the initiation of this study, the US EPA released the chemical components in August 2010.(32) The identified anionic and nonionic surfactant component are described in Chapter 3.

Dispersant Surfactants as Emerging Contaminants

Oil dispersant surfactants were studied previously for toxicity, both alone and in combination with dispersed oil. The majority of these studies focused on whole (intact) mixtures in controlled systems. However the individual components of a dispersant formulation are likely to have different chemical fate and transport properties in the environment. The surfactants contained in the Corexit 9500 and Corexit 9527 dispersant formulations qualify as emerging contaminants because of the unprecedented volume and unique sub-surface application of the dispersant on the Deepwater Horizon oil spill. During the application, there was no existing information that could be used to predict the environmental fate and/or impact of the dispersants used. For this reason, an analytical technique was developed to quantitatively track dispersant chemicals in seawater.

Current Analytical Methods

Analytical methods for the detection of hydrocarbon surfactants in environmental matrices exist.(29, 36-39) While these methods can sufficiently detect a variety of surfactants with a wide range of properties, methods for the detection of surfactants in Corexit dispersant formulations in seawater were only developed this past year.(40-42) Seawater is a difficult matrix for mass spectrometric detection, due to the presence of a high concentration of nonvolatile salts that can cause sample cone fouling (resulting in a loss of sensitivity) and other instrumental issues. Methods developed prior to this study focused on a single surfactant component in the Corexit dispersant formulations, bis-(2-ethylhexyl) sulfosuccinate [DOSS]. As such, DOSS was used as an indicator of dispersant occurrence.(40-42)

In order to fully embrace the complexity of dispersant formulations, analytical methods that evaluate multiple components in the dispersant are necessary. Each surfactant compound may exhibit different behavior in the environmental and during analysis, which will impact the method development process. The developed method must accommodate for the analytical difficulty of analyzing seawater samples and overcome many confounding factors such as a compound's instability during storage or the presence of systemic contamination. The surfactant components contained in the Corexit dispersants are common commercial surfactants. For this reason, surfactants can occur in common laboratory products, which would unintentionally contaminate samples and result in artificially increased concentrations. Large volume injection liquid chromatography (LVI-LC) is a technique that has been previously used for complex environmental matrices, such as wastewaters (43) and surface waters,(44) for the ultra trace detection of environmental contaminants without the need for extensive sample preparation. This approach reduces sample analysis time, the potential for analyte contamination, and the use of expensive consumables that then become solid waste. LVI-LC provides a high-throughput tool for the sensitive detection of surfactant components in seawater.

Fullerene Nanomaterials

Fullerene Production and Use

Fullerene nanomaterials are a class of carbon allotropes that are comprised of entirely sp^2 hybridized carbons that form a spherical shape. As an example, the structure of C_{60} is similar to that of a soccer ball.(45) The existence and laboratory synthesis of C_{60} was first reported in 1985 by Kroto et al.,(45) followed by a multitude of subsequent studies that further discovered additional processes that produce fullerenes. Meteorite strikes,(46) graphite pyrolysis,(45) and even burning candles (47) can be sources of fullerenes.

In addition to being produced by natural means, fullerenes are being engineered and manufactured for numerous commercial uses, including those for medical (48) and cosmetic (49) purposes. In 2007, the fullerene market was estimated to be worth $58.5 million and this amount is expected to grow significantly over the next decade.(50)

Fullerenes as Emerging Contaminants

The discovery of fullerenes in a variety of geologic materials indicates that the compounds are not ‘emerging’ in the sense of being newly-developed compounds. Instead, the nature of fullerenes’ emerging status comes from the recent research related to their use and interesting properties as nanomaterials. Fullerenes also possess direct and indirect properties that could be toxic to a variety of biota.(51-53) Fullerenes have electron donor-acceptor behavior,(54) are known to produce and quench reactive oxygen species (ROS),(52) and can form water-soluble aggregates over time.(55) In addition, fullerenes can act as sorptive surfaces that will impact the transport of other legacy or emerging contaminants in environmental systems.(56, 57) The various sources of fullerenes, both natural and industrial, along with their increased and unprecedented use, promotes fullerenes to the category of compounds of emerging environmental interest.

Current Analytical Methods

Isaacson et al. reported the use of LVI-LC with tandem mass spectrometry for the sensitive detection of fullerenes.(58) Due to the nature of fullerene production in combustion-based solids, analytical methods for the quantitative extraction of fullerenes from these solids are needed for understanding the environmental presence of fullerenes. The critical review by Isaacson et al.(59) discussed the current status of the complex nature of extracting fullerenes from solids. Most studies report low and/or variable extraction recovery (60-63) and there is no current standard protocol for fullerene extraction. The greatest challenege in the extraction of fullerenes from carbonaceous materials is due to a limited understanding of the intermolecular forces that exist between fullerenes and carbon-rich matrices. In Chapter 4 the structural characteristics of black carbon-like material are taken into consideration, along with its interactions with fullerenes, and the result is an extraction method that provided higher and more consistent extraction of fullerenes from a broad range of solid materials.

The following studies represent the culmination of many years of research regarding the development of analytical methods for emerging contaminants. Each chapter examines different classes of compounds that exist at varying stages of their 'emerging' status and in different environmentally-related matrices. Although the topics of each study may seem disparate, they each come down to a single overall theme: the use of novel analytical chemistry techniques to fill in the inherent data gaps related to emerging contaminants in the environment.

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CHAPTER 2: IDENTIFICATION OF NOVEL FLUOROCHEMICALS IN AQUEOUS FILM-FORMING FOAMS USED BY THE US MILITARY

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Abstract

Aqueous film-forming foams (AFFFs) are a vital tool to fight large hydrocarbon fires and can be used by public, commercial, and military firefighting organizations. In order to possess these superior firefighting capabilities, AFFFs contain fluorochemical surfactants, of which many of the chemical identities are listed as proprietary. Large-scale controlled (e.g., training activities) and uncontrolled releases of AFFF have resulted in contamination of groundwater. Information on the composition of AFFF formulations is needed to fully define the extent of groundwater contamination, and the first step is to fully define the fluorochemical composition of AFFFs used by the US military. Fast atom bombardment mass spectrometry (FAB-MS) and high resolution quadrupole-time-of-flight mass spectrometry (QTOF-MS) were combined to elucidate chemical formulas for the fluorochemicals in AFFF mixtures, and, along with patent-based information, structures were assigned. Sample collection and analysis was focused on AFFFs that have been designated as certified for US military use. Ten different fluorochemical classes were identified in the seven military-certified AFFF formulations and include anionic, cationic, and zwitterionic surfactants with perfluoroalkyl chain lengths ranging from 4 to 12. The environmental implications are discussed, and research needs are identified.

Introduction

Aqueous film-forming foams (AFFF) formulations are chemical mixtures that are used to effectively extinguish hydrocarbon fuel-based fires and have a secondary benefit of preventing reignition.(1) Due to their surface-tension lowering properties, AFFF containing fluorinated surfactants have superior firefighting capabilities compared to nonfluorinated fire extinguishing methods.(2) Fluorinated surfactants have other unique properties that cause some of these compounds to be classified as persistent, bioaccumulative, and toxic.(3) Historical reports of uncontrolled spills and the repeated use of AFFF during fire training and for AFFF performance testing have been correlated to higher concentrations of fluorochemicals, including perfluoroalkyl carboxylates, perfluoroalkyl sulfonates, and fluorotelomer sulfonates, in biota, surface water, or groundwater.(4-8) These studies did not report the fluorochemical composition of the AFFF released, and therefore there is no direct connection between the AFFF product spilled and the resulting contamination.

The US military possesses the largest stockpile (almost 11 million liters) of AFFF in the United States, accounting for approximately 29% of all AFFF in the US in 2004.(9) Unlike general commercial AFFF formulations, AFFF sold to the US military must conform to military-specific performance and quality control requirements as prescribed by the military specification (Mil-Spec) MIL-F-24385, which specifies characteristics such as extinguishment time, corrosion rate, environmental impact as indicated by short-term toxicity (LC50 (Fundulus herteroclitus)), biological oxygen demand (BOD), and chemical oxygen demand

(COD)), and total fluorine content (no specific methodology is required).(10) Nonmilitary AFFF must comply with other performance standards. Once an AFFF product has been shown to perform to MIL-F-24385 requirements, the product is listed on the US military's AFFF Qualified Products Listing (QPL).

Since the initial development of AFFF materials in 1966, seven different manufacturers have developed AFFF that have passed military specifications, and a subset was purchased on contract in large quantities by the military (Figure 2.1).(1) The fluorochemicals contained in the AFFF formulations can be the result of electrochemical fluorination or telomerization processes. These AFFF formulations sold by 3M containing fluorochemicals synthesized by electrochemical fluorination accounted for 75% of the total AFFF stockpiled on military bases.(9) The remaining stockpiled AFFF contain telomerization-based fluorochemicals,(9) which are structurally distinct from those made by electrochemical fluorination, a process dominated by 3M.(11, 12) Telomerization-based fluorochemicals possess carbon chains that are not fully fluorinated and typically have homologues of varying $-C_2F_4-$ units, while electrofluorination-based fluorochemicals possess fully fluorinated carbon chains with homologues of varying $-CF_2-$ units.(13) Although 3M voluntarily removed their AFFF products from manufacture due to the rising concern about PFOA/PFOS-based products in 2002,(13, 14) currently there is no restriction by the US government on the use of stockpiled 3M AFFF.(14) However, both the European Union and Canada have set forth regulations to cease use of and remove PFOS-based AFFF stockpiles.(15, 16) Other fluorochemical and AFFF manufacturers have agreed to comply with the EPA PFOA/PFOS Stewardship program to cease production of all C8-based fluorinated compounds before 2015.(17)

Both MSDS and patents pertaining to the AFFFs used by the military list that these mixtures contain fluorinated surfactants, although the exact elemental composition of these compounds is proprietary. The single exception is the presence of perfluoroalkyl sulfonate salts, as indicated in MSDS for 3M AFFFs.(18) For this reason, analytical tools are needed to determine (e.g., reverse engineer) the composition of AFFFs sold to the military. Fast atom bombardment mass spectrometry (FAB-MS) with unit mass resolution is an established qualitative technique that requires minimal sample preparation and that favorably ionizes hydrocarbon and fluorocarbon surfactants in commercial and environmental mixtures.(8, 19-21) As opposed to most LC-MS/MS methods, FAB-MS does not require prior knowledge of analytes of interest in order to analyze the samples (e.g., mass ranges, acidity/basicity, mixture composition, and concentration). In contrast, high resolution mass spectrometry (HRMS) with chromatographic separation allows for the accurate determination of ion masses, which can be used to determine specific elemental compositions.(22) However, the major obstacle is that full scan HRMS provides a large quantity of data that must be reduced in order to identify compounds of interest.(23-25) For this reason, multiple samples of AFFF formulations spanning a range of manufacturing years were first screened by FAB-MS to identify target analytes for further analysis by HRMS in order to determine the final elemental compositions of the fluorochemicals (Figure 2.2). Finally, the information on chemical structure was compared to structures given in patents.

Experimental Section

Materials

All solvents used for sample preparation and analysis by FAB-MS were HPLC-Grade quality or better from Sigma Aldrich (St. Louis, MO). Laboratory water at Oregon State University was deionized and cleaned with a Millipore Synergy UV Water System (Bedford, MA) that included a LC-Pak C18 polisher. For FAB-MS analysis, MS-grade 3-nitrobenzyl alcohol (3-NBA) was purchased from Sigma Aldrich.

UPLC/QTOF-MS analysis was performed at the Waters Corporation Facility in Pleasanton, CA. Solvents used for mobile phases and sample dilutions included Fisher Optima LCMS grade methanol from Fisher Scientific (Fair Lawn, NJ) and Millipore Milli-Q laboratory water (Bedford, MA). Ammonium acetate buffer was made using laboratory deionized water and high purity ammonium acetate (Sigma Aldrich).

Sample Collection

Sample containers (60 mL HDPE Nalgene bottles) purchased from VWR International (Radnor, PA) were shipped to 21 different US Navy and Air Force military bases within the United States. Sampling instructions also were sent that included sample handling and recording of pertinent AFFF formulation information. Sampling instructions specifically stated to sample AFFF from their original product container in order to avoid mixtures of products. Additional AFFF samples were sent by Bradley Williams of the US Naval Research Laboratory. In total, 74 QPL-listed AFFF samples were received with manufacturing dates ranging from 1984 to 2011. AFFF product names have changed over time; therefore, products were categorized by their manufacturer rather than product name and were reported as such (“3M AFFF”, “Chemguard AFFF”, “National Foam AFFF”, etc.). After receipt, AFFF samples were stored in the dark at room temperature until analysis.

Fast Atom Bombardment Mass Spectrometry

FAB-MS analyses were performed with a JEOL MS-ROUTE JMS-600H magnetic sector mass spectrometer that was equipped with a FAB interface (JEOL, Ltd., Peabody, MA). Prior to analysis, the instrument was calibrated using a polyethylene glycol mixture (with average molecular weight of 300 g/mol) over the m/z 100–1000, and the ionization energy was set to 5 keV, while xenon gas was used as the ionization gas.

Each AFFF sample was diluted at least 10:1 with HPLC-grade methanol, and an aliquot was mixed with 3-NBA on the FAB probe. Samples were scanned over an m/z range from 100–1000 in both positive and negative ionization mode. A minimum of 7 scans were performed for each sample, and the mass spectra were calculated as an average of the 7 scans. Blank samples, consisting of only 3-NBA, were also analyzed to provide background mass spectra and to verify no compound carryover and/or contamination between AFFF samples. A number of AFFF samples from each AFFF manufacturer were analyzed in order to cover the entire range of available lot numbers and manufacturing dates.

Multiple parameters were used to identify target masses for subsequent screening by high resolution mass spectrometry. Ions in a series characterized by spacings of ±m/z 50, which corresponds to −CF2– units, were selected because they are indicative of fluorochemicals produced by electrochemical fluorination. Ions with spacings of m/z 100 correspond to −C2F4– units were selected because they can be characteristic of fluorochemicals produced by telomerization or electrofluorination (Figures 2.5, 2.6).(11, 20) In addition, other masses that were identified in the FAB-MS spectra of multiple lots of the same AFFF were also added to the list of target masses.

Ultra Performance Liquid Chromatography/Quadrupole-Time of Flight Mass Spectrometry

For analysis by UPLC/QTOF-MS, all AFFF formulations were prepared in HPLC-grade methanol and diluted to 12 ppb concentrations of fluorochemical surfactants as estimated from information provided by the available MSDS. Blank samples (consisting of 50% 0.5 mM ammonium acetate in water and 50% methanol) were injected regularly throughout the sequence to verify that there was neither system contamination nor analyte carryover.

Separations were performed on a Waters Acquity H-Class UPLC (Waters Corp., Milford, MA); the chromatographic conditions are reported in the

Supporting Information (SI). The chromatographic conditions selected provided the minimum resolution required to separate the suspect ions of interest. A Waters Xevo G2 Quadrupole-Time of Flight (QTOF) mass spectrometer with electrospray ionization (ESI) was operated as the high resolution mass spectrometer. Voltages for the cone and capillary were 30 V and 1.50 kV, respectively. Additional parameters included a source temperature of 130 °C, a desolvation temperature of 350 °C, a cone gas flow of 25 L/h, and a desolvation gas flow of 1000 L/h. MS scan time was 0.1 s with an MS scan range of 150–1000 m/z. Every 15 s, the system was recalibrated using leucine-enkelphalin as the lockmass, and the resolution was set to be 20,000 (unitless, defined as the peak width at half-maximum). All samples were analyzed in both positive and negative ionization modes.

UPLC/QTOF chromatograms for each of the AFFF formulations were first screened for only compounds that had mass defects from −0.100 to +0.150, which is typical of fluorochemicals. Mass defects are the difference between the actual/theoretical ion mass from the nominal ion mass. For example: PFOS has an actual ion m/z 499.9375 and a nominal ion m/z 500.0000, for a mass defect of m/z −0.0625. The low-to-negative mass defects of fluorochemicals are due to the cumulative negative mass defect of multiple fluorine atoms (m/z −0.0016) and can be compared to the positive mass defect created by multiple hydrogen atoms (m/z +0.0079).

Chromatograms were extracted for each target mass. High accuracy masses (to the ten-thousandth of a mass-to-charge unit) were calculated as an average over the entire peak width, which has been reported to give the most reproducible results (Figures 2.7, 2.8).(26) Possible elemental compositions of the high-accuracy masses were calculated along with the error, which is reported as the deviation of the detected mass from the calculated elemental composition's mass (in parts-per-million [ppm]). In addition, the elemental composition of the +1 and +2 isotopes were used to rank the likely parent elemental compositions. The elemental composition constraints include an error limit of ±5 ppm and elemental limits of carbon: 0–50; hydrogen: 0–50; oxygen: 0–7; nitrogen: 0–7; sulfur: 0–7; and fluorine: 0–25.

Patent Information and Structure Confirmation

US Patents related to AFFF formulations contain limited information on the functional groups and possible perfluoroalkyl chain lengths of fluorochemical components. A database was compiled, which contained the masses and elemental formulas for all potential AFFF fluorochemicals identified in patents. The high accuracy masses detected by the UPLC/QTOF analysis and their calculated elemental composition were then matched to those in the structural database derived from patents to confirm the final structures of the identified fluorochemical compounds.

Results and Discussion

Electrochemical Fluorination-Based AFFF

3M AFFF

From the sampling program, 19 samples of 3M AFFF were received from US Air Force and Navy bases within the United States. The samples had a range of manufacturing dates from 1988 to 2001. Although 3M AFFFs were placed on the QPL in 1976, attempts to locate samples older than 1988 were unsuccessful. Six representative 3M AFFF samples were qualitatively analyzed by FAB-MS. The FAB-MS spectra of 3M AFFF obtained in negative ionization mode contained spacings of m/z 50, which is characteristic of compounds synthesized from electrochemical fluorination.(20) In the 3M AFFF, C6–C8 perfluoroalkyl sulfonates (Figure 2.3A) were identified components in all the 3M AFFF tested (Table 2.1), and this is consistent with the frequent detection of perfluoroalkyl sulfonates found in AFFF-impacted groundwater.(4, 5, 7, 8, 27) Contrary to these findings, however, no perfluoroalkyl carboxylates were detected in any AFFF product, with dates that ranged from 1988 to 2001. However, PFCAs are reported as primary components in early 3M AFFFs.(11) A limitation of the FAB-MS/QTOF-MS method is that it can only capture the major components and that minor (approximately <0.1%) fluorochemical compounds may go undetected; therefore, if PFCAs were an impurity and/or minor component of the analyzed AFFF products they could not be detected with the current method. Current research using LC/MSMS to determine trace components in AFFF has determined PFCAs are present in some 3M AFFF (unpublished work). While chemical degradation could occur during long-term storage of any AFFF product, it was beyond the scope of the study to determine the stability of fluorochemicals in commercial AFFF mixtures during long-term (e.g., decades) storage.

In addition, 3M AFFF were comprised of zwitterionic C4–C6 perfluoroalkyl sulfonamides containing carboxylic acid and tertiary amine functionalities (Figure 2.3B), which are consistent with patent information(28) and Material Safety Data Sheets (MSDS) that list “amphoteric fluoroalkylamide derivatives”.(29) The identification of these compounds was made in positive ionization mode, an uncommon method of mass spectrometric ionization for fluorochemical detection. Of the six 3M AFFF analyzed, the zwitterionic compounds were found only in AFFFs manufactured in 1993, 1998, and 2001 but not in those dating 1988 or 1989. The 3M AFFFs were recertified in 1992, but the addition of zwitterionic fluorochemicals to 3M AFFFs is not well documented.(30, 31) AFFF formulation recertification would occur if there were changes to military specifications or if the AFFF formulation itself was significantly changed (i.e., a change in chemical components). An additional set of ions of lower abundance was observed in positive ionization FAB-MS that corresponded to the zwitterionic sulfonamide class but with masses that were ± m/z 72 different (Table 2.1) from the chemical class shown in Figure 2.3B. The addition of m/z 72 indicate C5–C6 perfluoroalkyl sulfonamide compounds with an additional propanoic acid branch (Figure 2.3C), and the loss of m/z 72 indicates the absence of the propanoic acid branch (Figure 2.3D). These derivatives are impurities from the synthesis as indicated in the AFFF patent.(28)

No C8-based homologues of the zwitterionic class (Figure 2.3B) or the corresponding impurities (Figure 2.3C-D) were identified.

Telomerization-Based AFFF

National Foam AFFF

Nineteen samples were collected from military bases with manufacturing dates ranging from 2003 to 2008. Although National Foam has AFFFs on the QPL since 1976 (Figure 2.1), no samples from 1976 to 2003 were acquired. Six representative samples were analyzed by FAB-MS.

The primary fluorochemicals of National Foam AFFF were detected by m/z 100 spacings in both positive and negative mode FAB-MS, which correspond to $-C_2F_4-$ units that are characteristic of telomer-based fluorochemicals. The targeted ions were then identified by QTOF-MS as the 4:2, 6:2, 8:2, and 10:2 fluorotelomer sulfonamide with dimethyl quaternary amine and carboxylic acid functional groups (Figure 2.4A; Table 2.1).(32) Less abundant ions were identified with m/z −58 differences from the 4:2 and 6:2 fluorotelomer ions, which are related to the same structure but without the terminal acetic acid functionality (Figure 2.4B). In the related patent, Norman et al. suggest that these compounds could result as a byproduct in the synthesis of the major betaine compound.(32)

Ansul AFFF

Ansul AFFF, along with 3M and National Foam, was placed on the AFFF QPL in 1976 (Figure 2.1). Fifteen samples of Ansul AFFF were collected from the sampling program, with manufacturing dates that ranged from 1984 to 2010 (Figure 2.1), of these eight representative samples were analyzed by FAB-MS.

Negative ionization mode FAB-MS analyses for Ansul AFFF revealed two abundant ions with characteristic fluorotelomer mass spacings of ±m/z 100 (Table 2.1). The primary components identified in the Ansul AFFF were the 6:2 and 8:2 fluorotelomer thioether amido sulfonates at m/z 586 and 686, respectively (Figure 2.4C). This structure is supported by multiple patents(33-35) and a limited number of other reports on AFFF composition.(8, 20) An ion of lower abundance was identified at m/z 602, corresponding to a mass difference of m/z 15.9940 from the 6:2 thioether amido sulfonate and is proposed to be the addition of an oxygen atom (structure not shown). The identity of this fluorochemical class could not be definitively determined from the mass spectral data nor from the patents and may be a synthetic impurity. The 6:2 fluorotelomer sulfonate was also reported as being detected by LC/MS/MS in Ansul AFFF,(8) but with the current method no fluorotelomer sulfonates (FTS) were detected. The lack of identification of FTS in AFFF formulations is most likely due to the aforementioned high detection limits, and current work developing a quantitative LC-MS/MS method will determine these trace components.

Angus AFFF

Only one sample of Angus AFFF was received and analyzed. Because there was no recertification from the time that the product met Mil-Spec in 1994 to present (Figure 2.1),(30, 31) and there were no formulation changes that necessitate recertification, the single sample may well represent the entirety of Angus AFFFs regardless of the year of manufacture.

In the Angus AFFF formulation, the 6:2 fluorotelomer thioether amido sulfonate (Figure 2.4C) and corresponding oxygenated impurity (structure not shown) were detected. In addition, two masses at m/z 496 and 596 were identified through positive ionization FAB-MS analysis. By QTOF-MS analysis, the structure was determined to be a 6:2 and 8:2 fluorotelomer thioether with hydroxyl and trimethyl quaternary amine functionalities (Figure 2.4D; Table 2.1).(33)

Chemguard AFFF

From the sampling program, 11 samples were received from US military bases, and the manufacturing dates ranged from 2006 to 2010. While this is a narrow range of dates, there was no AFFF sample recertification, and therefore there have been no official formulation changes.(30, 31) Therefore these samples are likely to be representative of the QPL-listed AFFF product. Five representative samples were analyzed by FAB-MS.

Within the samples analyzed by FAB-MS, there were distinct differences between Chemguard products with manufacturing dates from 2006 to 2007 and 2008–2010. The FAB-MS spectra of the later manufacturing years had no patterns characteristic of fluorochemicals detected through positive and negative ionization FAB-MS, but there was a single strong peak detected at m/z 586, which was previously identified as the 6:2 fluorotelomer thioether amido sulfonate (Figure 2.4C) and verified by QTOF-MS. The other homologues of the fluorotelomer thioether amido sulfonate (4:2, 8:2, 10:2) may be present at concentrations below the above-specified detection limit. In the earlier manufacturing years, fluorochemical patterning was identified for m/z 602, 702, and 802, which was identified by QTOF-MS to be the sodium-adducted compounds of compounds with m/z 581, 681, and 781. These compounds were identified as 6:2, 8:2, and 10:2 fluorotelomer thioether amido amino carboxylic acid (Figure 2.4E; Table 2.1).(36)

Buckeye AFFF

Buckeye AFFF was initially certified for military use in 2004, making it the second most recent product to be added to the QPL (Figure 2.1).(30, 31) Only one sample of QPL-listed Buckeye AFFF was received from a military base, and an additional sample was supplied by the US Naval Research Laboratory; both of these samples were analyzed by FAB-MS.

No characteristic mass spacings of fluorochemicals were identified by analysis under negative ionization FAB-MS. Two different series of fluorotelomer-based homologues (m/z 100 spacing) were detected in positive ionization mode at m/z 432, 532, and 632 and m/z 414, 514, and 614 (Table 2.1). Based on AFFF patent information,(33) the fluorochemicals were identified as fluorotelomer betaines with quaternary amine and carboxylic acid functionalities (Figure 2.4F and 2.4G). The difference between the two series of homologues is 18 mass units, which is identified as the substation of a hydrogen atom with a fluorine atom near the fluorotelomer chain. Both compounds have perfluoroalkyl chains with lengths of 5, 7, and 9. The compounds with the additional fluorine atom near the fluorotelomer chains are referred to as x:y:z fluorotelomer betaine (Figure 2.4F), indicating that the compound has x fully fluorinated carbons, y singly fluorinated carbons, and z nonfluorinated carbons prior to the first functional group (quaternary amine) (Table 2.1). These compounds do not follow the typical telomerization pattern of even fluorocarbon chain lengths.(11) In addition, the structure of the x:y:z fluorotelomer betaine does not follow the typical telomerization paradigm of a fully fluorinated carbon chain (with the singly fluorinated carbon linkage). The synthesis of this unique structure results from the use of an unsaturated fluoroalkyl amine.(37, 38)

Fire Service Plus AFFF

No Fire Service Plus AFFF samples were received from the sampling program, which was expected as the AFFF joined the military QPL in 2011. However, two Fire Service Plus samples (from the same manufacturing batch) were received from the Naval Research Laboratory and analyzed.

Positive ionization mode FAB-MS analysis of Fire Service Plus AFFF showed fluorotelomer characteristic spacings (m/z 100) at the same masses as the National Foam AFFF. This was verified as the fluorotelomer sulfonamide betaine class with perfluoroalkyl chain lengths of 4, 6, 8, and 10 (Figure 2.4A). In addition, the 4:2 and 6:2 fluorotelomer sulfonamide amine impurities were also identified in the formulation (Figure 2.4B).

As the newest addition to the AFFF QPL for US military use, it is very unlikely that there has been any environmental exposure of this AFFF due to uncontrolled or controlled releases of the material.

Environmental Implications and Research Needs

This is one of the first studies to report the identities of per- and polyfluorinated surfactants contained in military-use AFFF. While the specific compounds are now known, the environmental behavior and toxicity of the individual fluorinated surfactants (and as mixtures) are still unknown.

Previous studies have examined the presence of PFOS and the other perfluoroalkyl sulfonic acids in environmental samples due to AFFF-use and have detected relatively high concentrations of these compounds in groundwater.(5, 7, 8) While Schultz et al. reported the identity of the fluorotelomer thioamido sulfonate in AFFF formulations, no data on its environmental occurrence were obtained.(8) Oakes et al. also included the 6:2 and 8:2 fluorotelomer compounds in their analytical method although no values for environmental presence were reported.(39) The scope of the current study was to qualitatively identify the fluorochemical components in AFFF, which are listed in various MSDS to range in concentrations of 0.5–25% (by weight) in the product concentrate. On-going research is underway to develop LC-MS/MS methods with the capability for quantifying trace levels all of the newly identified fluorochemicals in groundwater, sediment, and soil. Such methodology can be applied to future studies on the fate of the newly identified fluorochemicals in natural and engineered systems and to evaluate their occurrence and effects in biota.

Of the 11 fluorinated surfactant classes reported in this study, 9 were determined to have cationic or zwitterionic functionalities at environmental conditions (Figure 2.3B-D, Figure 2.4A, B, D-G). The nature of these fluorinated surfactants in the environment has not been investigated in the peer-reviewed literature. Cationic (nonfluorinated) surfactants have different environmental transport characteristics than anionic surfactants. For instance Lee et al. reported that the studied cationic surfactants would cation-exchange onto the negatively charged surfaces of sediments and therefore retard the transport of the compounds through the environmental system.(40) In addition, the adsorbed cationic surfactants could act as a carbon loading surface that further retained other hydrocarbon compounds at the source of contamination.(40) Cationic and zwitterionic fluorinated surfactants may also behave in a similar manner, suggesting that groundwater sampling may not be sufficient in the detection of these compounds in the environment. Furthermore, the cationic fluorocarbon surfactants may act as a sink to retain fluorochemicals or other priority pollutants and create long-term source zones of high fluorocarbon contamination.

Most of the studies also found detectable levels of perfluoroalkyl carboxylic acids (PFCAs) in AFFF-impacted groundwater,(5-8, 27, 39, 41) but none of the analyzed AFFF contained PFCAs as a major component. As previously alluded, PFCAs may have been major components of 3M AFFF prior to 1988 or are minor (e.g., < 0.1%) components of current AFFF at trace levels. In addition, the presence of PFCAs may be due to the degradation of other fluorochemicals. Wang et al. reported the degradation of fluorotelomers to the corresponding carboxylates through aerobic biotransformation in activated sludge.(42) Work by Houtz and Sedlak has shown, through the advanced oxidation process, that more functionalized fluorocarbon surfactants can be degraded down to the more oxidation-resistant fluorinated carbon backbone, resulting in the production of corresponding perfluoroalkyl carboxylates.(43) This has important implications toward the application of in situ chemical oxidation (ISCO) remediation processes that may be used to clean up contaminated sites that may also contain these AFFF-based fluorochemicals. These examples suggest that not only do the AFFF compounds present their own environmental and toxiocological concerns, they also could be potential sources of perfluoroalkyl carboxylates through environmental and anthropogenic transformation.

Future research studying the fate of the fluorochemicals during biodegradation and upon exposure to chemical remediation approaches (e.g., ISCO) is needed. The data from these experiments will have important ramifications toward the site closure of fluorochemical-contaminated military bases. The targeted approach based on FAB-MS described in this study may be useful in the identification of transformation products of the fluorochemicals identified in this study if they continue to exhibit surface-active properties. However, FAB-MS analysis has poor sensitivity (approximately mg/L levels) compared to that of LC-MS/MS (ng/L), which is necessary to detect trace levels of intermediates. Therefore, LC-MS/MS combined with QTOF analyses may be more suitable for environmental transformation and/or bioaccumulation studies.

In addition to understanding the environmental behavior of these fluorochemicals, it is mportant to understand the implications of remedial strategies applied in the field. For example, 'pump and treat' remediation may not be able to access the positively charged fluorochemicals that could cation-exchange to the sediments. In addition, advanced oxidation could potentially result in the increase of 'dead end products' (such as the perfluorinated carboxylates), some of which are compounds of concern. Development of new approaches to fluorochemical remediation may be important to fully account for the various classes identified in this research.

As previously noted, 3M ceased production of their PFOS-based AFFF in 2002, while the rest of the AFFF manufacturers agreed to the voluntary regulations of the EPA PFOA/PFOS Stewardship Program, which calls for the complete phase-out of C8-based products from materials. As reported in this study, while most AFFF formulations did contain C8 and above fluorinated surfactants, the major homologue (identified as the most intense signal via FAB-MS) in the telomerization-based AFFF were of perfluoroalkyl chain lengths less than 8, although fluorochemical homologues of chain length 8 or greater were identified at lesser intensities. The method described in this research could be applied to future AFFF formulations, after the 2015 deadline, to verify the removal of C8-based fluorochemicals from these products.

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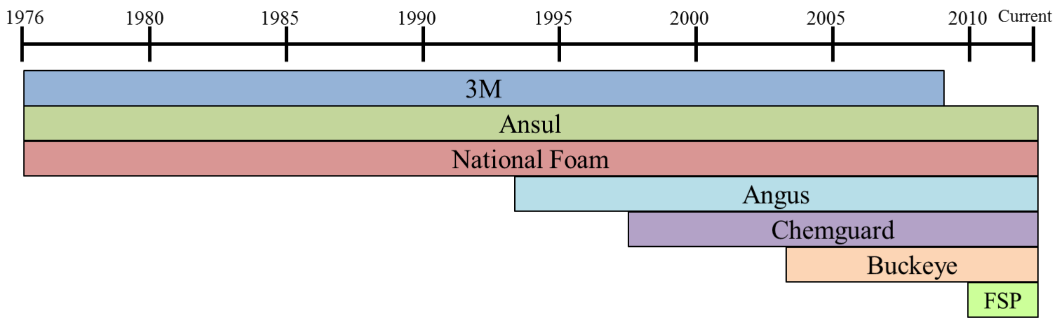

Figure 2.1. Timeline of AFFF product addition to the Department of Defense Qualified Products Listing (QPL) that were certified to MIL-F-24385 specifications. While the US military used AFFF since the development in 1963, the records of AFFF on the US military QPL are only available up to 1976. Although 3M remained on the QPL until 2010, the company ceased production of their AFFF product in 2002. "FSP" indicates the AFFF manufacturer Fire Service Plus, Inc.

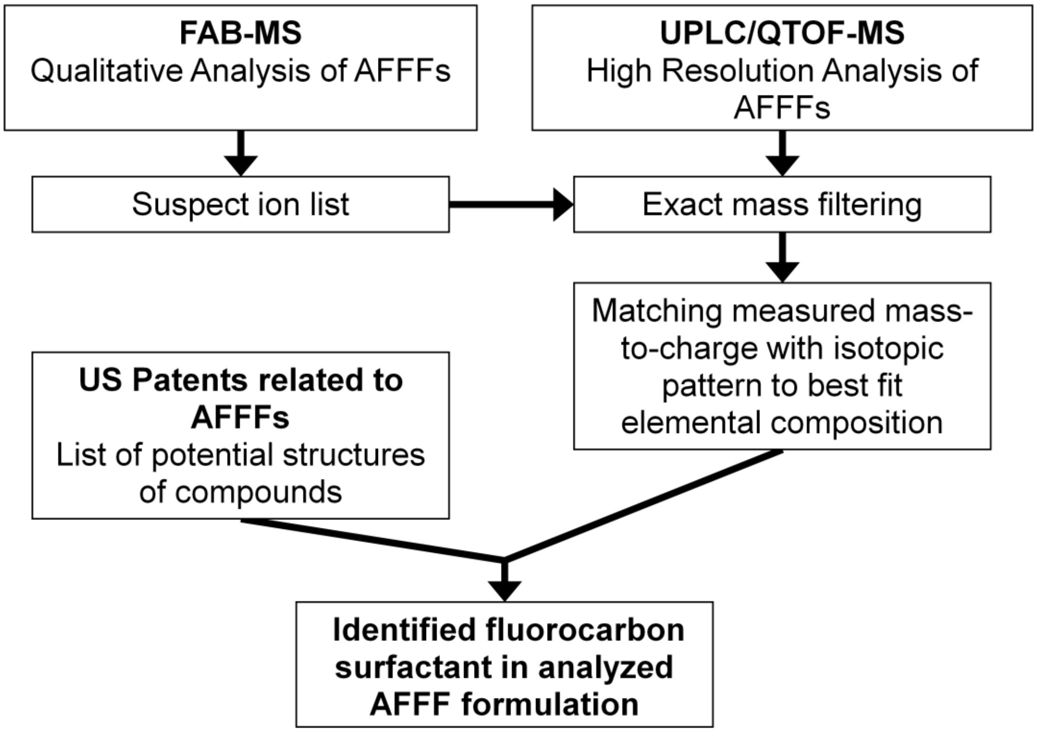

Figure 2.2. Workflow scheme for the elucidation of fluorochemical surfactants in AFFF formulations.

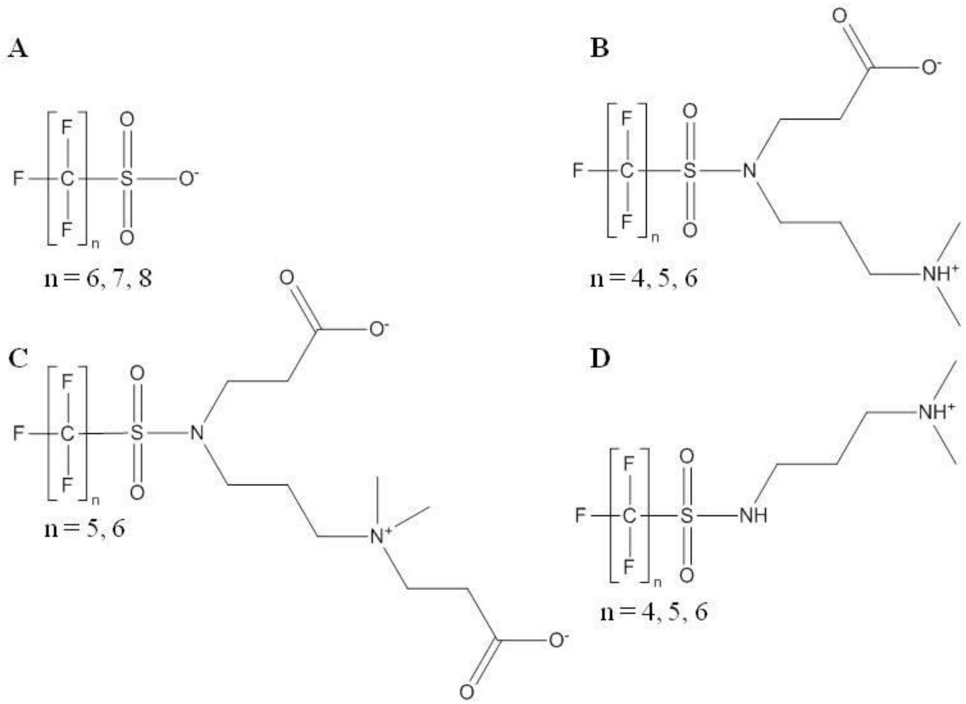

Figure 2.3. Electrofluorination-based fluorinated surfactants identified in AFFF. The perfluoroalkyl chain lengths identified in AFFF are shown as the number of n fluorocarbons. The ionic species shown are estimated at an environmentally relevant pH.

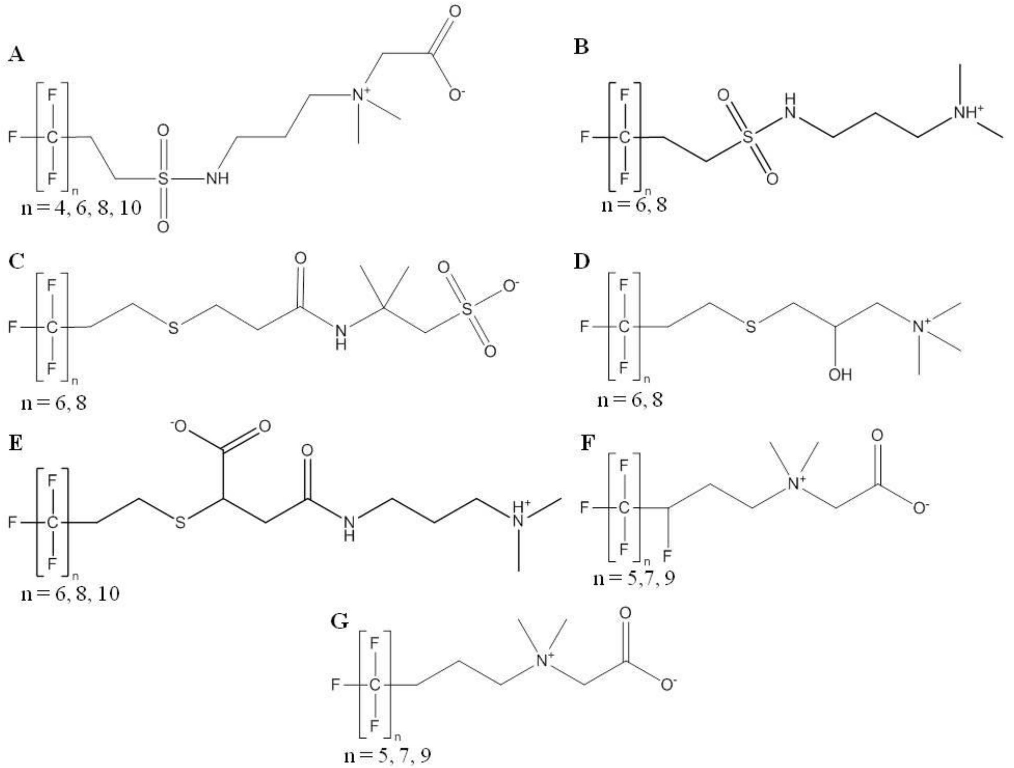

Figure 2.4. Telomerization-based fluorinated surfactants identified in AFFF. The perfluoroalkyl chain lengths identified in AFFF are shown as the number of n fluorocarbons. The ionic species shown are estimated at an environmentally relevant pH.

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SUPPORTING INFORMATION
IDENTIFICATION OF NOVEL FLUOROCHEMICALS IN AQUEOUS FILM-FORMING FOAMS (AFFF) USED BY THE US MILITARY

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Materials and Methods

High Performance Liquid Chromatography Quadrupole-Time-of-Flight Mass Spectrometry

Separations were performed on a Waters Acquity H-Class UPLC (Waters Corp., Milford, MA) using a Acquity BEH C18 column (2.1 mm ID x 100 mm, 1.7 µm particle size). The mobile phase consisted of 0.5 mM ammonium acetate in water (A) and methanol (B) and the flow rate was maintained at 0.3 mL/min for the entirety of the separation. The gradient program began with a mobile phase of 20% B followed by a slope to 95% B in 10 min, held at 95% B for 5 min then a downward slope to 20% B over 0.5 min and held at 20% B for 2.5 min to re-equilibrate the column. The column was maintained at a constant temperature of 60 ^{0} C. The sample injection size was 20 µL.

Figure 2.5. Example FAB-MS mass spectra of a 3M AFFF product.

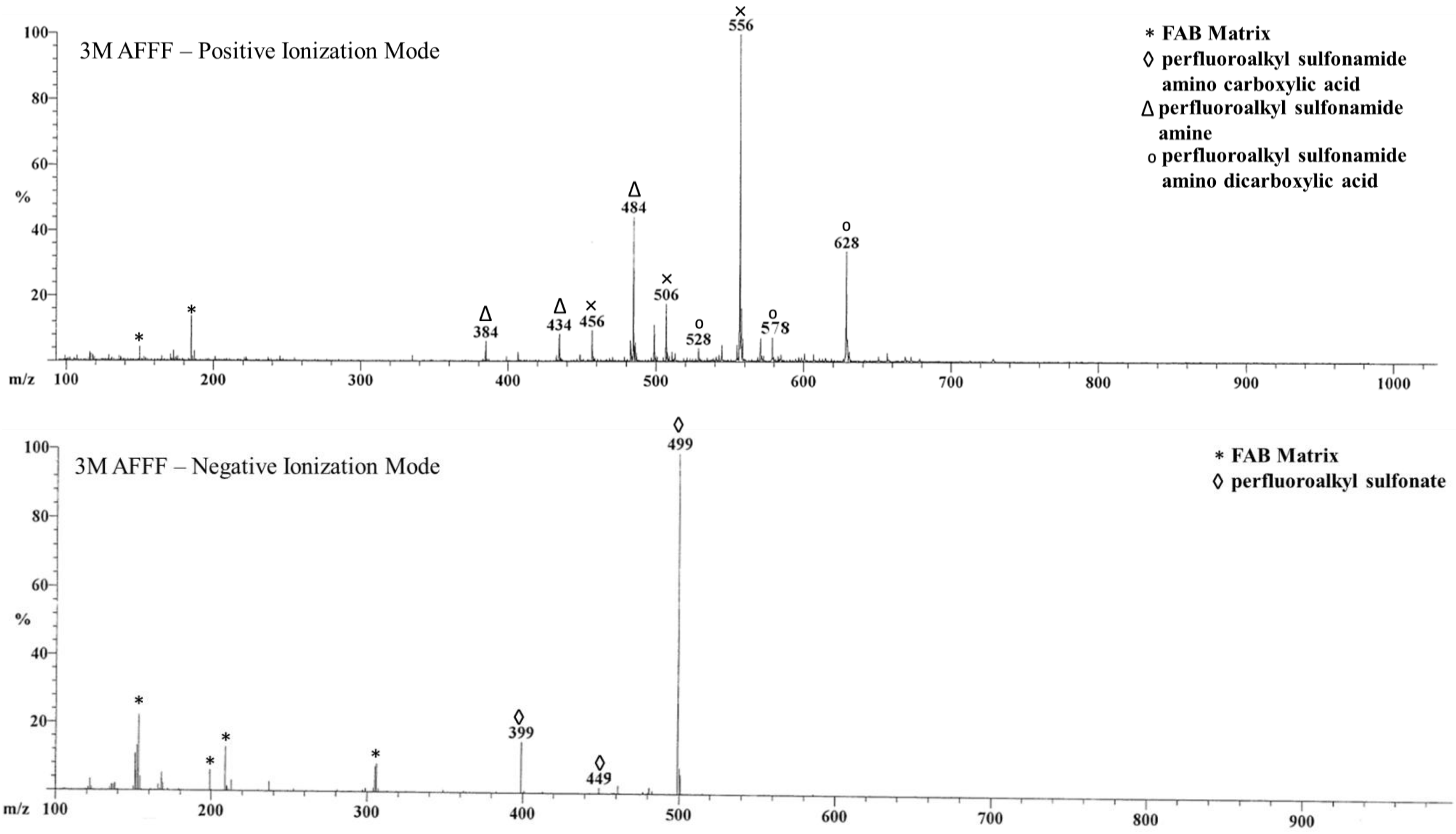

Figure 2.6. Example FAB-MS mass spectra of a Buckeye product.

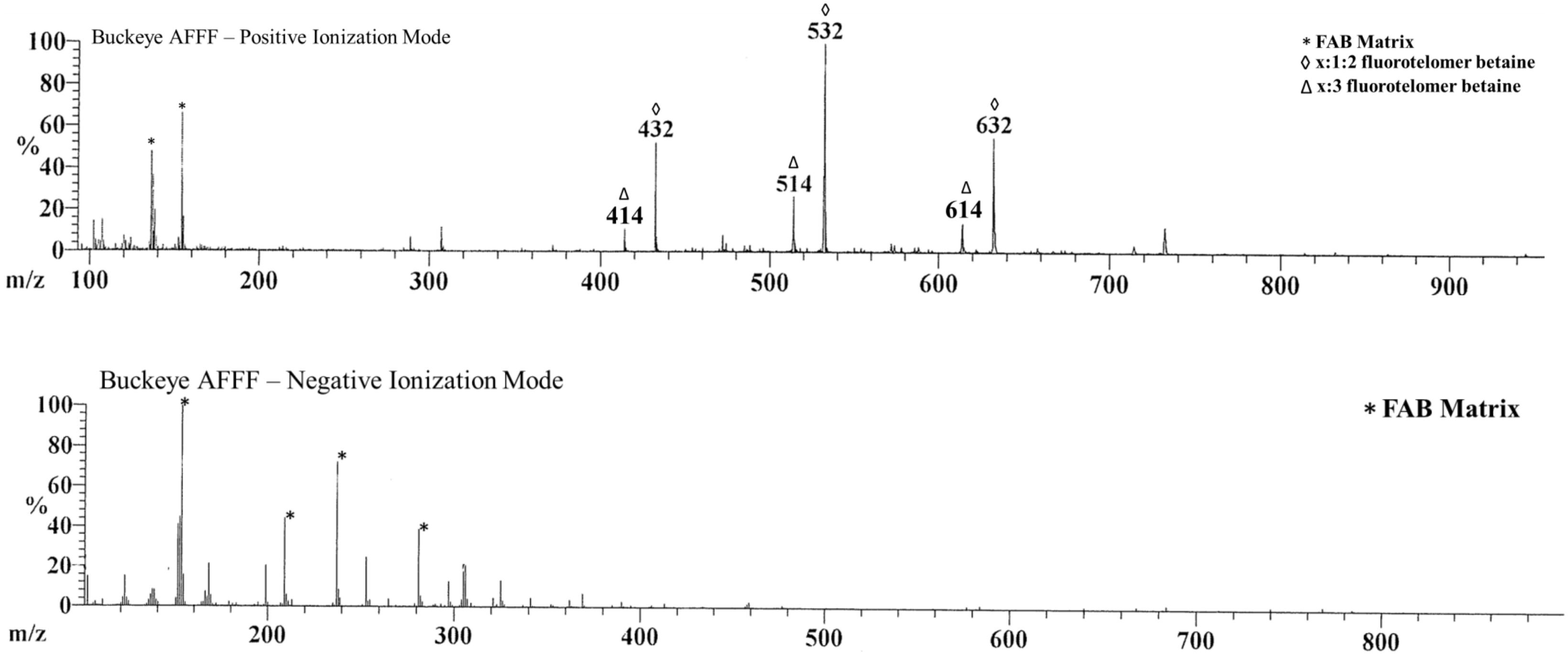

Figure 2.7. Example QTOF-MS high resolution chromatogram and mass spectrum of a 3M AFFF product, specifically isolating peak *m/z* 499. This high resolution mass (*m/z* 498.9297) was determined to be perfluorooctane sulfonic acid.

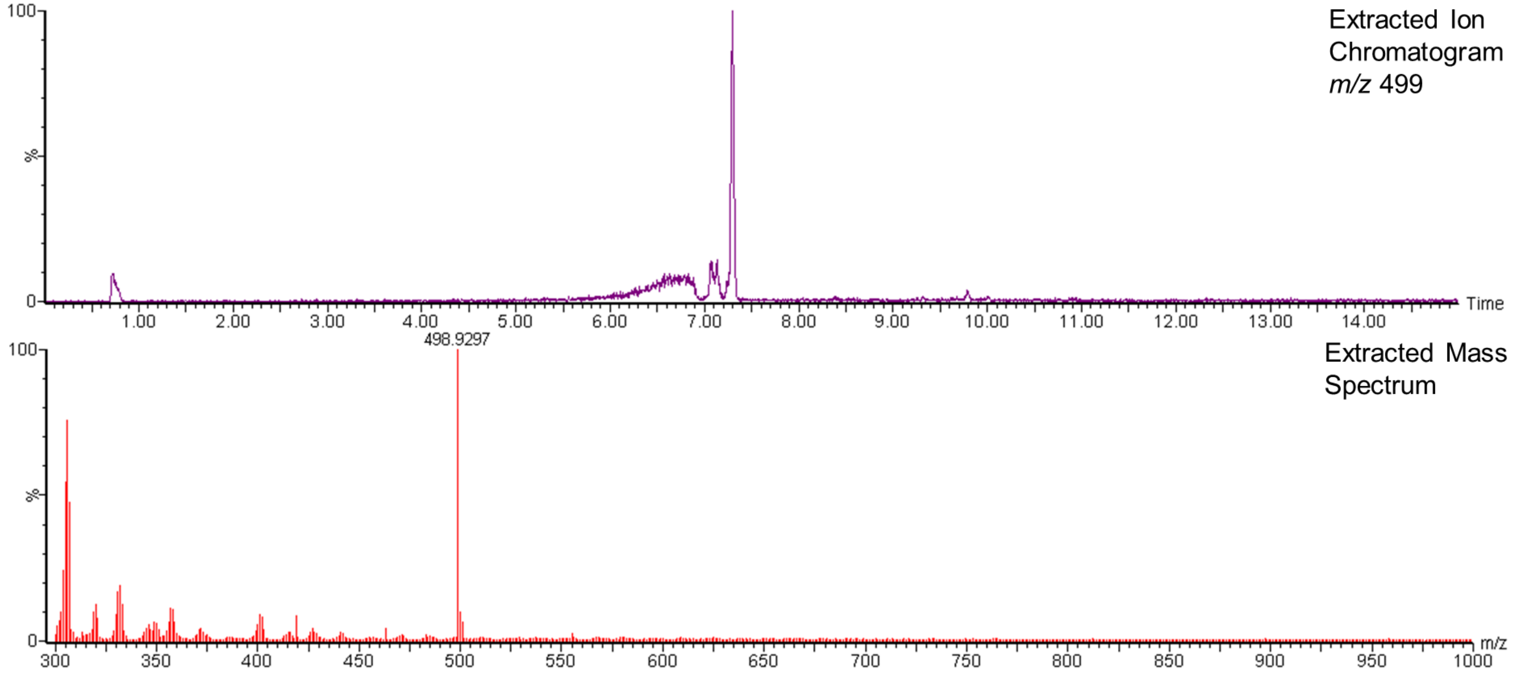

Figure 2.8. Example QTOF-MS high resolution chromatogram and mass spectrum of a Buckeye AFFF product, specifically isolating peak *m/z* 532. This high resolution mass (*m/z* 532.0759) was determined to be 7:1:2 fluorotelomer betaine.

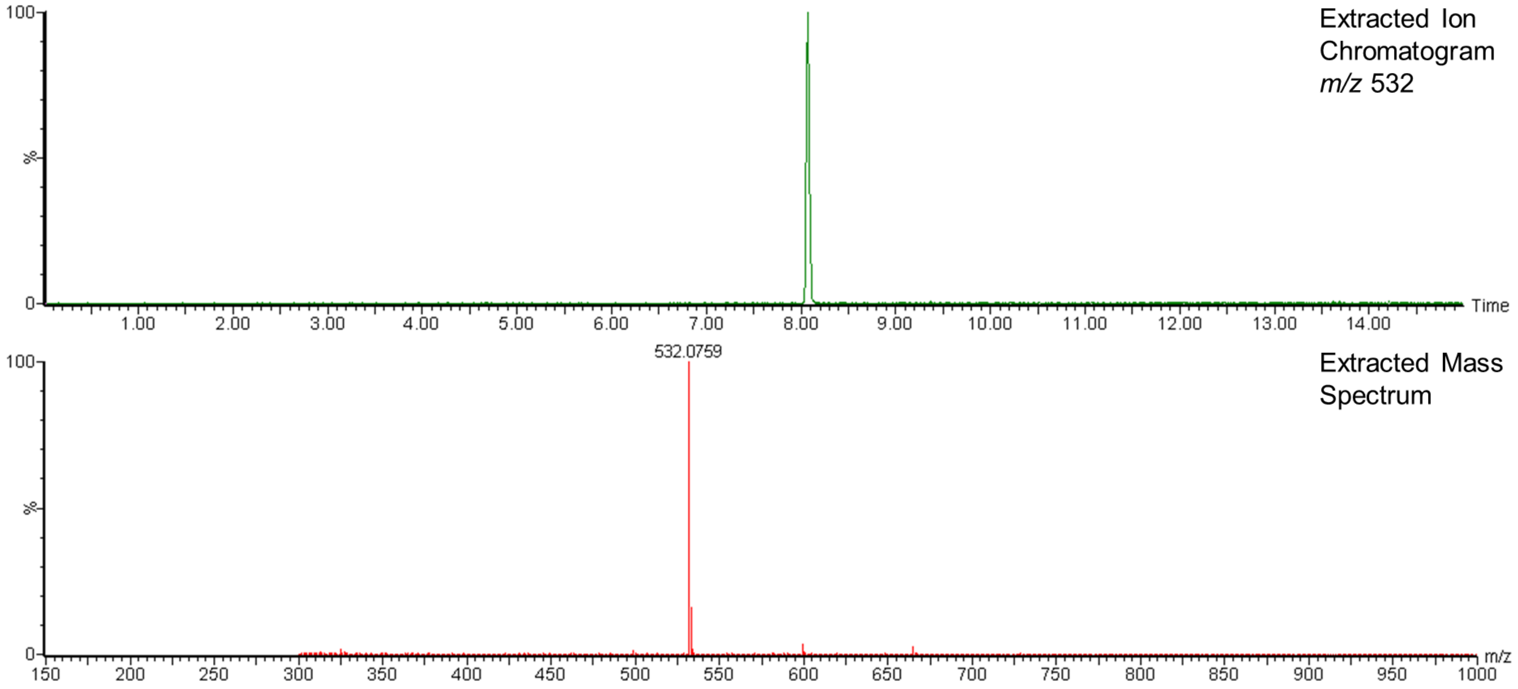

Table 2.1. High mass accuracy measurements for all identified AFFF fluorochemicals. ‡ High mass accuracy identification codes, Identification code ranking: 1 = Formula identified from accurate mass and isotopic match, 2 = Formula identified only from accurate mass, 3 = Formula alternatively identified (e.g. FAB-MS fluorochemical mass spacing from identified fluorochemical)

AFFF Product	Nominal Mass	Accurate Mass	Measured Mass	Error (ppm)	Isotopic Fit Conf %	Ion State	Ion Formula	ID Code‡
3M	385	385.0632	385.0632	0	96.88	[M+H]+	C9H14N2O2S1F9	1
	399	398.9361	398.9378	4.3	65.69	[M-H]-	C6O3S1F13	1
	435	435.0600	435.0586	-3.2	99.12	[M+H]+	C10H14N2O2S1F11	1
	449	448.9329	448.9328	-0.2	89.07	[M-H]-	C7O3S1F15	1
	457	457.0844	457.0829	-3.3	94.3	[M+H]+	C12H18N2O4S1F9	1
	485	485.0568	485.0567	-0.2	99.62	[M+H]+	C11H14N2O2S1F13	1
	499	498.9297	498.9303	1.2	97.34	[M-H]-	C8O3S1F17	1
	507	507.0812	507.0807	-1	3.33	[M+H]+	C13H18N2O4S1F11	2
	557	557.0780	557.0768	-2.2	94.27	[M+H]+	C14H18N2O4S1F13	1
	579	579.1023	579.1034	1.9	51.45	[M+H]+	C16H22N2O6S1F11	1
	629	629.0991	629.0967	-3.8	61.06	[M+H]+	C17H22N2O6S1F13	1
National Foam	513	513.0881	513.0890	1.8	99.99	[M+H]+	C13H18N2O2S1F13	1
	571	571.0936	571.0930	-1.1	99.81	[M]+	C15H20N2O4S1F13	1
	613	613.0818	613.0818	0	49.55	[M+H]+	C15H18N2O2S1F17	1
	671	671.0872	671.0861	-1.6	96.62	[M]+	C17H20N2O4S1F17	1
	771	771.0808	771.0818	1.3	91.47	[M]+	C19H20N2O4S1F21	1
	871	871.0745	871.0771	3	15.18	[M]+	C21H20N2O4F25S	2
Ansul	586	586.0391	586.0394	0.5	84.23	[M-H]-	C15H17N1O4S2F13	1
	602	602.0341	602.0332	-1.5	6.63	[M-H]-	C15H17N1O5S2F13	2
	686	686.0328	686.0312	-2.3	13.65	[M-H]-	C17H17N1O4S2F17	2
Angus	496	496.0980	496.0967	-2.6	14.49	[M]+	C14H19N1O1S1F13	2
	586	586.0391	586.0381	-1	0.17	[M-H]-	C15H17N1O4S2F13	2
	596	596.0916	nd	nd	nd	[M]+	C16H19N1O1S1F17	3
	602	602.0341	602.0332	-1.5	6.63	[M-H]-	C15H17N1O5S2F13	2
Chemguard	586	586.0391	586.0375	-2.7	9.64	[M-H]-	C15H17N1O4S2F13	2
	581	581.1144	581.1125	-3.3	98.2	[M+H]+	C17H22N2O3S1F13	1
	681	681.1080	681.1093	1.9	40.34	[M+H]+	C19H22N2O3S1F17	1
	781	781.1016	781.1032	2.0	3.99	[M+H]+	C21H22N2O3S1F21	2
Buckeye	414	414.0927	414.0909	-4.3	0.66	[M]+	C12H15N1O2F11	2
	432	432.0833	432.0844	2.5	29.64	[M]+	C12H14N1O2F12	2
	514	514.0863	514.0852	-2.1	7.41	[M]+	C14H15N1O2F15	2
	532	532.0769	532.0756	-2.4	3.77	[M]+	C14H14N1O2F16	2

	614	614.0799	614.0792	-1.1	0.42	[M]+	C16H15N1O2F19	2
	632	632.0705	632.6900	-2.4	19.75	[M]+	C16H14N1O2F20	2
Fireade	513	513.0881	513.0866	-2.9	9.36	[M+H]+	C13H18N2O2S1F13	2
	571	571.0936	571.0931	-0.9	98.54	[M]+	C15H20N2O4S1F13	1
	613	613.0818	613.0799	-3.1	0.01	[M+H]+	C15H18N2O2S1F17	2
	671	671.0872	671.0879	1	99.75	[M]+	C17H20N2O4S1F17	1
	771	771.0808	771.0811	0.4	44.04	[M]+	C19H20N2O4S1F21	1
	871	871.0745	871.0724	-2.5	77.66	[M]+	C21H20N2O4F25S	1

Table 2.2. Structural identification of all identified AFFF fluorochemicals. * x:y:z fluorotelomer indicates an alkyl chain with x carbons completely fluorinated, y carbons partially fluorinated, and z carbons non-fluorinated . †Refer to the generic structures in **Figure 2.3** and **2.4**; numbers in parentheses indicate completely fluorinated chain length. 'Pat.' refers to patent used for structural determination, the numbers correspond to the reference.

AFFF Product	Nominal Mass	Generic Name	IUPAC Name	Generic Structure †	Pat.
3M	385	perfluorobutane sulfonamide amine	N-(3-(dimethylamino)propyl)-perfluorobutane-1-sulfonamide	3D (4)	1
	399	perfluorohexane sulfonic acid	1-perfluorohexane sulfonic acid	3A (6)	1, 2
	435	perfluoropentane sulfonamide amine	N-(3-(dimethylamino)propyl)-perfluoropentane-1-sulfonamide	3D(5)	1
	449	perfluoroheptane sulfonic acid	1-perfluoroheptane sulfonic acid	3A (7)	1, 2
	457	perfluorobutane sulfonamide amino carboxylic acid	3-(N-(3-(dimethylamino)propyl)-perfluorobutylsulfonamido)propanoic acid	3B (4)	1
	485	perfluorohexane sulfonamide amine	N-(3-(dimethylamino)propyl)-perfluorohexane-1-sulfonamide	3D(6)	1
	499	perfluorooctane sulfonic acid	1-perfluorooctane sulfonic acid	3A (8)	1, 2
	507	perfluoropetane sulfonamide amino carboxylic acid	3-(N-(3-(dimethylamino)propyl)-perfluoropentylsulfonamido)propanoic acid	3B (5)	1
	557	perfluorohexane sulfonamide amino carboxylic acid	3-(N-(3-(dimethylamino)propyl)-perfluorohexylsulfonamido)propanoic acid	3B (6)	1
	579	perfluoropentane sulfonamide ammonio dicarboxylic acid	N-(2-carboxyethyl)-3-(N-(2-carboxyethyl)-perfluoropentylsulfonamido)-N,N-dimethylpropan-1-aminium	3C (5)	1
	629	perfluorohexane sulfonamide ammonio dicarboxylic acid	N-(2-carboxyethyl)-3-(N-(2-carboxyethyl)-perfluorohexylsulfonamido)-N,N-dimethylpropan-1-aminium	3C(6)	1
National Foam	513	6:2 fluorotelomer sulfonamide amine	N-[3-(dimethylamino) propyl]-1H,1H,2H,2H-perfluoro-1-octanesulfonamide	4B (6)	3
	571	4:2 fluorotelomer sulfonamide betaine	N-(carboxymethyl)-N,N-dimethyl-3-(1H,1H,2H,2H-perfluoro-1-hexanesulfonamido)propan-1-aminium	4A (4)	3
	613	8:2 fluorotelomer sulfonamide amine	N-[3-(dimethylamino) propyl]-1H,1H,2H,2H-perfluoro-1-decanesulfonamide	4B (8)	3
	671	6:2 fluorotelomer sulfonamide betaine	N-(carboxymethyl)-N,N-dimethyl-3-(1H,1H,2H,2H-perfluoro-1-octanesulfonamido)propan-1-aminium	4A (6)	3
	771	8:2 fluorotelomer sulfonamide betaine	N-(carboxymethyl)-N,N-dimethyl-3-(1H,1H,2H,2H-perfluoro-1-decanesulfonamido)propan-1-aminium	4A (8)	3
	871	10:2 fluorotelomer sulfonamide betaine	N-(carboxymethyl)-N,N-dimethyl-3-(1H,1H,2H,2H-perfluoro-1-dodecanesulfonamido)propan-1-aminium	4A (10)	3
Ansul	586	6:2 fluorotelomer thioether amido sulfonic acid	2-methyl-2-(3-((1H,1H,2H,2H-perfluoro-1-octyl)thio)propanamido)propane-1-sulfonate	4C (6)	4, 5, 6
	602	6:2 fluorotelomer thioether amido sulfonic acid (+ oxygen)	n/a	n/a	
	686	8:2 fluorotelomer thioether amido sulfonic acid	2-methyl-2-(3-((1H,1H,2H,2H-perfluoro-1-decyl)thio)propanamido)propane-1-sulfonate	4C (8)	4, 5, 6
Angus	496	6:2 fluorotelomer thio hydroxy ammonium	2-hydroxy-N,N,N-trimethyl-3-((1H,1H,2H,2H-perfluoro-1-octyl)thio)propan-1-aminium	4D (6)	4
	586	6:2 fluorotelomer thioether amido sulfonic acid	2-methyl-2-(3-((1H,1H,2H,2H-perfluoro-1-octyl)thio)propanamido)propane-1-sulfonate	4C (6)	4, 5, 6
	596	8:2 fluorotelomer thio hydroxy ammonium	2-hydroxy-N,N,N-trimethyl-3-((1H,1H,2H,2H-perfluoro-1-decyl)thio)propan-1-aminium	4D (8)	4
	602	6:2 fluorotelomer thioether amido sulfonic acid (+ oxygen)	n/a	n/a	
Chemguard	586	6:2 fluorotelomer thioether amido sulfonic acid	2-methyl-2-(3-((1H,1H,2H,2H-perfluoro-1-octyl)thio)propanamido)propane-1-sulfonate	4C (6)	4, 5, 6
	581	6:2 fluorotelomer thioether amido amino carboxylic acid	4-((3-(dimethylamino)propyl)amino)-4-oxo-2((1H,1H,2H,2H-perfluorooctyl)thio)butanoic acid	4E (6)	7
	681	8:2 fluorotelomer thioether amino carboxylic acid	4-((3-(dimethylamino)propyl)amino)-4-oxo-2((1H,1H,2H,2H-perfluorodecyl)thio)butanoic acid	4E (8)	7
	781	10:2 fluorotelomer thioether amino carboxylic acid	4-((3-(dimethylamino)propyl)amino)-4-oxo-2((1H,1H,2H,2H-perfluorododecyl)thio)butanoic acid	4E (10)	7
Buckeye	414	5:3 fluorotelomer betaine	N-(carboxymethyl)-1H,1H,2H,2H,3H,3H -N,N-dimethylperfluorooctan-1-aminium	4F (5)	4
	432	5:1:2 fluorotelomer betaine*	N-(carboxymethyl)-1H,1H,2H,2H,3H -N,N-dimethylperfluorooctan-1-aminium	4E (5)	4
	514	7:3 fluorotelomer betaine	N-(carboxymethyl)-1H,1H,2H,2H,3H,3H -N,N-dimethylperfluorodecan-1-aminium	4F (7)	4
	532	7:1:2 fluorotelomer betaine*	N-(carboxymethyl)-1H,1H,2H,2H,3H -N,N-dimethylperfluorodecan-1-aminium	4E (7)	4
	614	9:3 fluorotelomer betaine	N-(carboxymethyl)-1H,1H,2H,2H,3H,3H -N,N-dimethylperfluorododecan-1-aminium	4F (9)	4
	632	9:1:2 fluorotelomer betaine*	N-(carboxymethyl)-1H,1H,2H,2H,3H -N,N-dimethylperfluorododecan-1-aminium	4G (9)	4
Fireade	513	6:2 fluorotelomer sulfonamide amine	N-[3-(dimethylamino) propyl]-1H,1H,2H,2H-perfluoro-1-octanesulfonamide	4D (6)	3
	571	4:2 fluorotelomer sulfonamide betaine	N-(carboxymethyl)-N,N-dimethyl-3-(1H,1H,2H,2H-perfluoro-1-hexanesulfonamido)propan-1-aminium	4A(4)	3
	613	8:2 fluorotelomer sulfonamide amine	N-[3-(dimethylamino) propyl]-1H,1H,2H,2H-perfluoro-1-decanesulfonamide	4B (8)	3
	671	6:2 fluorotelomer sulfonamide betaine	N-(carboxymethyl)-N,N-dimethyl-3-(1H,1H,2H,2H-perfluoro-1-octanesulfonamido)propan-1-aminium	4A (6)	3
	771	8:2 fluorotelomer sulfonamide betaine	N-(carboxymethyl)-N,N-dimethyl-3-(1H,1H,2H,2H-perfluoro-1-decanesulfonamido)propan-1-aminium	4A (8)	3
	871	10:2 fluorotelomer sulfonamide betaine	N-(carboxymethyl)-N,N-dimethyl-3-(1H,1H,2H,2H-perfluoro-1-dodecanesulfonamido)propan-1-aminium	4A (10)	3

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CHAPTER 3: TRACE ANALYSIS OF ANIONIC AND NONIONIC SURFACTANTS FROM OIL DISPERSANTS IN GULF OF MEXICO SEAWATER USING LARGE VOLUME INJECTION LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY

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Abstract

After the April 2010 explosion on the Deepwater Horizon oil rig, and subsequent release of millions of barrels of oil, two Corexit oil dispersant formulations were used in unprecedented quantities both on the surface and sub-surface of the Gulf of Mexico. Although the dispersant formulations contain four classes of surfactants, current studies to date focus solely on the anionic surfactant, bis-(2-ethylhexyl) sulfosuccinate (DOSS). For this reason, a quantitative analytical method was developed for the detection of all four classes of surfactants, as well as the hydrolysis products of DOSS α- and β-ethylhexyl sulfosuccinate (α-/β-EHSS). Large volume, direct injection of seawater, followed by liquid chromatography tandem mass spectrometry (LC-MS/MS), minimized analytical artifacts, analysis time, and chemical and solid waste. This method was applied to the analysis of the Corexit formulations and Gulf of Mexico seawater. Whole method performance characteristics included accuracy, as indicated by recovery from 88 – 119%; precision, as indicated by relative standard deviations of 10 – 23%; and limits of quantification of 49 – 3000 ng/L. Concentrations of DOSS in the seawater samples ranged from 71 – 13,000 ng/L, while the nonionic surfactants, including Span 80, Tween 80, Tween 85, were infrequently (26% of samples) detected at concentrations from 840 – 9100 ng/L. The DOSS hydrolysis products were detected (< LLOQ) in seawater and in both Corexit formulations, indicating that the hydrolysis products were applied at the time of Corexit application. Additional research is required to provide unequivocal evidence of DOSS transformation.

Introduction

On April 20, 2010, an explosion on the Deepwater Horizon oil rig resulted in a rupture at the oil well site and the subsequent release of approximately 780 million liters of oil until the flow was effectively stopped on July 15, 2010.(1) Multiple response tools were deployed to mitigate the environmental impact of the released oil, including mechanical removal, controlled burns, and the use of chemical dispersants to break up the oil slick and allow for faster biodegradation of the toxic oil constituents. During the spill, 7.0 million liters of Corexit 9500 and 9527 oil dispersant were applied to both the surface oil slick (4.1 million liters) and at the wellhead (2.9 million liters).(2) Multiple studies show low to moderate toxicity of Corexit oil dispersants, both as the dispersant alone and when mixed with crude oil.(3-6)

Corexit 9500 and Corexit 9527, contain one class of anionic surfactant (bis-(2-ethylhexyl) sulfosuccinate, DOSS) and three classes of nonionic surfactants (sorbitan monooleate, Span 80; sorbitan monooleate polyethoxylate, Tween 80; and sorbitan trioleate polyethoxylate, Tween 85).(3) The United States Environmental Protection Agency (USEPA) set the aquatic life benchmark for chronic exposure of DOSS to 40,000 ng/L and a reporting limit of 20,000 ng/L. Benchmarks were not set for the other surfactant components of Corexit dispersants.(1) Although the dispersant contains multiple components, only DOSS has received attention as a marker for the presence of Corexit dispersant. (11-13)

There is little information about the fate of these dispersants in aquatic environments. The chemical and biological transformation pathways, and the resultant toxicity of these transformation products, have not been characterized. Hales proposed the biodegradation pathway of linear dialkyl sulfosuccinates, in activated sludge, that included the oxidation of both esters, resulting in two monoester sulfosuccinate regioisomers and sulfosuccinic acid.(7) DOSS, a branched dialkyl sulfosuccinate, would hydrolyze to form α-/β-ethylhexyl sulfosuccinate (α-/β-EHSS) (Figure 3.2). In addition, α-/β-EHSS can occur as intermediates in the synthesis of DOSS. For this reason, data on the occurrence of α-/β-EHSS would yield further insight into the composition of the Corexit formulations applied to the Gulf and to differentiate the chemicals added from those that form as a result of transformation processes after application. To fully characterize the complex mixture of the dispersant formulations, analytical methods are needed for detecting a broad range of dispersant surfactants with varying chemical properties. These methods would aid in the determination of the presence and fate of parent and transformation products.

Large-volume injection liquid chromatography (LVI-LC) is a technique that reduces the number of sample preparation steps, which is important for minimizing sample analysis time and analysts' labor costs, the potential for contamination, and for reducing liquid and solid waste.(8) LVI-LC is an alternative to solid phase extraction that has been demonstrated for environmental contaminants in surface water and wastewater systems,(9, 10) but not yet for seawater. Analytical methods for the detection and quantification of only DOSS (as an indicator of the whole Corexit dispersant formulation) were previously developed to analyze Gulf of Mexico seawater.(11-13) Methods by Kujawinski et al. and Gray et al. utilized offline sample preparation techniques to concentrate DOSS from seawater samples, with detection limits of 3 ng/L and 250 ng/L, respectively.(11, 13) Mathew et al. directly injected 50 µL of 50:50 seawater:acetonitrile with the detection limit of 3,000 ng/L.(12) Only Gray et al. reports DOSS contamination at sub-µg/L concentrations from unknown sources during analysis, which resulted in an increased limit of detection for the method.(13) The current study utilizes large volume injection liquid chromatography (LVI-LC) for the detection of all the surfactant components in Corexit oil dispersant, including DOSS. To the best of our knowledge, analytical methods for nonionic surfactants, including Tween 80 and Tween 85,(14-16) have not been developed nor applied to seawater.

The purpose of this study was to develop an analytical method for the quantitative detection of the anionic and nonionic surfactant classes, as well for α-/β-EHSS, the two potential degradation products or synthetic impurities of DOSS in the two Corexit oil dispersant formulations. Large-volume injection, combined with LC-MS/MS, offers the benefit of minimal sample preparation that, along with decreasing total analysis time, also reduces the potential sources of DOSS contamination. For a demonstration of the capabilities of this analytical method, seawater samples collected during the oil spill and dispersant application were analyzed along with the Corexit formulations.

Experimental Method

Chemicals and Analytical Standards.

Standards of bis-(2-ethylhexyl) sodium sulfosuccinate (DOSS), sorbitan monooleate (Span 80), sorbitan monooleate polyethoxylate (Tween 80), and sorbitan monooleate polyethoxylate (Tween 85) were obtained from Sigma Aldrich (Saint Louis, MO). A standard containing 13C4-labeled DOSS was provided by Ed Furlong and James Gray at the United States Geological Survey National Water Quality Laboratory (Denver, CO) that was synthesized by Cambridge Isotope Laboratories, Inc (Andover, MA). Qualitative standards for the DOSS hydrolysis products, α- and β-ethylhexyl sulfosuccinate (α-/β-EHSS) were synthesized in laboratory by base-catalyzed hydrolysis of DOSS and qualitatively purified (Supporting Information (SI); "α-/β-EHSS Synthesis Method").

HPLC-grade isopropanol, acetonitrile, acetone, and methanol were purchased from Sigma Aldrich. Laboratory 18-MΩ, deionized (DI) water was obtained by an in-house Millipore Synergy unit with an LC-Pak polisher (EMD Millipore Corp, Billerica, MA). High purity ammonium acetate was also purchased from Sigma Aldrich. Instant Ocean® salt mix (Spectrum Brands Company, Madison, WI) was provided by Robert Tanguay at Oregon State University.

Parent stock standards were prepared from solid or concentrate in solvent; DOSS standards were prepared in methanol and Span 80, Tween 80, and Tween 85 were prepared in isopropanol. Although others report DOSS standards are unstable in solution for longer than 24 hours,(11) preliminary work found all organic solvent-based standards were stable for over 1.5 months at 4 ^{0}C (Figure

3.4). Analytical standards were prepared in 25% isopropanol and 75% ocean salt solution (created by mixing 15.2 g of Instant Ocean® in DI water). These analytical standards were found to be stable in the glass HPLC autosampler vials for up to 12 h, although most samples were prepared and analyzed within 8 hours (Figure 3.5).

Corexit Formulation Analysis

In order to determine the estimated concentration of each analyte in the concentrated Corexit 9500 and Corexit 9527 formulations, samples of each mixture were received from Ronald Tjeerdema of the University of California – Davis. Highly concentrated samples were created by diluting the pure formulations in methanol. Analytical samples were made in 25% isopropanol, 75% Instant Ocean at 1 mg/L and 100 µg/L concentrations. The higher concentration was used to determine the α-/β-EHSS concentrations and the lower concentration was used to determine parent analyte concentrations. All samples were analyzed using the same method as for field samples.

Field Sample Collection and Preparation.

Gulf of Mexico seawater samples were collected on the R/V Walton Smith between May 25, 2010 and June 6, 2010. The samples were collected by a CTD-Niskin rosette system at multiple sites and varying depths.(17) The collected water was then split into BD Falcon 50-mL polypropylene centrifuge tubes (BD Biosciences, San Jose, CA) and frozen immediately. The samples were kept frozen until they were shipped with blue ice to Oregon State University. Field blanks consisting of laboratory water (using a MilliQ Advantage A10 water purification system) were made on the ship and frozen until shipment. Samples were shipped frozen and stored at -20 C upon receipt.

To reduce or eliminate analyte loss, the frozen seawater samples (in the 50 mL centrifuge vials) were first weighed to determine volume and then transferred (while frozen) into a 250 mL HDPE bottle. The centrifuge vials were then rinsed with 3 aliquots of isopropanol (final isopropanol volume equivalent to 25% of the final sample volume) and the rinsate was added to the frozen seawater sample in the 250 mL bottle. Field sample preparation steps significantly impacted the recovery of analytes in the seawater samples. The addition of isopropanol to eliminate analyte loss was selected because in non-sterile seawater the nonionic analytes gave irreversible loss and filtration gave lower recoveries of all analytes except α-/β-EHSS. The experimental details that established the use of isopropanol to eliminate analyte loss are reported in the SI (“Sample and Storage Stability”, Figure 3.5).

High Performance Liquid Chromatography.

Chromatographic separations were performed using an Agilent 1100 HPLC system (Agilent Technologies, Inc., Santa Clara, CA). The HPLC was upgraded with large volume injection and multidraw kits for sample injection sizes of up to 1,800 µL. An Agilent Zorbax C18 guard column (4.6 mm ID x 12.5 mm length x 5-µm particle size) was placed in front of the a Targa C18 analytical column (2.1 mm ID x 150 mm x 5-µm particle size; Higgins Analytical, Inc., Mountain View, CA) and the guard column was replaced approximately every 100 injections. Because the HPLC gave significant background levels of DOSS, an additional Agilent Zorbax C18 guard column, with the same dimensions as described above, was placed in the flow path after the solvent mixer and purge valve but prior to the autosampler as described by Powley et al.(18) With this setup, DOSS contamination originating from within the HPLC eluted after the DOSS analyte peak (Figure 3.9).

The HPLC mobile phase included 0.5 mM ammonium acetate in DI water (A) and acetonitrile (B). The gradient program followed: a starting composition of 5% B that was held for the first 7 min, and then increased to 50% B in 0.5 min, and then to 60% B in 9.5 min, and followed by an increase to 97.5% B that was then held for 10 min before the composition returned to 5% B in 1 min for a total run time of 36 min. In addition to the solvent gradient, the flow rate was 0.5 mL/min for the first 17 min before it was increased to 0.75 mL/min for the rest of the analytical run. In order to reduce solvent dwell time (the time it takes for changes in the gradient to reach the analytical column) the autosampler switch valve was set to bypass the autosampler injector system at 7 min, in order to reduce analyte carryover, the autosampler switch valve switched back to send the mobile phase through the injector system at 17.5 min (Figure 3.10, Figure 3.11). Without this “main-pass” switch, nonionic analyte carryover ranged from 4 – 40% of the original concentration. With the switch, the nonionic analytes retained in the injection system were pushed onto the column with the 97.5% acetonitrile mobile phase and eluted with the analytes retained on the column.

Tandem Mass Spectrometry.

To prevent fouling of the sample cones by the nonvolatile salts in seawater, the initial flow from the column was diverted to waste, after 9.5 min the flow was switched to the mass spectrometer. In addition, from 16 to 23.5 min the flow was diverted to waste during the injector system cleaning step (the first 7.5 min of the main-pass switch). The entire LC-MS/MS timeline is visually shown in Figure 3.10.

Mass spectrometric detection was performed with a Waters Acquity Triple Quadrupole Mass Spectrometer (Waters Corporation, Milford, MA). DOSS, 13C4-DOSS, and α-/β-EHSS were detected in negative ionization mode with multiple reaction monitoring (MRM) mode. A single MRM transition was identified and scanned for α-/β-EHSS (m/z 309 > 81), while two MRM transitions were scanned for DOSS (quantitative: m/z 421 > 81, qualitative: m/z 421 > 227). The negatively ionized analytes were detected during the first 17 min. Span 80 was detected in positive ionization mode with MRM mode with two MRM transitions (quantitative: m/z 429 > 411, qualitative: m/z 446 > 429). The qualitative transitions for DOSS and Span 80 were monitored to verify presence, although there were no ratio requirements due to poor sensitivity of the qualitative transitions. Tween 80 and Tween 85 represent a homologous series of compounds with varying polyethoxylate chain lengths and therefore cannot be identified by a single MRM transition. Alternatively, a common fragment ion (m/z 309) was identified for both Tween 80 and Tween 85, as reported by Borisov et al.(19) Therefore, precursor ion scanning (positive ionization) was used to scan for all precursor masses (m/z 400-1300) that fragmented into m/z 309 in order to quantify the homologous series of Tween 80 and Tween 85. MS parameters for all analytes are reported in Table 3.3.The positively ionized analytes were detected for the latter 16 min of the analytical run.

Analyte Quantification.

Preliminary observations found that, even with a 95% aqueous wash step, residual salts suppressed ionization of the nonionic analytes (Figure 3.7). For purposes of compensating for the strong ion suppression of seawater, due to the high ionic strength, all analytical standards were made in 25% isopropanol and 75% Instant Ocean for matrix-matched calibration. A calibration curve consisted of at least 5 calibration standards and required a correlation coefficient of 0.99 or greater in order to be used for quantification. All calibration curves were 1/x weighted, and standards whose calculated concentrations were beyond 30% of the intended concentration were removed from the calibration curve calculation. The lowest standard concentration was determined by the calculated lower limit of quantification (LLOQ; discussed below) and the highest standard (upper limit of quantification ULOQ) was within 4 orders of magnitude for DOSS and α-/β-EHSS and 2 orders of magnitude for Span 80, Tween 80, and Tween 85 (Table 3.1). Each calibration standard was spiked with the internal standard 13C4-DOSS to give a final concentration of 100 ng/L. Because there are no commercial analytical standards available for α-/β-EHSS and the synthesis was qualitative, no independent quantitative calibration curve could be developed for α-/β-EHSS. Therefore, quantification of α-/β-EHSS was performed assuming an equimolar response ratio for α-/β-EHSS and the internal standard 13C4-DOSS (see SI: “Calculation of α-/β-EHSS Concentration”).

Accuracy and Precision.

To determine accuracy of the whole method four samples of blank Oregon Coast seawater were spiked with all analytes (including EHSS) at low concentration levels (equivalent to the second lowest standard). Recovery was determined as the ratio of calculated analyte concentration to spiked analyte concentration. Precision was reported as the relative standard deviations (RSD) of the four replicate analyses (Table 3.1).

Limits of Detection/Quantification.

First, ten blank samples, consisting of 25% isopropanol and 75% Oregon Coast seawater, were analyzed to determine a baseline background signal (i.e. noise) for all of the analytes. The area of the background signal for each analyte was integrated and a standard deviation of the area was calculated. A calibration curve spanning ≤ 2 orders of magnitude and consisting of relatively low concentrations for all analytes was then developed with analytical standards prepared in 25% isopropanol and 75% Instant Ocean solution. The limit of detection (LOD) and lower limit of quantification (LLOQ) were estimated by multiplying the background peak area standard deviation by 3.3 and 10, respectively, and dividing this value by the slope of the calibration curve.(20)

Quality Control.

Blank and check standards were used for quality control throughout the sample analysis and consisted of at least 20% of the total samples run in any given sequence. Check standards consisted of 25% isopropanol:75% Instant Ocean solution that was spiked with all analytes. For DOSS quantification, the calculated concentration for the check standards must be within 30% of the spiked concentration. For Span 80, Tween 80, and Tween 85 there were no internal standards available; therefore, the check standard criteria required concentrations to be within 35%. Due to concerns about DOSS contamination, blanks, consisting of isopropanol:Instant Ocean solution and spiked with 13C4-DOSS, were used regularly to verify that background DOSS concentration levels were below the LLOQ and that there was no carryover of any of the analytes. Failure to meet QC criteria required corrective action until QC checks were brought back into control before proceeding with sample analysis

Results and Discussion

To the best of our knowledge, this is the first study to quantitatively detect multiple surfactant analytes in dispersant formulations by the large volume, direct injection of seawater. Although samples consisted of 75% seawater, all analytes were chromatographically separated (Figure 3.1) without instrumental concerns related to the high salt concentration. DOSS, α-/β-EHSS, Span 80 are single compounds that could be identified using the common multiple reaction monitoring (MRM) mode. The detection of the homologous series of Tween 80 and Tween 85 was more challenging because the varying chain lengths of the polyethoxylates made MRM detection for each individual compound difficult. This was also complicated by the fact that analytical standards for any single homologue are not commercially available, so that only the mixture standards were available for purchase. The precursor ion scanning technique, detecting all compounds that produce the m/z 309 fragment ion, provided an alternative to MRM for the detection of Tween 80 and Tween 85 (Figure 3.12).

LVI-LC is a tool for the sensitive detection of analytes in environmental aqueous samples that avoids extensive sample preparation. While there are often concerns related to the direct injection of seawater for mass spectrometric detection, during the extent of the method development and sample analysis there were no observed instrumental issues.

The injection of non-volatile salts is of a concern for any analytical method utilizing mass spectrometric detection because salt sprayed into the ionization chamber can lead to sample cone fouling and corrosion. Utilizing the post-column divert valve built into the Waters Acquity TQD mass spectrometer, the initial flow, containing most of the salt, was diverted to waste away from the mass spectrometer (Figure 3.10, Figure 3.11). This was a vital step in the protection of the MS system during sample analysis. After months of analyses there was no significant deposition of salt on the sample cones. While column fouling is also a concern with large volume injection, a single analytical column was used for approximately 1 year (~ 2500 large volume injections) without observing diminishing chromatographic peak quality. Guard columns could be used for approximately 100 injections before peak shape deterioration (primarily peak tailing and splitting). Even with the above described instrumental protection procedures, ionization suppression was observed for the nonionic analytes (Figure 3.7). We propose that the decrease in sensitivity is due to the formation of sodium-adducted compounds, which result from low levels of residual salts that were retained with the analytes and co-eluted into the mass spectrometer. Sodium-adducted compounds have been previously reported to decrease fragmentation efficiency.(21, 22)

Method Accuracy and Precision.

Whole method accuracy, as indicated by percent recovery, ranged from 81 – 119% (Table 3.1). Whole method precision, as indicated by relative standard deviation (RSD), ranged from 10 – 23% (Table 3.1). Higher RSD values were observed with Tween 85 (17%) and Span 80 (23%), which is due to higher detection limits for these compounds as well as the lack of internal standards to accommodate for between-injection differences in ionization efficiency. The developed method provides similar recovery of DOSS (88 ±10%, mean ± 95% CI) as those for previously reported methods (80 – 100% recovery).(11-13) In contrast, this LVI method required no sample preparation other than the addition of isopropanol. The addition of isopropanol, which ensured analyte stability in seawater, was half of the dilution used by Mathew et al.(12) The use of $^{13}C_4$-DOSS as an internal standard for the nonionic compounds was evaluated and the labeled compound did not adequately describe the variation of any of the nonionic compounds. Therefore it could not function as an internal standard for any nonionic analyte. Future research examining the presence and fate of the nonionic analytes will require analytical standards for the individual Tween 80 and Tween 85 polyethoxylate homologues and isotopically labeled internal standards for these analytes.

Recoveries for the nonionic analytes were better in the isopropanol:Instant Ocean solution than in an ammonium acetate buffer solution, suggesting that the high salt content of the seawater is the primary source of ion suppression and requires matrix-matched calibration (SI “Salt Elution Timeline”, Figure 3.3, Figure 3.7).

Limits of Detection/Quantification

Limits of detection (LOD) and lower limits of quantification (LLOQ) ranged from 1 to 1,300 ng/L and 49 to 3,000 ng/L, respectively (Table 3.1). The background contamination level of DOSS had a mean estimated concentration of 10 ng/L. Due to the high variability (130% RSD) of the DOSS background contamination the LOD was conservatively raised to be equal to the LLOQ at 67 ng/L (Table 3.1). The use of laboratory blanks, travel blanks, and sample blanks were extremely important for eliminating the sources of DOSS contamination, which were found to occur on container surfaces and in solvents. The DOSS LOD (67 ng/L) is higher than that reported by Kujawinski et al. (3 ng/L),(11) although it is below other published methods (250 ng/L(12, 13) and 20,000 ng/L10). Comparisons of the LOD and LLOQs of α-/β-EHSS or nonionics in seawater was not possible because comparable methods do not exist.

The sensitivity of DOSS and EHSS were multiple orders of magnitude better than those of the nonionic analytes (Span 80, Tween 80, Tween 85). This is most likely due to the poorer ionization efficiency of the analytes and broader peak shape. In addition, the peaks designated as Tween 80 and Tween 85 represent a broad series of polyethoxylate compounds, which results in a broader overall peak.

Method Demonstration

Corexit 9500 and 9527 Formulations

Whole Corexit 9500 and Corexit 9527 formulations were determined to contain 18% and 17% (w/w) DOSS, respectively. Both Corexit 9500 and Corexit 9527 contained detectable quantities of α-/β-EHSS at 0.0052% (w/w) and 0.012% (w/w), respectively. The ratios of β-EHSS:α-EHSS in the Corexit formulations were 0.4 and 0.2 for Corexit 9500 and Corexit 9527, respectively (Figure 3.13). These ratios are only applicable to the products available for analysis and the actual ratio could vary between lot numbers. It was beyond the scope of the current study to determine whether these concentrations are due to synthetic impurities or the degradation DOSS during storage of the Corexit formulations.

The nonionic surfactants were detected in the Corexit 9500 at 4.4% (w/w, Span 80), 18% (w/w, Tween 80), and 4.6% (w/w, Tween 85) and in the Corexit 9527 formulation at 2.7% (w/w, Span 80), 11% (w/w, Tween 80), and 4.3% (w/w, Tween 85). It should be noted that these concentrations could vary between batches and the reported values will not be representative of all Corexit formulations used in the Gulf. The formulations consisted of other components outside of the scope of the current work, including petroleum distillates, 1,2-propanediol, 2-butoxy-ethanol, and 1-(2-butoxy-1-methoxy)-2-propanol.(3)

DOSS

Quantifiable concentrations of DOSS were detected in over half of the seawater samples analyzed, with concentrations ranging from 71 to 13,000 ng/L (Table 3.2). The majority of the samples containing detectable DOSS concentrations were at depths deeper than or equal to 1,000 m, with a mean concentration of 4,100 ng/L (n=8). The mean concentration at the more shallow depths was 110 ng/L (n=4). The measured DOSS concentrations of depth seawater samples are consistent with those previously reported by Kujawinski et al.(11) but are at concentrations below the detection limits reported by Mathew et al.(12)

In the absence of isopropanol, DOSS loss from seawater samples was due to sorption to the HDPE container walls, suggesting that DOSS will associate with organic particulate in an aqueous environment. Future work should examine the concentrations of DOSS in ocean sediments as a potential environmental compartment for the accumulation of DOSS.

α-/β-EHSS

While no quantifiable concentrations of α-/β-EHSS were detected in any of the analyzed seawater samples, there were multiple detections that were above the LOD of 1 ng/L (n=12; Table 3.2). Although most α-/ β-EHSS detections corresponded with DOSS detections, there were samples that contained detectable quantities of α-/ β-EHSS without DOSS and vice versa. The greatest limitation on α-/ β-EHSS quantification of the samples was due to the dependence of the α-/β-EHSS LLOQ on the lowest DOSS standard. Current work is focused on the quantitative synthesis of an analytical standard for α-/β-EHSS and other DOSS transformation products, which will improve the ability to quantify DOSS transformation products in seawater samples.

While the other analytes portrayed sample stability issues in laboratory seawater standards, α-/β-EHSS compounds did not display any loss of concentration in seawater. This observation suggests that α-/β-EHSS are more water soluble and will be in the aqueous phase longer than any of the parent analytes. Because detectable quantities of α-/β-EHSS were measured in the Corexit formulations, the detection of α-/β-EHSS in seawater cannot be used as an unambiguous indicator of DOSS degradation in the environment.

α-EHSS, but not β-EHSS, was also at detectable concentrations in the DOSS analytical standards, most likely as a synthetic impurity (approximately

400 ppm concentration in 96% pure solid DOSS standard). The β-EHSS:α-EHSS ratio for the EHSS standard synthesized in laboratory was 0.5 (Figure 3.13). As stated above, α-EHSS was present in the stock DOSS solution, therefore the increase in β-EHSS:α-EHSS from 0 to 0.5 suggests that the hydrolysis reaction primarily synthesized β-EHSS. The synthesis of α-/β-EHSS from the DOSS standard was the result of a 16 h base-catalyzed hydrolysis with 5 M LiOH and the yield of α-/β-EHSS was low. In seawater, with a pH generally around 8, the hydrolysis reaction rate (if solely mediated by ocean basicity) would be much less than the synthesis reaction rate. In the seawater samples, the β-EHSS:α-EHSS ratios varied from 0.02 to 1.5. The ratios that are greater than 0.4 (the highest observed in the analyzed Corexit formulation) suggest that β-EHSS (and α-EHSS to a lesser extent) is produced in seawater due to the hydrolysis of DOSS, which results in the increased β-EHSS:α-EHSS ratio. Further work to determine the chemical and biodegration of DOSS for the formation of β-EHSS and α-EHSS, and the resulting β-EHSS:α-EHSS ratio, is necessary to determine the environmental implications of these measurements.

Nonionic Compounds

There were no detectable quantities of Span 80 in any of the analyzed samples (Table 3.2). Samples that were positive for the nonionic analytes contained concentrations for Tween 80 that ranged from 3,500 to 9,100 ng/L (n=4) and Tween 85 that ranged from 840 to 2,900 ng/L (n=3, Table 3.2). There was no significant correlation between concentrations of Tween 80 and Tween 85 (correlation coefficient r2=0.48, n=6). While there was a greater number of analyte detections observed at the lower depths, the purpose of the sampling program was not to obtain sufficient monitoring data to develop a correlation between depth and analyte concentration.

The difficulty of stabilization of the nonionic compounds in seawater, combined with relatively high LLOQs, is consistent with the relatively few observations of the nonionic analytes in seawater. The degradation of the nonionic surfactants in various conditions has been previously reported by many researchers, and have found the fatty acid ester structure of the compounds to be readily oxidizable by both base- and acid-catalyzed reactions, as well as by autooxidation.(23) Others report the rapid biological loss of sorbitan polyethoxylates, such as Tween 80 and Tween 85, due to the degradation by esterase enzymes.(24) This is consistent with the rapid loss of the nonionic analytes in non-sterilized laboratory seawater. Additional research is required to better understand the fate and potential degradation of the nonionic analytes in seawater systems.

Acknowledgements

The authors would like to thank Nancy Kerkvliet (OSU) for providing space for the EHSS synthesis; James Gray and Ed Furlong (USGS) for collaborative discussions and provision of the $^{13}C_4$-DOSS standard; Samantha Joye and Kim Hunter (UGA) for the collection and provision of Gulf of Mexico seawater samples; and Ronald Tjeerdema (UCD) for early collaborations related to Corexit oil dispersant. In addition, the others would like to acknowledge Paul Blakemore of Oregon State University for his advice on the synthesis of α-/β-EHSS.

Gulf of Mexico sample collection, aboard the R/V Walton Smith, was supported by a grant from the National Science Foundation OCE-1043224 given to Samantha B. Joye. In addition, this study was supported, in part, by a grant from the BP/The Gulf of Mexico Research Initiative (as part of the University of Mississippi-lead consortium for research entitled ‘Ecosystem Impacts of Oil and Gas Inputs to the Gulf (ECOGIG)’), the OSU Superfund Research Program Award Number P42ES016465 from the National Institute of Environmental Health Sciences, and the N.L. Tartar Research Fellowship from Oregon State University.

Table 3.1. Whole method performance indicated by recovery, precision, limit of detection (LOD), lower limit of quantification (LLOQ), upper limit of quantification (ULOQ), and quantification method.

Compound	LOD (ng/L)	LLOQ (ng/L)	ULOQ (ng/L)	Recovery (% ± 95% CI)	RSD (%)	Quantification Method
DOSS	67*	67	34,000	88 ± 10	10	ISC: $^{13}C_4$-DOSS
α-/β-EHSS	1†	49†	25,000	86 ± 11	11	ISC: $^{13}C_4$-DOSS
Span 80	1,250	3,000	60,000	91 ± 21	23	Ext. Cal
Tween 80	987	2,700	400,000	119 ± 13	10	Ext. Cal
Tween 85	99	700	150,000	106 ± 20	17	Ext. Cal

* DOSS LOD is equal to DOSS LOQ due to background variability. † α-/β-EHSS concentrations were determined assuming equal molar response as that of DOSS. ISC: 13C4-DOSS - internal standard calibration using 13C4-DOSS as internal standard, Ext. Cal. - external standard calibration

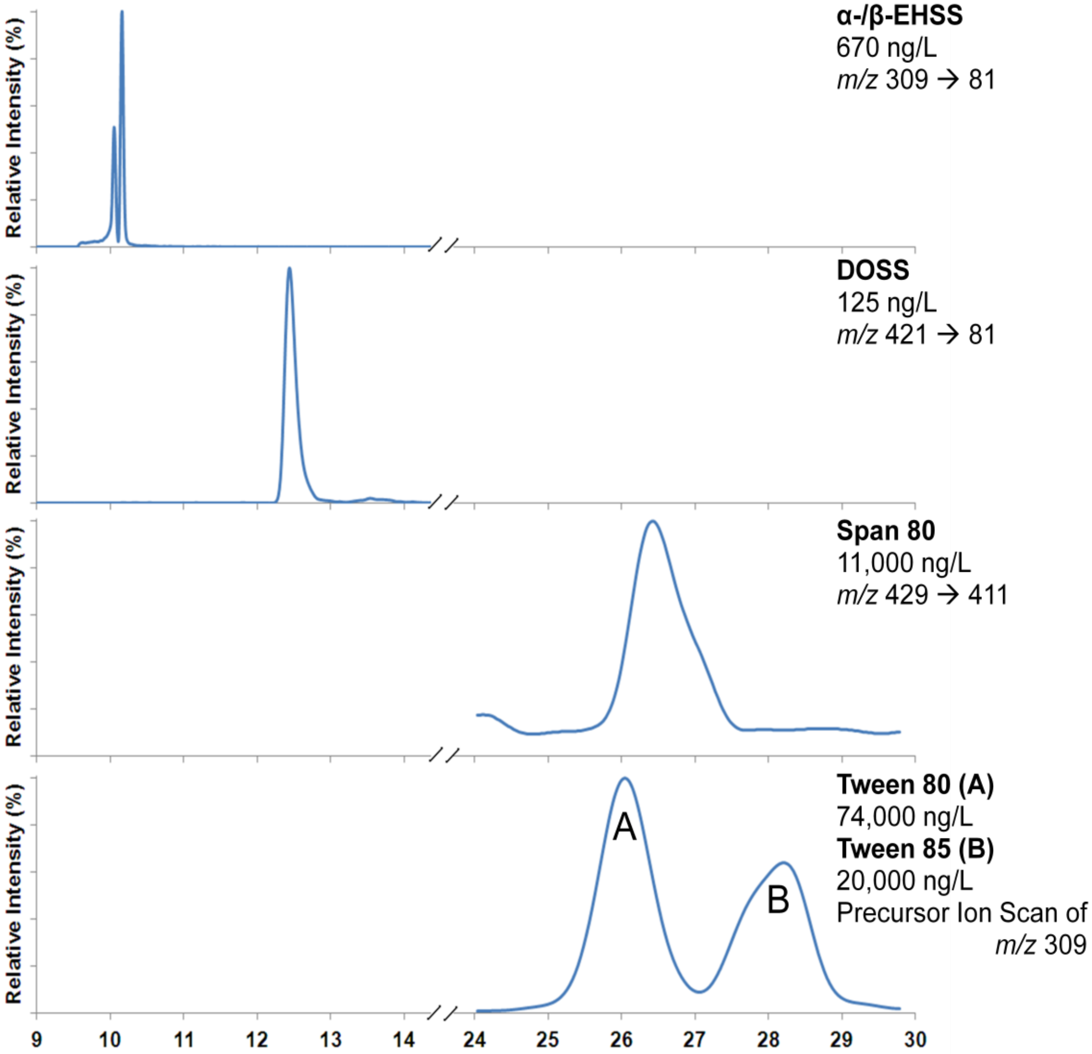

Figure 3.1. An LVI-HPLC/MSMS chromatogram of all analytes in an analytical standard consisting of 25% isopropanol and 75% Instant Ocean.

Table 3.2. Concentrations of DOSS, α-/β-EHSS, Span 80, Tween 80, and Tween 85 for each sampling location, with sample conditions of depth and distance from the Deepwater Horizon well head (designated MC252).

Sample Station	Cast	Distance to MC252 well head (m)	Depth (m)	[DOSS] ng/L	[α-/β-EHSS] ng/L	[SPAN80] ng/L	[TWEEN80] ng/L	[TWEEN85] ng/L
WS58	75	410	600	nd	nd	nd	nd	nd
			900	nd	nd	nd	nd	nd
			1210	7,700	< LLOQ	nd	nd	860
			1400	nd	nd	nd	nd	nd
WS6	73	610	1180	13,000	nd	nd	4,800	840
WS76	86	1290	50	nd	nd	nd	nd	nd
			1000	nd	nd	nd	9,100	nd
			1100	100	nd	nd	5,900	nd
			1200	11,000	< LLOQ	nd	nd	2,900
WS78	90	13320	90	nd	< LLOQ	nd	nd	nd
			600	95	< LLOQ	nd	nd	nd
			1130	nd	nd	nd	nd	nd
WS79	91	15790	90	71	< LLOQ	nd	nd	nd
			90	110	nd	nd	nd	nd
			600	nd	< LLOQ	nd	3,500	nd
			900	76	< LLOQ	nd	nd	nd
			1050	170	< LLOQ	nd	nd	nd
WS16	89	17700	100	nd	nd	nd	nd	nd
			600	170	< LLOQ	nd	nd	nd
			1025	76	< LLOQ	nd	nd	nd
			1100	nd	< LLOQ	nd	nd	nd
			1200	220	< LLOQ	nd	nd	nd
			1300	200	nd	nd	nd	nd

< LLOQ designates the analyte was below the lower limit of quantification but above the limit of detection, while “nd” indicates the analyte was below the limit of detection.

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SUPPORTING INFORMATION

TRACE ANALYSIS OF ANIONIC AND NONIONIC SURFACTANTS FROM OIL DISPERSANTS IN GULF OF MEXICO SEAWATER USING LARGE VOLUME INJECTION LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY

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EXPERIMENTAL METHODS

α-/β-EHSS Synthesis Method.

Materials. The DOSS solution was made to be a concentration of 0.5 M in THF (Sigma Aldrich). A solution of 5 M lithium hydroxide (Sigma Aldrich) was made in Millipore deionized laboratory water. For quenching the reaction, 0.5 M HCl (Sigma Aldrich) was made in Millipore deionized laboratory water.

Synthesis Procedures. A 5 mL aliquot of the 0.5 M DOSS solution was added to a 15 mL graduated conical vial, followed by an addition of 1 mL 5 M LiOH. The mixture was then stirred for a set amount of time (4, 6, and 16 hours) in a hood. After the time, the solution was quenched with 0.5 M HCl to bring to a neutral pH. The top (THF) layer was then removed from the solution and was stored at 4 C until analysis.

Purification. The degradation solution (in THF) was allowed to come to room temperature, then 25 µL of the solution was diluted with 0.5 mM ammonium acetate buffer to 25 mL for a 1:1000 dilution. The solution was then filtered through a styrene divinylbenzene (SDB) solid phase extraction membrane (3M Company, St Paul, MN) and the aqueous solution was captured.

Calculation of α-/β-EHSS Concentration.

Due to the lack of an analytical standard for α-/β-EHSS, the concentration of the analyte was estimated assuming equal molar ionization of DOSS to α-/β-EHSS.

Using the response ratio of α-/ β-EHSS:$^{13}C_4$-DOSS and the slope DOSS:$^{13}C_4$-DOSS calibration curve, the concentration of α-/ β-EHSS was calculated by the following equations:

(α-/ β-EHSS:$^{13}C_4$-DOSS ratio)/(DOSS: $^{13}C_4$-DOSS slope) = [α-/ β-EHSS] (ng DOSS / L)

[α-/ β-EHSS]*(421 g DOSS/mol)$^{-1}$*(309 g α-/ β-EHSS/mol) = [α-/ β-EHSS] (ng α-/ β-EHSS / L)

Salt Elution Timeline.

In order to determine the required time for the complete elution of the salts in the seawater samples, an eluent precipitation experiment was developed. A sample of 1,800 µL (25% Isopropanol, 75% Instant Ocean sample) was injected using the developed analytical HPLC parameters except with the post-column eluent collected instead of injected into the MS. After the injection, 30 s (0.25 mL) fractions of eluent were collected over the first 10 min of the analytical run.

A solution of 1 M $AgNO_3$/0.6 M HNO_3 was made in deionized water for the precipitation of solid AgCl (Cl^- as a broad indicator of seawater). Preliminary experiments found samples containing > 0.1% seawater showed visible AgCl precipitate. A 100-µL aliquot of the $AgNO_3$ solution was added to each eluent and the samples were briefly shaken.

Sample and Storage Stability.

Standard Stability. Standards made from pure solid (for DOSS) or liquid (for Span 80, Tween 80, and Tween 85) were made in 25-mL volumetric flasks with methanol (for DOSS) or isopropanol (for Span 80, Tween 80, and Tween 85). Standards were made at three different dates to compare the long term stability of the stock and stored at 4 ^{o}C until analysis. Standards that were 31 days old and 44 days old were compared to standards made on the day of analysis. Working standards, consisting of 25% isopropanol and 75% Instant Ocean or 100% Instant Ocean, were made in 6-mL glass autosampler vials and analyzed over time while left at 4 ^{o}C on the autosampler tray. Each vial was sample was analyzed at least 4 times over 12 hours.

Short Term Sample Stability. Standards of all analytes were made in 100% Oregon Coast seawater at the second lowest concentration level in order to mimic seawater samples. The samples were stored in 50-mL HDPE centrifuge tubes and three samples of each standard were stored at room temperature (20 ^{o}C), 4 ^{o}C, and -20 ^{o}C. At three time points over 13 hours, one sample at each temperature was prepared as described in the *Field Sample and Preparation* section of the **Experimental Methods**.

Long Term Sample Stability. To determine the long term stability of seawater samples, open ocean water collected from the Oregon coast was spiked with all analytes and the mixture was separated into multiple 50-mL centrifuge tubes. All long term stability samples were then stored at -20 ^{o}C until analysis. During each analysis, for a total of 7 months, individual samples were thawed with isopropanol, as described above.

RESULTS AND DISCUSSION

Salt Elution Timeline.

AgCl precipitate was observed for samples from 1 min to 7.5 min, suggesting majority of the salts eluted during this time. The post-column divert valve was set to divert the eluent flow to the mass spectrometer only after 9.5 min, which verified that no high concentrations of salt would be directed to the MS (**Figure 3.2**).

Storage and Sample Stability

Standard Stability. Parent stock standards were stable within an acceptable range over 44 days of analysis when stored at 4 ^{o}C (**Figure 3.3**). It was therefore assumed that all standards would be stable for long term storage in 100% organic solvent when stored at the designated temperature. The addition of 25% isopropanol to Instant Ocean was necessary for the stability of all analytes in the working standards (**Figure 3.4**).

Short Term Sample Stability. All analytes did not show significant loss when prepared by thawing the frozen sample in 25% isopropanol (**Figure 3.5**). Spiked ocean water in HDPE bottles showed rapid loss of all parent analytes (not EHSS) when sitting at room temperature. Originally, it was thought that the loss of these analytes was due to sorption to the plastic walls, therefore an isopropanol rinse was used to recover the sorbed analytes. While this IPA rinse did recover DOSS analytes, suggesting sorption was one mechanism of loss, it did not recover the nonionic surfactants. This indicates that sorption to the vial wall may not be the only mechanism of analyte loss. Biodegradation may be a source of irretrievable loss of the nonionic surfactants, as the seawater was not sterilized.

In addition, analytes spiked into non-sterilized seawater showed irreversible loss of the nonionic analytes (Span 80, Tween 80, and Tween 85) over a short period of time at room temperature (significant loss occurred after 1 hour, **Figure 3.5**).

The method of sample thawing into 100% isopropanol for the final sample composition produced the most consistent results with full recovery of all analytes. If the loss was due to biodegradation, the isopropanol sterilizes the solution and therefore ceases any further biodegradation activity upon thawing.

Long Term Storage Stability. For Tween 80 and Tween 85, there were no significant changes in concentration after 7 months at -20 °C in seawater (as determined by the slope, $p > 0.05$). For Span 80, there was a significant negative slope ($p < 0.05$) that would result in a 64% decrease in concentration over the 7 months of analysis. For DOSS, there was a significant negative slope ($p < 0.05$) that would result in a 21% decrease in concentration over the 7 months of analysis.

Figure 3.2. Hydrolysis transformation pathway for DOSS to α- and β-EHSS.

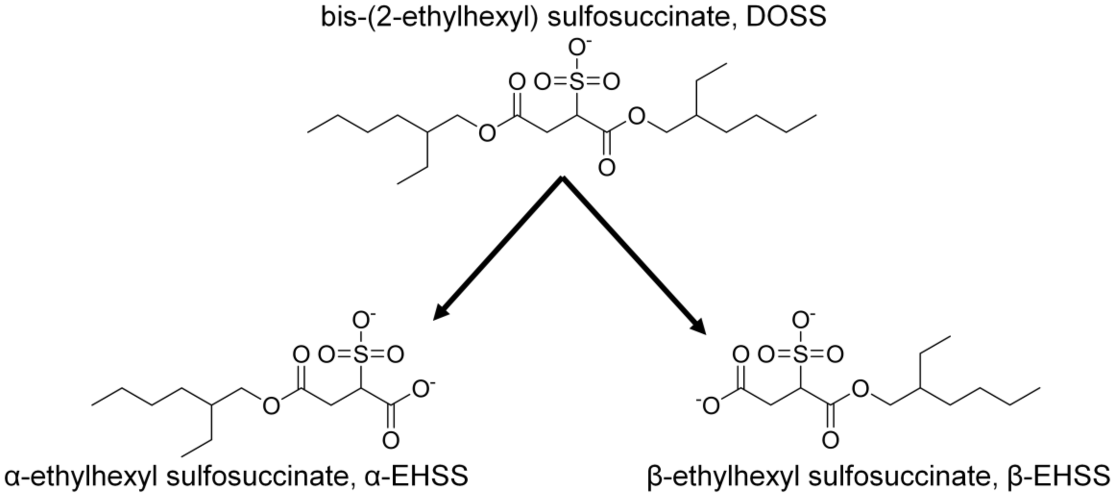


Figure 3.3. Visual demonstration of salt elution from the analytical column using $AgNO_3$ as a precipitation indicator of Cl^- ions.

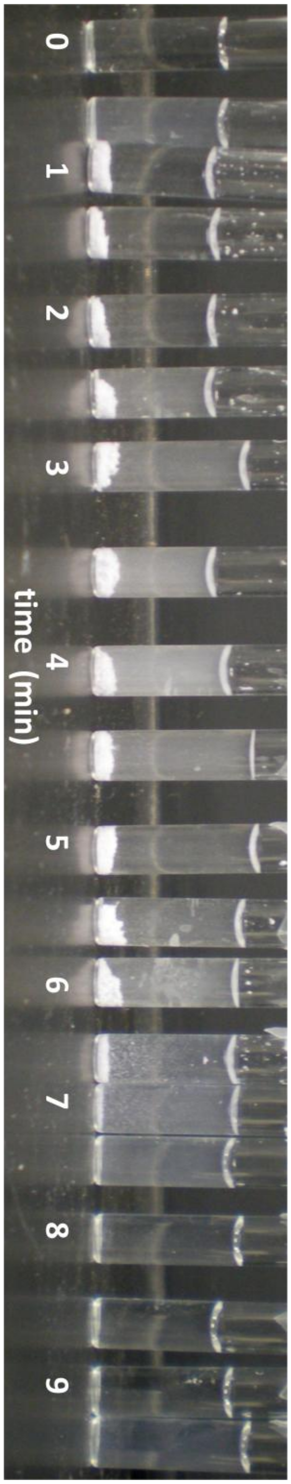


Figure 3.4. Stability of analytes in parent stock standards prepared in methanol (DOSS) and isopropanol (Span 80, Tween 80, Tween 85).

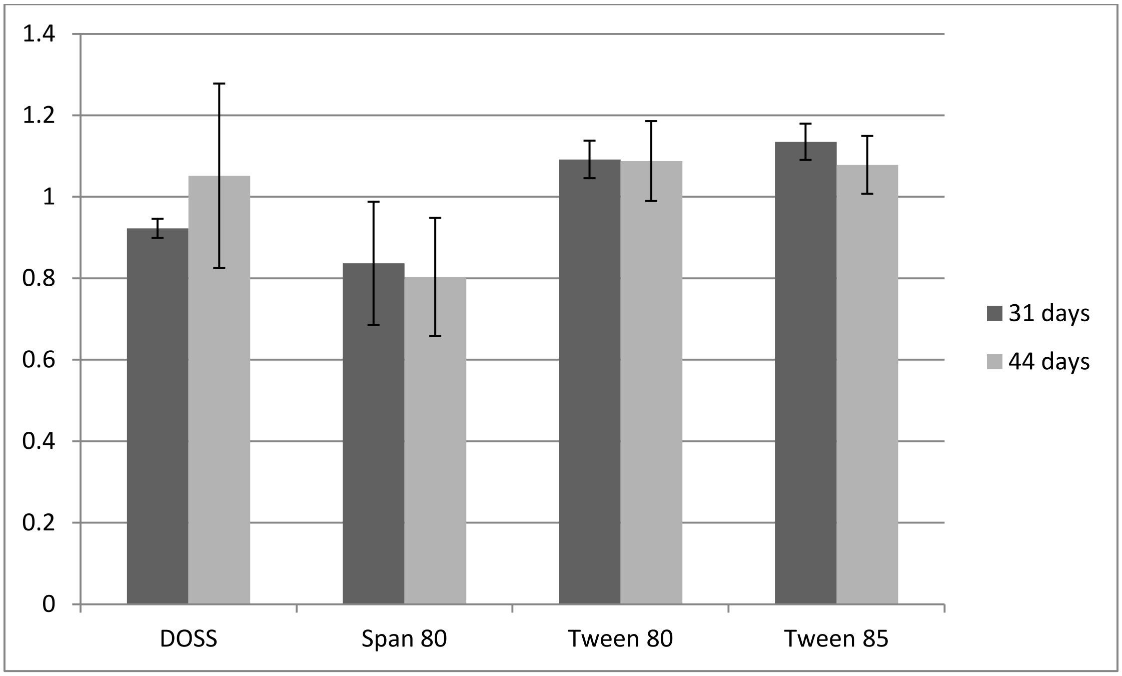
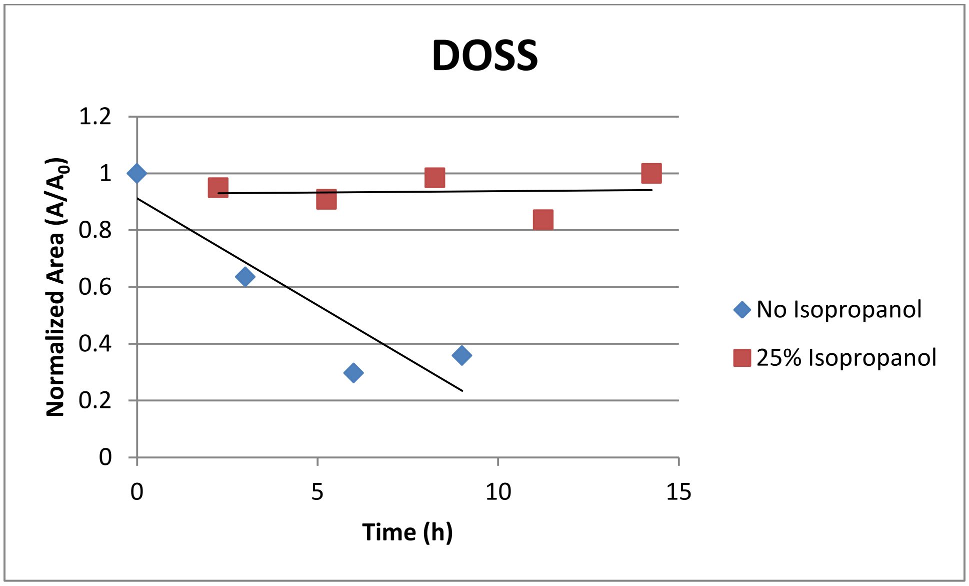


Figure 3.5. Stability of analytical standards in HPLC autosampler vials with/without 25% isopropanol. Normalized area was calculated by dividing the resultant area counts by the largest area counts for that compound.



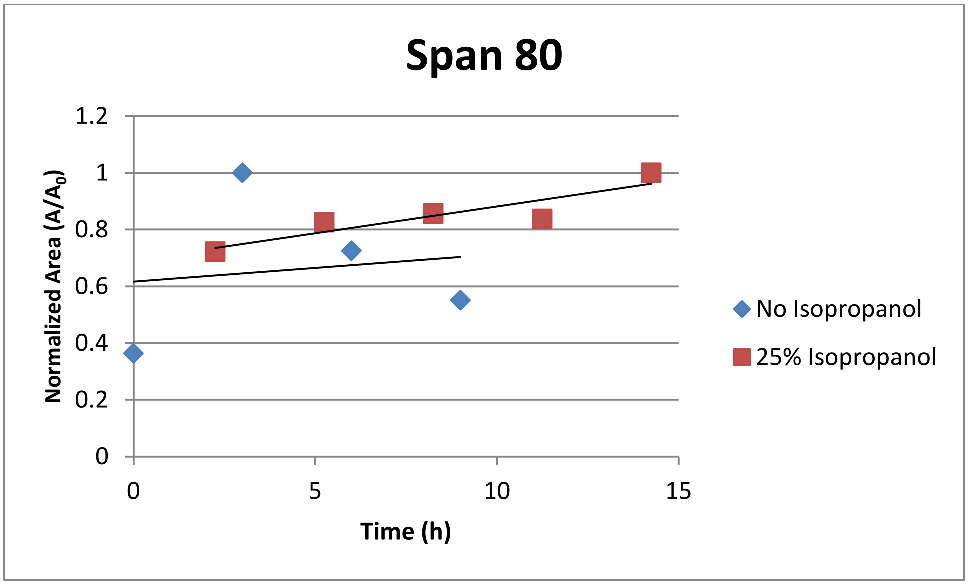
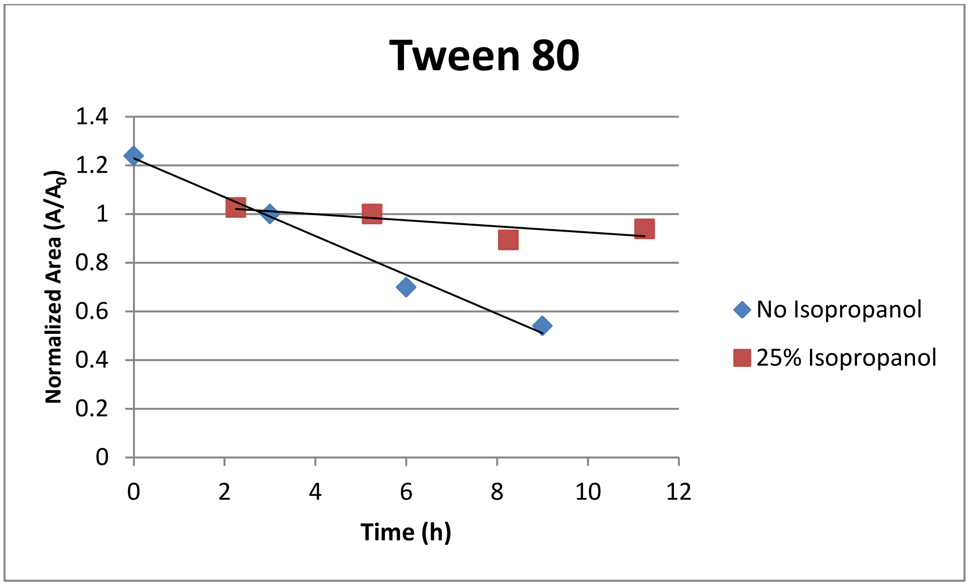


Figure 3.5 cont'd. Stability of analytical standards in HPLC autosampler vials with/without 25% isopropanol. Normalized area was calculated by dividing the resultant area counts by the largest area counts for that compound.



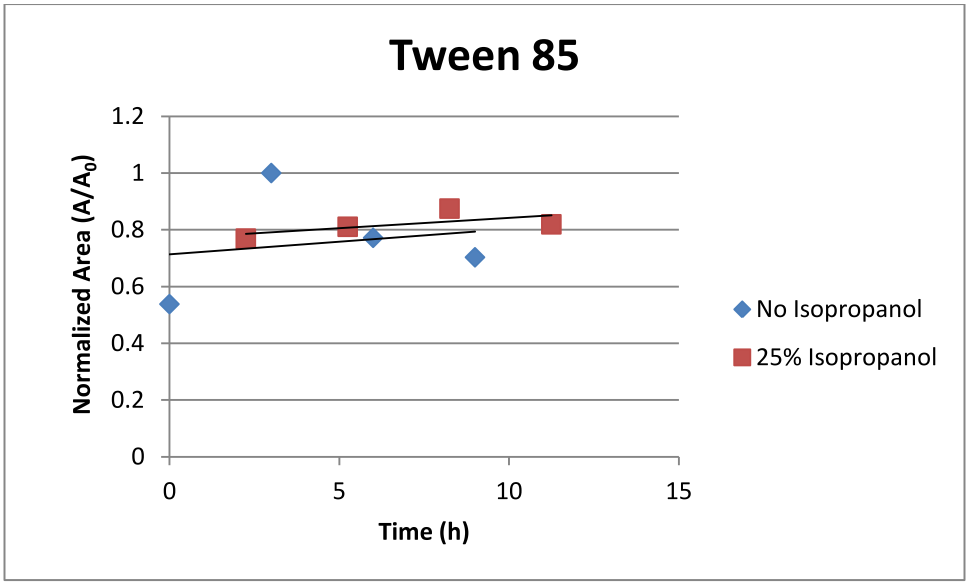


Figure 3.6. Short term stability of the analytes in seawater sitting in HDPE centrifuge tubes at various temperatures. After storage, but prior to analysis, all samples were transferred to a 250 mL HDPE bottle and the centrifuge tube was rinsed with isopropanol.

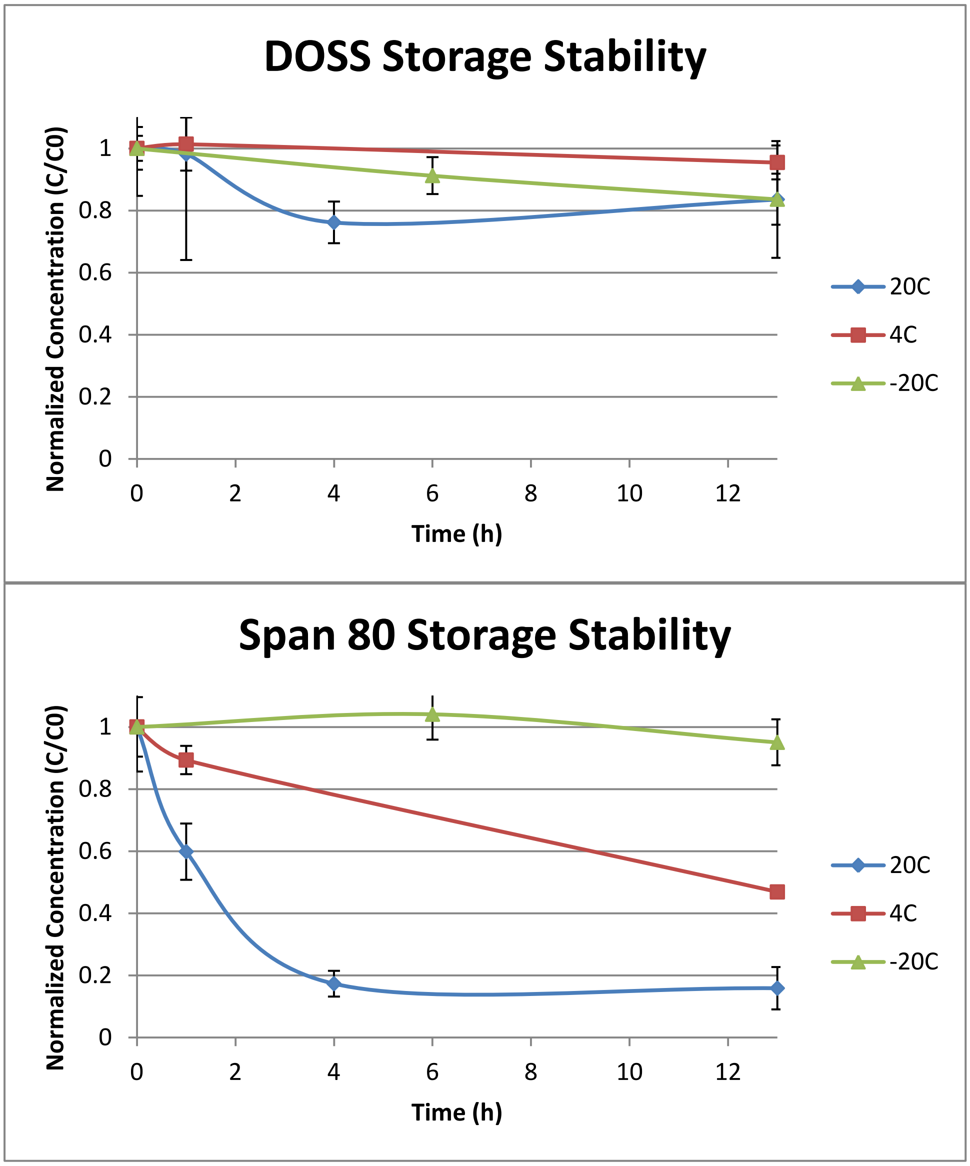


Figure 3.6 cont'd. Short term stability of the analytes in seawater sitting in HDPE centrifuge tubes at various temperatures. After storage, but prior to analysis, all samples were transferred to a 250 mL HDPE bottle and the centrifuge tube was rinsed with isopropanol.

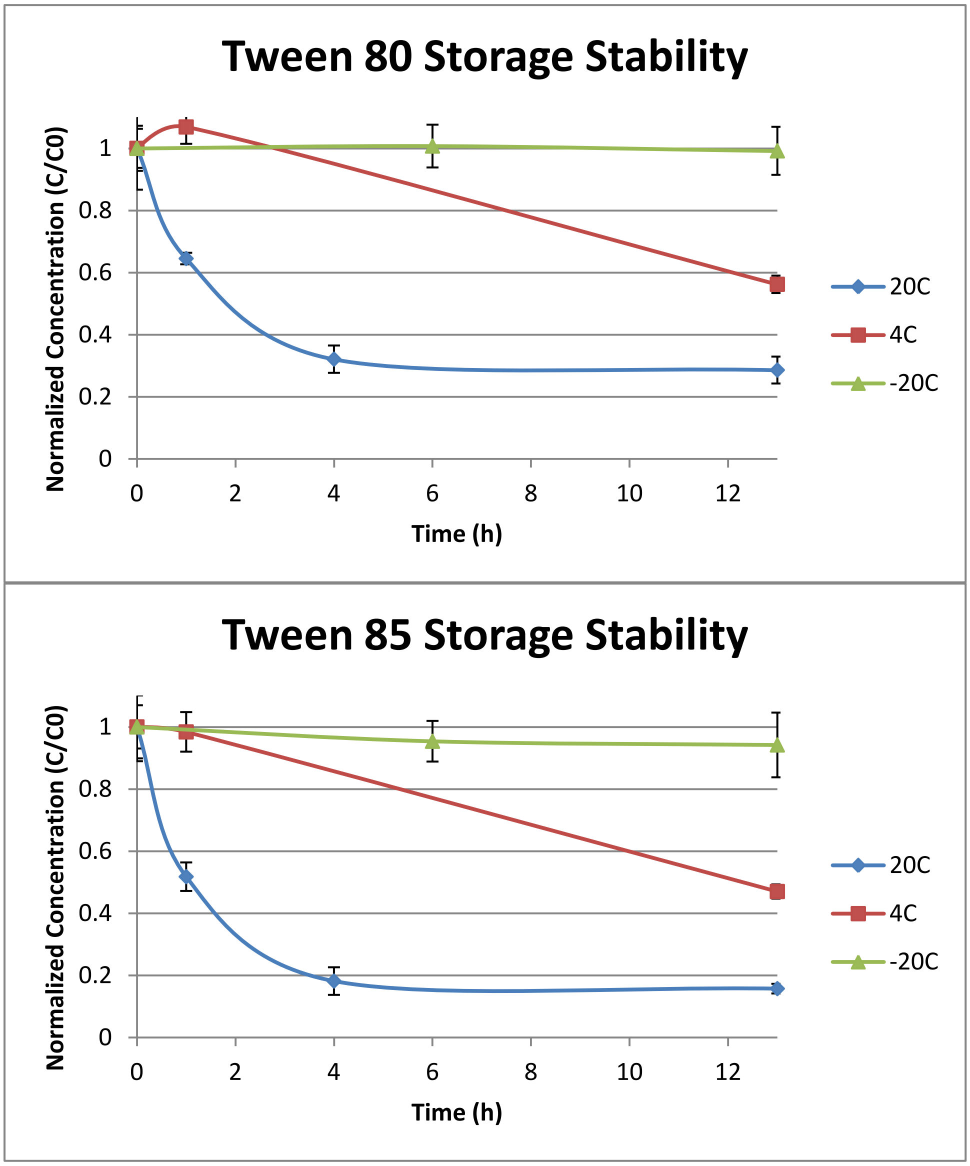


Figure 3.7. Matrix effects, as indicated by normalized peak area counts, of nonionic analytes prepared in buffered water (0.5 mM ammonium acetate), Instant Ocean, and Oregon Coast water.

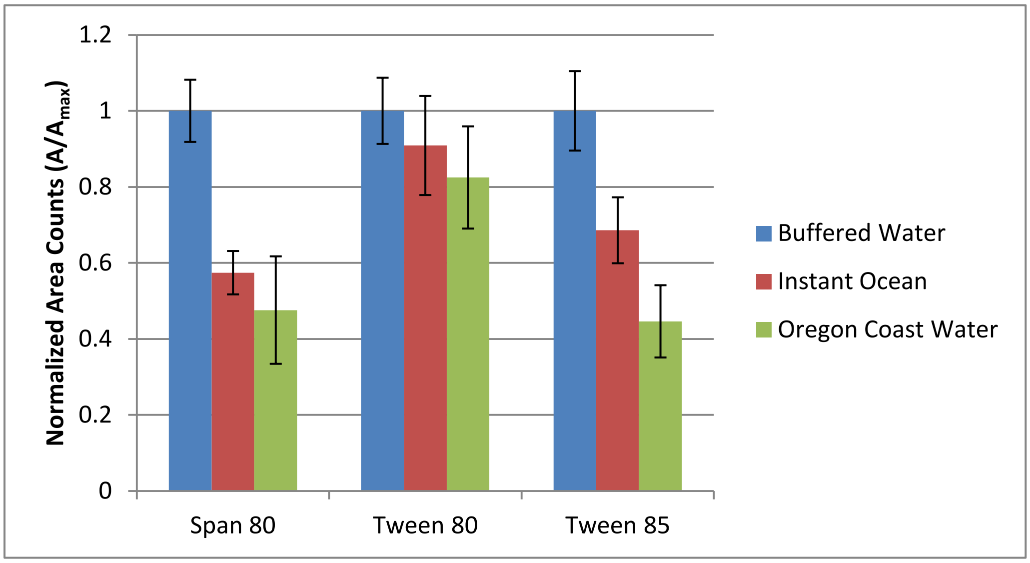


Figure 3.8. Recovery of all analytes before and after filtration through a Sterivex 0.2-µm sterilization filter.

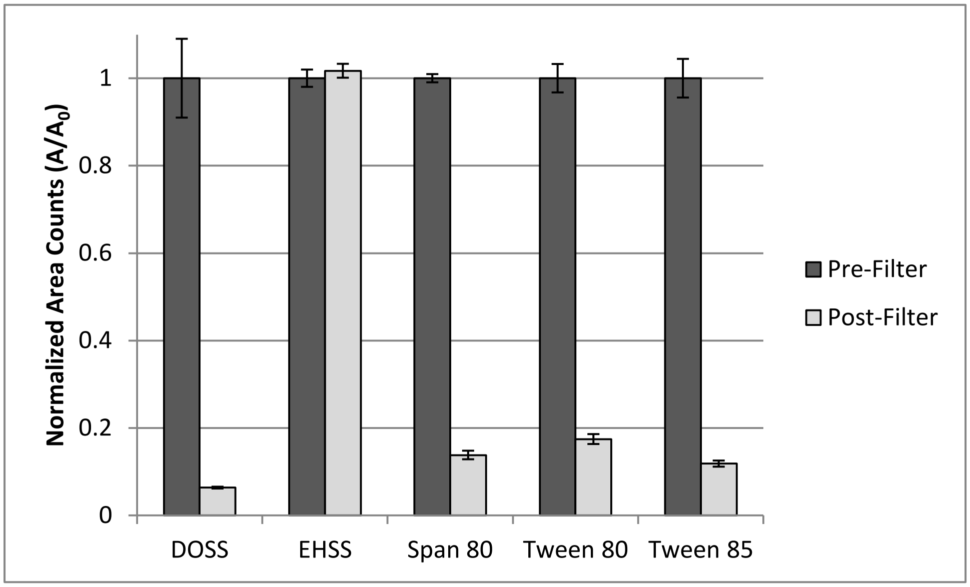


Figure 3.9 Chromatographic separation of DOSS analyte in sample (A) and the system background contamination (B) using a guard column placed after the pump mixer, but before the autosampler.

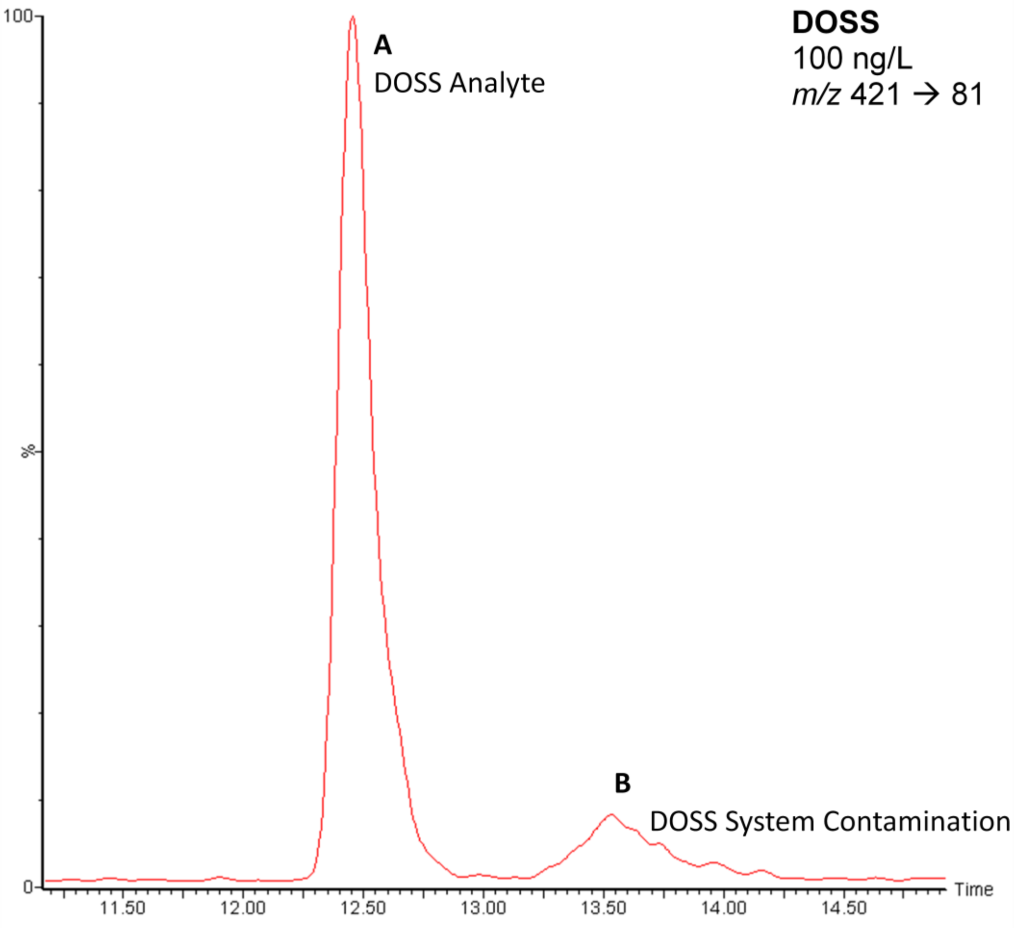


Table 3.3. MS Parameters for the detection of all analytes. * indicates secondary MRM transitions used for analyte verification.

Analyte	**Retention Time (min)**	**MS Ionization**	**Scan Mode**	**Parent Ion (*m/z*)**	**Daughter Ion (*m/z*)**	**Cone Voltage (V)**	**Collision Energy (V)**
DOSS	12.7	Negative	MRM	421	81	44	26
		Negative	MRM	421	227*	42	18
$^{13}C_4$-DOSS	12.7	Negative	MRM	425	81	44	26
α-/β-EHSS	10.1	Negative	MRM	309	81	44	26
Span 80	26.8	Positive	MRM	429	411	40	13
		Positive	MRM	446	429*	40	13
Tween 80	26.0	Positive	Parent Ion	400 - 1300	309	30	40
Tween 85	28.2	Positive	Parent Ion	400 - 1300	309	30	40

Figure 3.10. Timeline of LC-MS/MS Parameters.

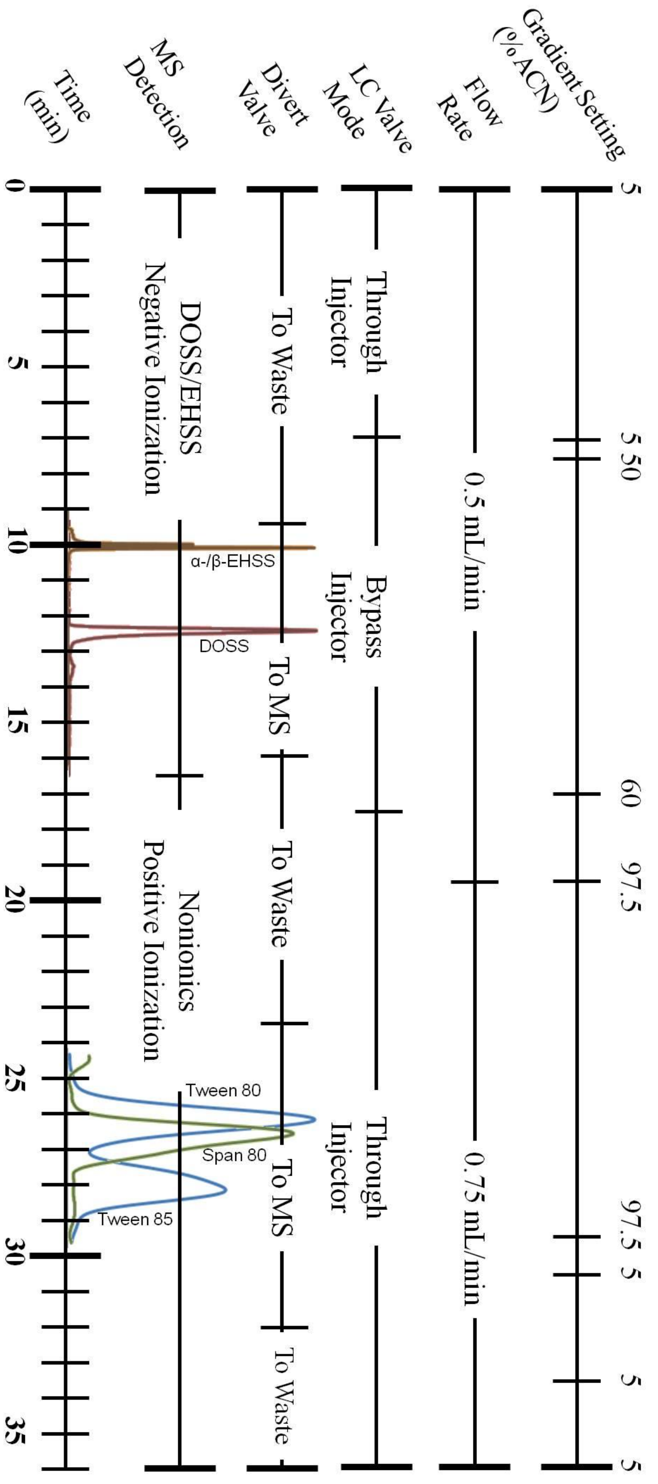


Figure 3.11. Schematic of LC/MS design. Top figure is the LC/MS in main pass LC mode and post-column solvent flow is diverted to the waste (column wash step). Bottom figure is the LC/MS in bypass LC mode with post-column solvent flow diverted to the MS (analyte elution step)

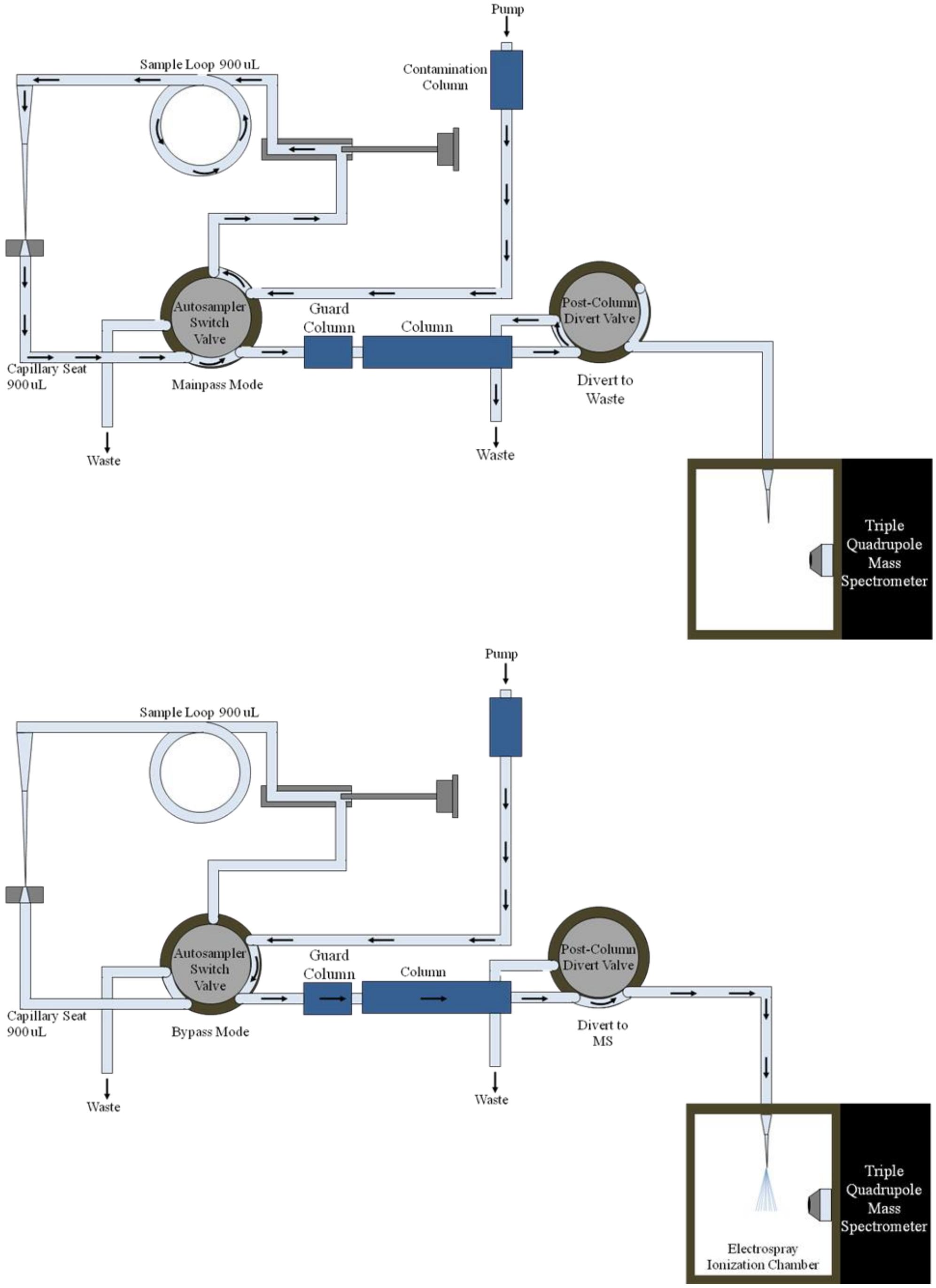


Figure 3.12. Top: Chromatogram of the parent ion scanning for *m/z* 309, Tween 80 and Tween 85 are designated as peaks A and B, respectively. Middle: Parent ions of product ion *m/z* 309 for peak A. Bottom: Parent ions of product ion *m/z* 309 for peak B.

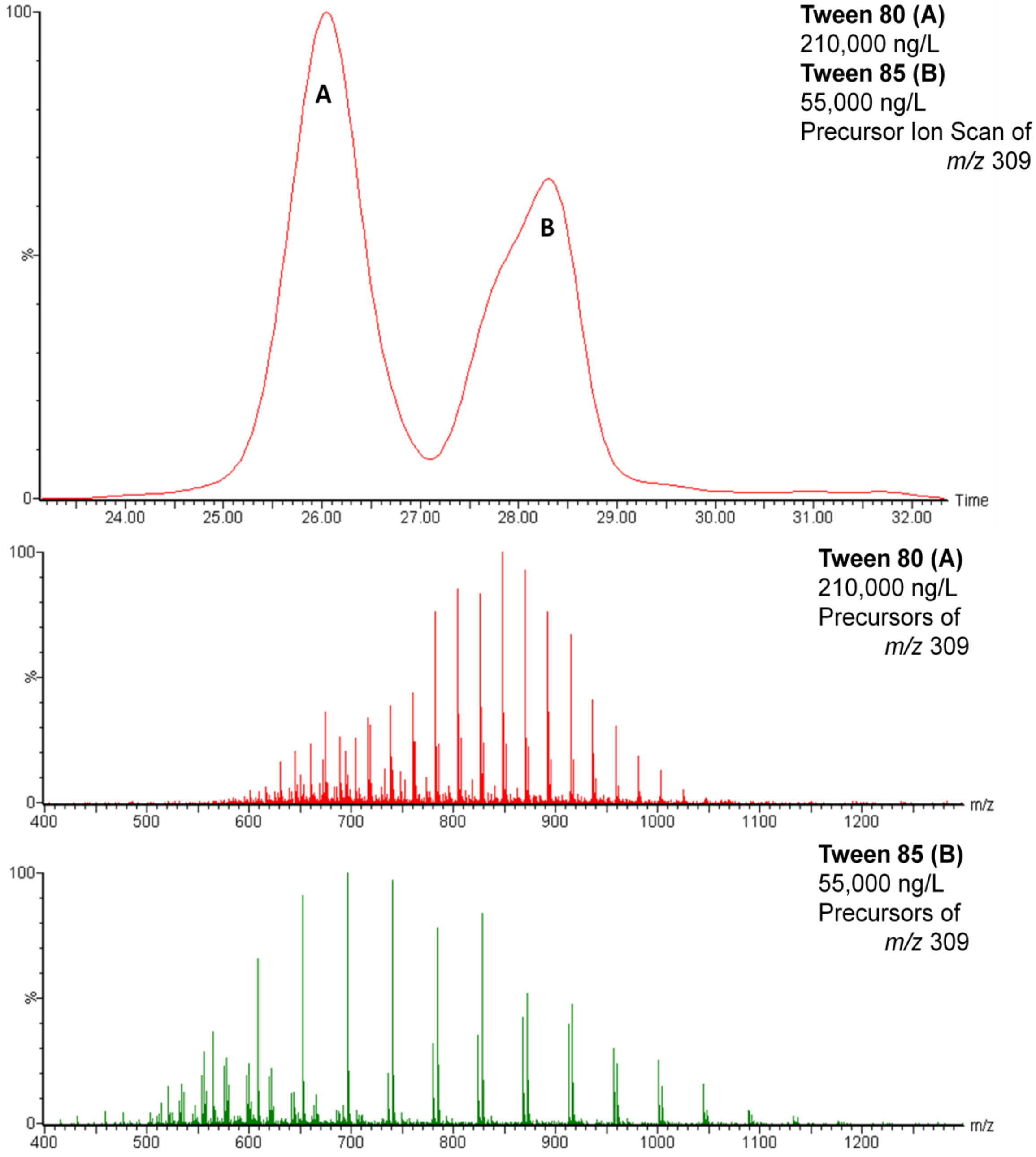
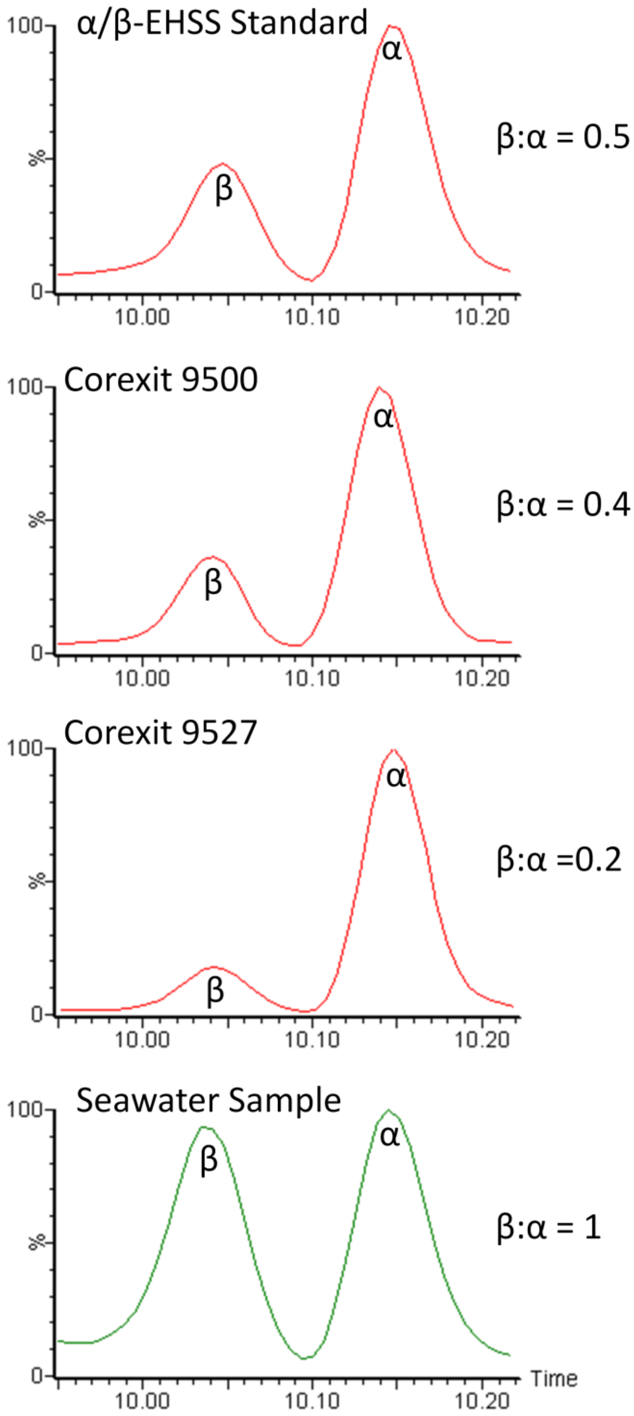


Figure 3.13. Chromatogram of α-/β-EHSS for the degradation standard synthesized in lab, Corexit 9500 formulation, Corexit 9527 formulation, and a Gulf of Mexico seawater sample. Area counts of β-EHSS to α-EHSS reported as a β:α ratio.



CHAPTER 4: IMPACT OF EXTRACTION SOLVENT AND ANALYTE CONCENTRATION ON THE EXTRACTION OF C60 FROM ENVIRONMENTAL MATRICES

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Abstract

Fullerenes possess unique chemical properties that make them important compounds for environmental and biomedical applications. Previous reports indicate that toluene-based extraction techniques may vary in their ability to extract C_{60}, especially from highly carbonaceous solid matrices. This research examined the effects of (i) solvent type (toluene alone versus a 80:20 mixture of toluene and 1-methylnaphthalene) and (ii) analyte concentration on the extraction efficiency of an isotopically-labeled surrogate compound, $^{13}C_{60}$. The toluene/1-methylnaphthalene mixture increased fullerene extraction efficiency 5-fold from carbon lampblack, but was not significantly different from toluene when applied to wood stove soot or montmorillonite. Recovery of the $^{13}C_{60}$ surrogate declined with decreasing analyte concentration. The usefulness of isotopically-labeled surrogate is demonstrated and the study provides a quantitative assessment regarding the dependence of fullerene extraction efficiencies on the geochemical characteristics of solid matrices.

Introduction

The production of the C_{60} fullerene by the laser vaporization of graphite was first reported by Kroto et al. in 1985.(1) Many studies also reported the synthesis of fullerenes through other laboratory-based processes.(2, 3) In addition to synthetic routes, fullerenes are produced by natural processes, including burning candles, meteorite strikes, and geologic processes.(4, 5) Sanchis et al. reported the presence of C_{60} and C_{70} in aerosols occurring over the Mediterranean Sea.(6) Fullerenes are strong adsorbents for organic compounds, including many environmental contaminants.(7, 8) In addition, C_{60} produces and quenches reactive oxygen species(9) and has other toxic characteristics.(10) These studies suggest a significant background level of fullerenes may be present in a variety of environmental systems and that they are environmentally relevant.

Previously described methods for the extraction of fullerenes from from solids of varying carbon contents indicate low and/or variable recovery of fullerenes, ranging from 1.2 % to 114 %.(6, 11-15) Other studies did not report extraction efficiencies for their methods.(5, 16-19) Most of the studies did not utilize an isotopically-labeled surrogate. With the commercial availability of isotopically-labeled $^{13}C_{60}$, the scientific community has been supplied with a promising tool for the development of more robust extraction procedures. Multiple parameters, including temperature,(14, 20) extraction solvent, extraction time,(15) extraction method,(12) and analyte concentration,(12) can impact the extraction efficiency of fullerenes in environmental solids. The focus of the current study was on the effects of solvent type, analyte concentration, and sample mass on the fullerene extraction efficiency.

Marcus et al. (21) reported that C_{60} solubility was dependent on polarizability and π-electron donor capacity. Toluene has relatively high values for both of these parameters, making it a common solvent for C_{60} extraction. However, while toluene is the conventional solvent used for fullerene extraction,(5, 11, 12, 14, 15, 17-19) other solvents, including polychlorinated benzenes and polycyclic aromatic hydrocarbons, have a greater predicted ability to solvate fullerenes due to greater polarizability and/or π-electron donor ability.(21) Higher solubility was used as a potential indicator of increased fullerene extraction ability, as increased solubility suggests increased favorable interactions between the analyte and solvent.

The objective of the current study was to evaluate the impact of various method parameters on C_{60} extraction from environmental solids. Our conceptual approach was twofold. We tested whether a solvent system containing a component with enhanced electron donor capacity would be superior to the standard toluene-only approach. We further examined how extraction efficiency would depend on analyte concentration and sample mass. To achieve environmental significance, we choose lampblack as a proxy for an aromatic matrix of high carbon content; wood stove soot as a proxy for an organic matrix with significant aromaticity but of lesser carbon content; and montmoorillonite, an organic matter-free solid phase that can possess some hydrophobic microsites. This study used $^{13}C_{60}$ as a surrogate to model the extraction of fullerenes.

Experimental

Materials and standards

Analytical fullerene standards were purchased from MER Corporation (Tucson, AZ), including C_{60}, C_{70}, and $^{13}C_{60}$ (35% ^{13}C-enriched). HPLC-grade methanol and toluene used for standards and mobile phases were purchased from Sigma Aldrich (St. Louis, MO) and 1-methylnaphthalene was purchased from Sigma Aldrich at 95% purity.

Stock analytical standards were created by dissolving solid C_{60}, C_{70}, and $^{13}C_{60}$ standards in toluene; all stock standards were sonicated briefly to fully dissolve the solid standards. The solvent composition of analytical standards consisted of 80% methanol, 20% extraction solvent to match the composition of the samples. As reported by Isaacson et al., all samples containing methanol were analyzed within 24 h after preparation.(22)

Three materials were chosen as test matrices for the experiment; carbon lamp black, wood stove soot, and montmorillonite. Carbon lampblack was purchased from Fisher Scientific (ThermoFisher Scientific, Waltham, MA). This petroleum-based soot was chosen as a suitable model matrix for a highly carbonaceous solid material. Wood stove soot was collected from a local resident’s wood stove, which was a secondary combustion stove. The wood stove soot was used to emulate a heterogeneous environmental matrix with high carbon concentrations.(23) Montmorillonite STx-1 was used to represent a solid mineral matrix with negligible content of organic carbon and was procured from the source clay mineral repository of the Clay Minerals Society (Chantilly, VA).

Sample extraction

Extractions were performed on three different matrices: carbon lampblack, wood stove soot, and montmorillonite. Each matrix was weighed and added to disposable 15-mL glass centrifuge vials. A standard of $^{13}C_{60}$ was added to each matrix sample, for a delivered mass of 37.5 ng of $^{13}C_{60}$ to each sample. The isotopically-labeled $^{13}C_{60}$ was used to quantify extraction efficiency. Extraction efficiency is defined as the percentage of labeled surrogate mass recovered [($^{13}C_{60}$,recovered/ $^{13}C_{60}$,added) * 100]. An aliquot of extraction solvent was added to the vial (the volume was dependent on sample mass) and the samples were briefly vortexed to mix the solid sample matrix and solvent thoroughly before being subjected to ultrasonication at room temperature using a VWR Model 75HT ultrasonic bath for 30 min. The vials were then centrifuged at 1625g using an IEC CL Clinical Centrifuge (Damon/IEC Division, Needham Heights, MA) for 15 min. After centrifugation, the supernatant was collected and all extracts were stored in the dark, at room temperature, until analysis. Extraction process blanks, consisting of only extraction solvent without matrix and isotopically-labeled surrogate, were used to verify no background contamination. Prior to analysis, extracts were diluted with methanol to a final sample composition of 80:20 (v/v) methanol:extraction solvent, which was either 100% toluene or 80:20 (v/v) toluene:1-methylnaphthalene.

Extraction solvent composition

The impact of final sample composition on chromatographic quality was first evaluated by comparing 80:20 methanol:toluene with increasing concentrations of 1-methylnaphthalene. Preliminary analysis indicated that the final injected sample solvent composition could not consist of more than 4% 1-methylnaphthalene. Therefore, solids extracted with 100% toluene were compared to solids extracted with 80:20 toluene:1-methylnaphthalene, which were subsequently diluted with methanol to give final sample composition of 80:20 methanol:toluene and 80:16:4 methanol:toluene:1-methylnaphthalene, respectively. For the extraction solvent evaluation experiment, the mass of the sample matrix was held constant at 50 mg and spiked with a standard of $^{13}C_{60}$ for a final solid concentration of 0.75 ng/mg. The extraction solvent volume was 1.5 mL for all extractions. A single extraction was performed on the samples and three replicates of each of the sample were extracted. It was determined that a single extraction would be adequate to generate meaningful information.

Surrogate concentration and sample mass of lampblack

Carbon lampblack was the matrix with the greatest retention potential and, thus, chosen as the test solid for the evaluation of variations in surrogate concentration and sample mass. Single samples of lampblack ranging from 50 mg to 500 mg were evaluated and all samples were spiked with 37.5 ng of $^{13}C_{60}$ to give a final surrogate concentration ranging from 0.75 to 0.075 ng/mg. In order to accommodate for the increased solid volume, the extraction solvent volume was increased from 1.5 to 3 mL (Table 4.1). The single sample at each lampblack mass was extracted three times with 80:20 toluene:1-methylnaphthalene, as determined from the previous experiment, and each extract was analyzed individually.

Instrumental analysis

The analytical method used to quantify $^{13}C_{60}$ and endogenous C_{60} and C_{70} was previously described by Isaacson et al.(22) Chromatographic separation of the analytes was performed on a Waters Alliance 2695 HPLC system (Waters Corporation, Milford, MA) that was modified with a 2,500-µL syringe and extended sample loop for large volume injections. The analytical column was a Targa C18 column with dimensions 2.1x150mm, 5-µm particle size (Higgins Analytical, Inc., Mountain View, CA). The injection size was 500 µL. The mobile phase composition was isocratic at 45:55 methanol:toluene at a flow rate of 0.3 mL/min for the entire 15-min run (Figure 4.1).

Mass spectrometric detection was performed with a Waters Acquity Triple Quadrupole Detector Mass Spectrometer (Waters Corporation). The fullerene analytes were detected in negative ionization mode with single ion monitoring for the negative charged molecular ion: m/z 720 (C_{60}), 735 ($^{13}C_{60}$), and 840 (C_{70}). The following settings were employed: a capillary voltage of 4.2 kV, a cone voltage of -275 V, a source temperature of 150 ^{0}C, a desolvation temperature of 450 ^{0}C, a cone gas flow of 50 L/hr, and a desolvation gas flow of 1000 L/hr.

Safety Caution: care should be taken when handling toluene with mass spectrometry, specific steps should be taken to reduce the chance for toluene vapor to enter the mass spectrometer including operating at a higher desolvation temperature, a higher cone and desolvation gas flow, ballasting the roughing pumps regularly, and changing the roughing pump oil more frequently.

Quantification of fullerenes

External standard calibration was used to quantify the concentration of all analytes (C_{60}, $^{13}C_{60}$, C_{70}) with a minimum 4-point calibration curve ranging from 0.1 to 5 µg/L, and a linearity of R2 > 0.95, with 1/x weighting. The quantification of $^{13}C_{60}$ was used to determine extraction efficiency, while C_{60} and C_{70} were measured as an indication of endogenous concentrations in environmental solids. In order to ensure data quality, blank and check standards consisted of at least 10% of the total number of samples analyzed within an analytical sequence. Check standards were required to be within 40% of the standard concentration in order to continue analysis. For this method, the limit of detection (LOD) was set equal to the lower limit of quantification (LLOQ) for all fullerenes, which was 0.1 µg/L for all analytes, as previously determined by Isaacson et al.(22)

Results and discussion

Method optimization

Extraction solvent

Toluene was the first solvent evaluated in this study, because it is the conventional solvent of choice when performing fullerene extractions or when solubilizing fullerenes in standard solutions.(5, 11, 12, 14, 15, 17-19) Toluene is a good solvent for fullerenesbecause it possesses both hydrophobic character and the conjugated π-electron system, which encourages solvent-solute interactions with highly conjugated fullerenes.(21) Initial extraction experiments using 100% toluene as the extraction solvent and $^{13}C_{60}$ as the surrogate revealed a broad range of extraction efficiencies that was dependent on the sample extracted. Lampblack gave the lowest extraction efficiency (5.7 ± 1.5%, mean efficiency ± standard deviation), followed by wood stove soot (52 ± 14%), and montmorillonite (88 ± 7.2%). The low extraction efficiency of the surrogate from the lampblack is similar to that reported in other studies using ultrasonication with toluene to extract endogenous C_{60} from various carbonaceous materials.(12, 15) This finding indicated that previous methods that relied on toluene as the extraction solvent, without first evaluating the extraction efficiency, may have significantly underestimated the total concentration of C_{60}.

The second extraction solvent consisted of 80:20 toluene:1-methylnaphthalene. The use of 1-methylnaphthalene was chosen due to its increased polarizability and similar electron pair donor ability when compared to toluene.(21) The calculated increased solubility of C_{60} was an order of magnitude higher in 1-methylnaphthalene compared to toluene.(21, 24) Extractions performed with 80:20 toluene:1-methylnaphthalene resulted in a 5-fold increase in extraction efficiency of $^{13}C_{60}$ from lampblack (24 ± 9.5%) when compared to that obtained by toluene alone. (Figure 4.2). The extraction efficiencies of toluene or 80:20 toluene:1-methylnaphthalene is not suspected to be limited by fullerene solubility in the two solvents. We speculate that increased recovery with the addition of 20% 1-methylnaphthalene in toluene is due to the recovery of a fraction of $^{13}C_{60}$ that was associated with the higher energy nature of the condensed aromatic rings of lampblack. No apparent increase in the extraction efficiency of $^{13}C_{60}$ from wood stove soot or montmorillonite was obtained with 80:20 toluene:1-methylnaphthalene. The consistently low recovery of $^{13}C_{60}$ from wood stove soot with both 100% toluene (52 ± 14%, mean recovery ± standard deviation) and 80:20 toluene:1-methylnaphthalene (52 ± 16%) would suggest that the associations of the surrogate with wood stove soot and lampblack cannot be overcome by the increased polarizability of 1-methylnaphthalene.

Surrogate concentration and sample mass of lampblack

Recovery of $^{13}C_{60}$ from lampblack ranged from 53% to less than 3% (below the $^{13}C_{60}$ limit of quantification) for $^{13}C_{60}$ concentrations from 0.75 to 0.075 ng/mg in the solid, respectively (Figure 4.3A). We propose that the increased mass of sample increases the abundance of higher energy sites for irreversible sorption relative to the mass of the spiked surrogate. Therefore, the mass of lampblack increases the greater irreversible sorption of $^{13}C_{60}$, which results in the decreased recovery. Using the $^{13}C_{60}$ m in the first extract and the calculated mass of retained $^{13}C_{60}$ on the solid, a linear sorption isotherm was developed. The computed lampblack:extraction solvent (80:20 toluene:1-

methylnaphthalene) partition coefficient of 29 L/kg indicates a strong partitioning favoring the lampblack matrix. The lampblack:toluene partition coefficient (calculated for a single sample mass) of 1800 L/kg is almost two orders of magnitude greater than the 80:20 toluene:1-methylnaphthalene solution. We speculate that this is due to the ability of 1-methylnaphthalene to disrupt the analyte:matrix interactions, resulting in a more favorable analyte partitioning into the solvent.

While the absolute mass of the endogenous C_{60} recovered increased with the mass of the solid extraction (Figure 4.3B), the apparent concentration of endogenous C_{60} (mass of C_{60} extracted / mass of matrix), without correcting for the added labeled surrogate, decreased from 0.44 ng/mg for the 50 mg sample of lampblack down to 0.12 ng/mg for the 500 mg sample (Figure 4.3B). One reason for this may be due to the fact that the extraction solvent volume did not increase proportionally with the mass of lampblack extracted. Even with the stronger 80:20 toluene:1-methylnaphthalene extraction solvent, there is still favorably partitioning to the lampblack matrix. In order to increase the C_{60} mass recovered from carbonaceous materials, the strength of the extraction solvent should be increased, although care should be taken with 1-methylnaphthalene as it has a high boiling point and its volume will be difficult reduce using a evaporative concentration step. Also, higher proportions of 1-methylnaphthalene has previously shown to hinder chromatographic quality.

Quantification of C_{60} and C_{70} in environmental solid matrices.

If the C_{60} concentration is corrected for $^{13}C_{60}$ extraction efficiency at 50 mg (53% $^{13}C_{60}$ recovery), the concentration of endogenous C_{60} in lampblack was 0.83 ng/mg. Assuming the partition coefficient for $^{13}C_{60}$ can be used to calculate the model C_{60} partitioning between the extract solvent and lampblack, the equilibrium concentration of C_{60} in the extraction solvent can be used to calculate the original C_{60} concentration in lampblack, which was 0.73 ng/mg. The detection of C_{60} in lampblack was an expected result, given the high carbon content and nature of the material. However, no C_{60} was detected in wood stove soot or the montmorillonite. The lack of detection in wood stove soot can be attributed to the much lower temperatures of formation and, therefore, less energy available for the formation of condensed aromatic ring structures in this material. Therefore, if C_{60} was present in wood stove soot, it would be at low concentrations (below the LLOQ). Montmorillonite, being a strictly mineral phase, would not be suspected to contain fullerenes and observations corroborated this hypothesis.

There were no detectable concentrations of C_{70} detected in any of the examined matrices. Preliminary studies examined the recovery of spiked C_{70} (non-endogenous) into the lampblack material (50 mg) and found no recoverable quantities could be extracted using the 80:20 toluene:1-methylnaphthalene extraction method. Although effective for the recovery of $^{13}C_{60}$, the more aromatic solvent combination was not able to overcome the intermolecular interactions between the matrix and any spiked C_{70}. This finding suggests that any prior method that reports detectable quantities of C_{70} may also be underestimating the actual concentration and that $^{13}C_{60}$ may not be an adequate surrogate to assess the extraction efficiency of C_{70} from carbonaceous materials.

Concluding remarks

The addition of 1-methylnaphthalene to toluene for the extraction of fullerenes gave significant increases in the extraction of $^{13}C_{60}$ from a solid matrix consisting of pure, polycondensed aromatic ring systems. Yet, there was no observed difference in other, more heterogeneous matrices. Consequently, we recommend that this combined solvent system (80:20 toluene:1-methylnaphthalene) be used when an environmental matrix contains significant amounts of aromatic structures of the type that occurs in lampblack. Isotopically-labeled surrogate compounds, such as $^{13}C_{60}$, are valuable tools for the evaluation of extraction methods as extraction surrogates. Our study further revealed a strong dependence of extraction efficiency on the fullerene's solid concentration, indicating that further work should focus on optimizing extraction conditions for samples with low level fullerene concentrations.

Acknowledgements

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Table 4.1. Lampblack sample mass, extraction solvent volume (80:20 toluene:1-methylnaphthalene), sample:solvent ratios and $^{13}C_{60}$ concentration.

Sample mass (mg)	**Extraction solvent volume (mL)**	**Sample to solvent ratio (mg/mL)**	**Tracer concentration in sample (ng/mg)**
52	1.5	35	0.72
100	1.5	67	0.38
160	1.5	107	0.23
260	2	130	0.14
500	3	170	0.075

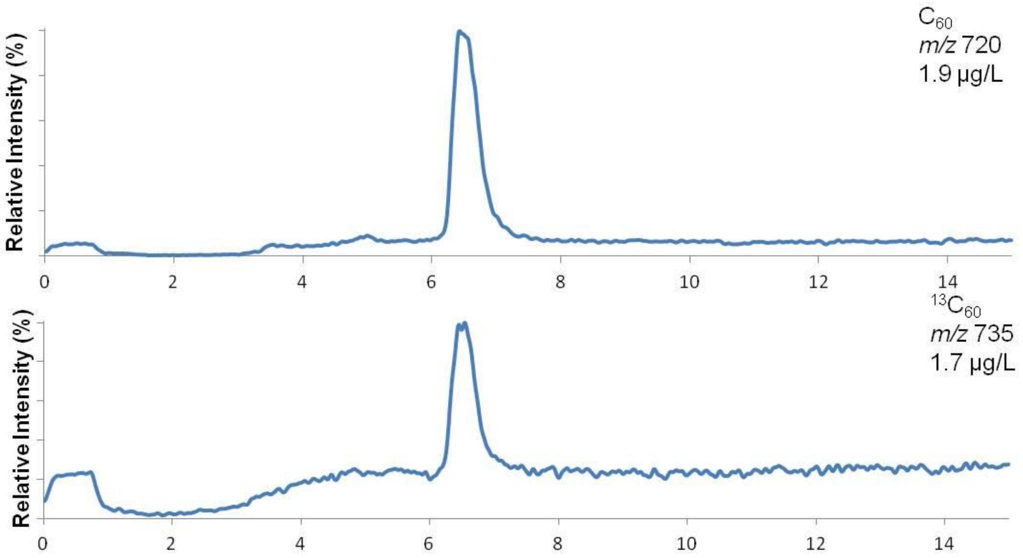

Figure 4.1. Typical chromatogram of C_{60} and $^{13}C_{60}$ in a 80:20 toluene:1-methylnaphthalene extract (80:16:4 methanol:toluene:1-methylnaphthalene final composition) of lampblack.

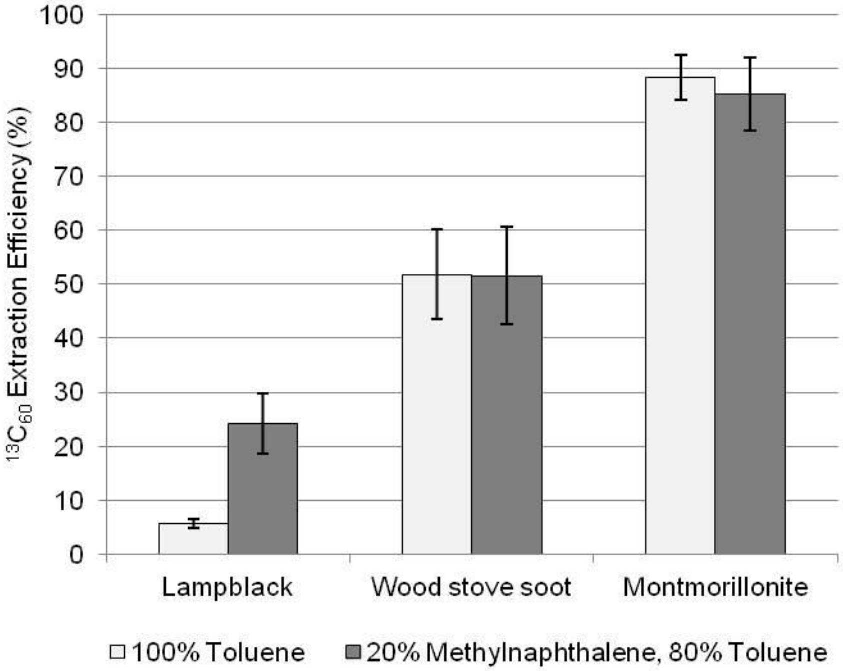

Figure 4.2. Extraction efficiency of a single extraction conducted with 100% toluene, 80:20 toluene:1-methylnaphthalene for three different environmental solids at 50 mg. Extraction efficiency was determined by percent recovery of spiked $^{13}C_{60}$ (n = 3).

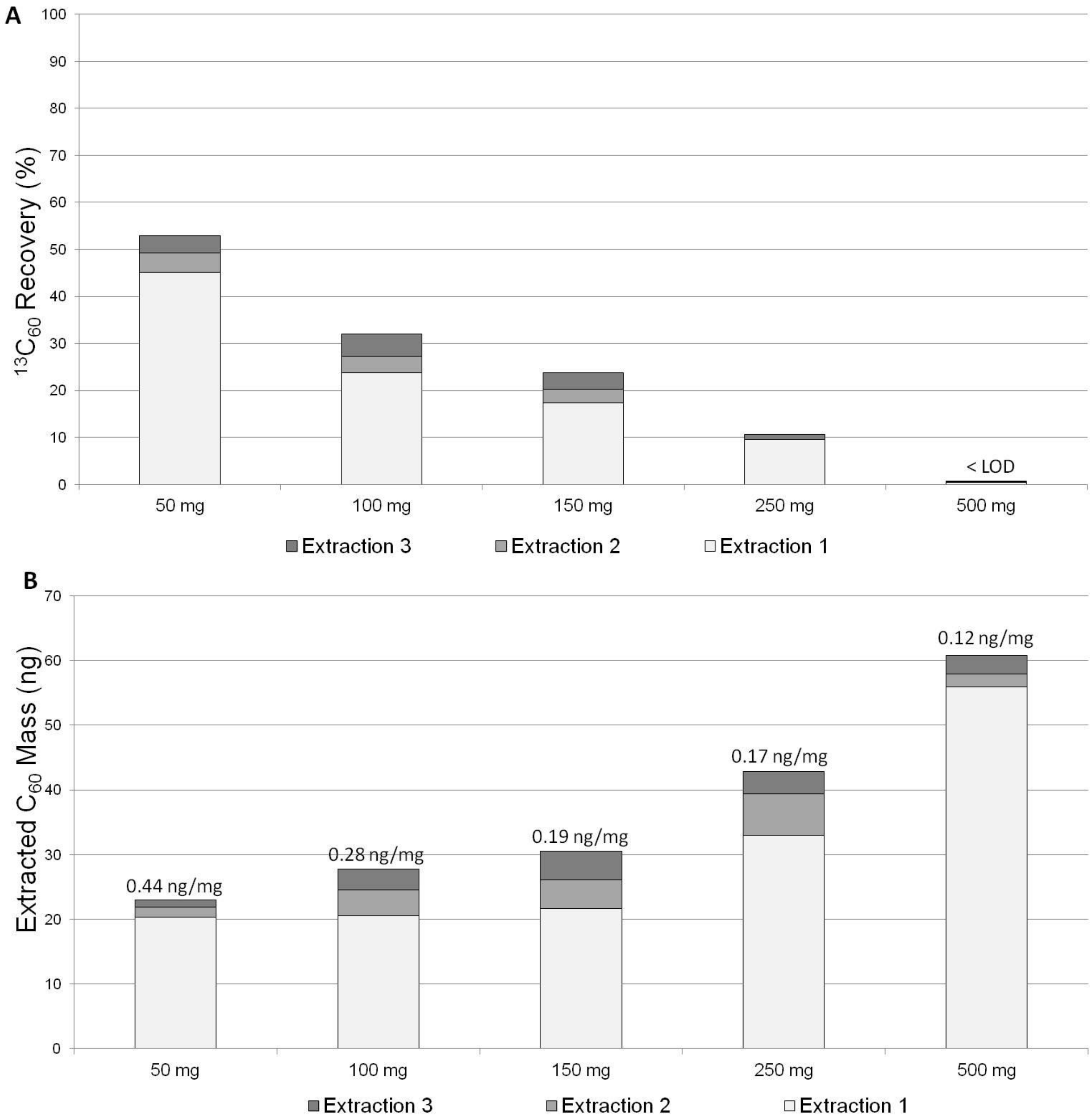

Figure 4.3. Extraction efficiency of $^{13}C_{60}$ (A) and mass of endogenous C_{60} (B) recovered from lampblack as a function of mass of solid sample with 80:20 toluene:1-methylnaphthalene. < LOD indicates the extracted concentration of $^{13}C_{60}$ was below the detection limit. Concentrations above each mass in B are the apparent C_{60} concentrations without any correction for extraction efficiency.

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CHAPTER 5: CONCLUSIONS

The study of emerging contaminants poses unique difficulties for analytical chemists due to the inherent lack of knowledge about these chemical compounds. New analytical methods must be developed for the analysis of emerging contaminants in order to understand their occurrence, fate, and potential adverse environmental impacts. To address existing data gaps over the identities, properties, and environmental behavior of emerging contaminants, analytical methods must use novel tools and exploit any existing background information related to the chemicals of interest. The analytical chemistry research presented in this dissertation represents a first step toward better understanding the environmental impact of multiple classes of emerging contaminants.

In Chapter 2, an analytical tool for the identification of previously unknown fluorochemicals was developed. A non-target screening approach was applied to military-use aqueous film-forming foams (AFFF), which were reported to contain fluorochemical surfactants of unknown composition and structure. In order to identify potential fluorochemicals of interest for a ‘suspect ion’ list, an additional screening tool was developed using the surfactant-selective aspect of fast atom bombardment mass spectrometry (FAB-MS). When FAB-MS was combined with the high resolution identification capabilities of quadrupole time-of-flight mass spectrometry (QTOF-MS), a list of potential elemental formulas for the suspect ions were identified. The vital and final step for the elucidation of structure was the development of a database of potential fluorochemical surfactants in AFFF and fluorochemical patents.

Using the described tools, eleven different fluorochemical classes were identified, ten of which did not have published data on their environmental presence or fate. These findings open up new data gaps related to the environmental impacts of AFFF use. Positively-charged fluorochemicals, both zwitterionic and cationic compounds, will have potentially different transport behavior in the environment when compared to that of anionic and nonionic fluorochemicals that are studied currently. From the findings in this study, future work should focus on evaluating the wide variety of AFFF-based fluorochemicals and their impact on environmental systems.

Some emerging contaminants become the focus of the scientific research due to events that bring the chemicals into the public spotlight. After the Deepwater Horizon oil spill, rapid research commenced to study the environmental impacts of the spill, including the use of oil dispersants. While the identities of the oil dispersant components were revealed months after the first application, the fate and impact of the dispersant is still not fully understood. Other methods exist for the detection of single compound in the oil dispersant, bis-(2-ethylhexyl) sulfosuccinate (DOSS), but they do not fully embrace the complex mixture of the dispersant formulations. In Chapter 3, an analytical method using large volume injection liquid chromatography with tandem mass spectrometric detection (LVI-LC-MS/MS) was developed in order to achieve trace level detection of multiple surfactant compounds contained in the Corexit oil dispersants. LVI-LC-MS/MS is an analytical tool that provides the benefit of minimal sample preparation, low consumables use, and decreased analyst time while offering low level detection limits.

In seawater, the analytical method described in this research was capable of detecting both anionic and nonionic surfactants with limits of quantification from 49 ng/L to 3,000 ng/L. In the Gulf of Mexico seawater samples, which were collected after the Deepwater Horizon oil spill, the anionic surfactant (DOSS) was detected more frequently in seawater samples than any of the nonionic surfactants. In addition, DOSS was detected at a much broader range of concentrations (71 to 13,000 ng/L). The research described above is the first study to look at the DOSS transformation products and/or synthetic impurities. α-/β-ethylhexyl sulfosuccinate (α-/β-EHSS). α-/β-EHSS was detected in multiple samples, as well as the original Corexit dispersants, indicating that the compounds should be included in future studies. In future studies the developed method will be applied to post-spill Gulf of Mexico samples in order to determine the occurrence, fate, and potential long-term environmental impacts of oil dispersant use.

Quantitative analytical methods are vital for understanding the environmental fate and presence of emerging contaminants. While fullerene nanomaterials have been around longer than most emerging contaminants, they have become the focus of research due to their unique properties and use in products of personal care, electronics, and medical uses. While quantitative LC-MS methods exist for measuring C_{60} and C_{70} in a variety of environmental matrices, previous studies involving the extraction of fullerenes from solids gave variable and/or low recovery. These findings suggested that further research was necessary to understand the intermolecular interactions between C_{60}, carbonaceous matrices, and the extraction solvent. The use of isotopically-labeled surrogate standards, such as $^{13}C_{60}$, was a useful tool for evaluating the extraction of C_{60} from environmental solids containing endogenous fullerene concentrations (such as lampblack). While conventional extractions performed with toluene under ultrasonication were suitable for low carbon-containing solids, the highly carbonaceous lampblack gave low extraction efficiency (5% recovery of spiked $^{13}C_{60}$). With the combination of 1-methylnaphthalene and toluene, the extraction efficiency of the $^{13}C_{60}$ surrogate from lampblack was increased 5-fold. In addition, the mass of the extraction sample decreased the extraction efficiency of the spiked surrogate and the apparent concentration of the endogenous fullerenes on solids. The findings reported in Chapter 4 indicate the importance of evaluating experimental variables, such as extraction solvent and sample mass, when attempting to develop a validated extraction method. In addition, the use of $^{13}C_{60}$ as a surrogate for C_{70} extraction may lead to significant underestimations of C_{70} in the environment. Future work should test these additional factors while determining the background presence of fullerenes in the environment. Such analytical advances are needed to understand the future environmental impact of fullerene use in commercial and industrial products.

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