Vitamins as Confounding Factors when Measuring PAHs by FACS

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Abstract

Crude oil contamination has commonly been tracked by measuring polycyclic aromatic hydrocarbons (PAHs) in fish bile and or tissues using fluorescence. Research on the planktivorous fish species, menhaden, raised questions about other types of fluorescent compounds measured in these assays- particularly vitamins A and E, which are obtained from phytoplankton. Vitamins are fluorescent compounds, and it is possible that these vitamins are confounding factors when trying to measure PAHs using fluorescence. In order to evaluate this, scanning fluorescence spectroscopy was used to detect PAH and vitamin standards alone and when combined with fish oil. Fish oil was obtained from an "over the counter brand" (Nature's Bounty), a prescription brand (Lovaza), a commercial product (menhaden oil from DayBrook Industries) and wild menhaden collected from the Delaware Bay, NJ and from Barataria Bay, LA in 2010. Vitamins and PAH standards were bought commercially. Fish oil and standards were analyzed in 75% ETOH using a SpectraMax M5. Results showed that fluorescence was a sensitive detection tool: PAH-like substances were quantified in wild fish at the ppb level. Major peaks in wild fish samples were at Em350/Ex280 and Em450/Ex350. For vitamin standards: peaks were at Em350/Ex290 for vitamin E and at Em450/Ex320 for vitamin A. Vitamin A and E peaks appeared to be present in wild fish oil and commercial fish oil products. However, commercial fish oil products also contained a major peak at Em450/Ex350. This peak was found in many 4-6 rings PAH standards but not vitamins. Results indicated that vitamins in fish may be confounding factors when detecting PAHs using fluorescence technologies.

Introduction

Cardiovascular disease has been a popular topic for the past few years. Research has shown that cardiovascular disease is one of the most lethal diseases, and is very common in the United States (Go, et al 2012). An effective way to prevent cardiovascular disease is by taking fish oil, which is rich in omega-3 fatty acid, and appears to lower cholesterol.

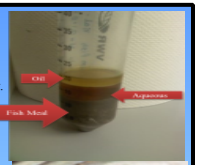
Crude oil contamination is an ongoing environmental issue that is likely to affect fisheries, with high potential of affecting human health. The release of crude oil from the British Petroleum's DeepWater Horizon Oil Rig on April 20, 2010 has caused concern for the Gulf of Mexico's fishery, in particular the impact of polycyclic aromatic hydrocarbons (PAHs). Menhaden is a marine teleost fish that is especially important to the commercial fisheries of the Gulf and Atlantic coasts. It is an oily, prey species used in the bait and reduction industries as well as for making fish oil. Due to their oily nature, menhaden will likely accumulate crude oil contaminants. Contaminated omega-3 from menhaden fish oil, may expose humans to PAHs. Some PAHs are known carcinogens.

For this project, fish oil was obtained from an "over the counter brand" (Nature's Bounty), a prescription brand (Lovaza), a commercial product (menhaden oil from DayBrook Industries) and wild menhaden collected in the fall 2010 from the Delaware Bay, NJ (MVNJ) and from Barataria Bay, LA (GILA). GILA fish were collected September 30th, 3 months after the DWH pipeline was capped. Menhaden oil was tested for vitamins A and E because it was anticipated that as first order consumers of phytoplankton, menhaden would bioaccumulate high levels of these vitamins. Since vitamins A and E contain an aromatic ring, they were likely to fluoresce under test conditions making them confounding factors when trying to measure PAHs using fluorescence. Analysis of fluorescent aromatic compounds (FACs) is commonly used to monitor PAHs following crude oil spills (Kreitsberg, et al, 2010).

Materials and Methods:

Materials:

Fish oil was obtained from an "over the counter brand": Nature's Bounty (NB) (lot number: 371123-01), Lovaza (a prescription brand provided by Dr. John Sowa, Seton Hall University) DayBrook (DB) (commercial product provided from DayBrook industries), GILA (wild menhaden collected from the Barataria Bay, LA in 2010) and MVNJ (wild menhaden collected from the Delaware Bay, NJ in 2010). Wild menhaden were collected by NJ Fish and Wildlife and LA Wildlife and Fisheries.



Methods:

Fish oil preparation:

The head and tail of the fish were cut off, and the fish filleted and de-boned. The filets were cut into smaller pieces and pounded into meal using a glass tube inside a round bottom centrifuge tube. The meal was centrifuged in a round bottom tube for six hours at 10,000 rpm. Following centrifugation, two top layers could be seen, one oil and one aqueous. The bottom of the tube was punctured to separate the two layers.

Vitamin Standards/PAHs Standard Analysis

To extract PAHs, the oil was thawed and mixed by vortexing. In a 1.5 ml microcentrifuge tube, 50 µl of fish oil and 1.15 ml of 75% ethanol (EtOH) were combined. The mixture was vortexed continuously for 1 minute and then the oil was separated from the EtOH by centrifuging for 20 minutes at 13,000 rpm. The oil went to the bottom of the tube. One milliliter of the EtOH was removed and placed in a quartz cuvette. Samples were analyzed for fluorescent compounds using two settings on a SpectraMax M5/M5 scanning fluorometer. The first setting involved holding the emission wavelength (Em) at 350 nm and scanning for excitation wavelengths (Ex) from 250 to 340 nm. This setting was best for aromatic hydrocarbons with one or two aromatic rings such as vitamin E (1 ring) and naphthol (2 rings). The second setting involved holding the Em at 450 nm and scanning for Ex from 250 to 430 nm. This setting was best for polycyclic aromatic hydrocarbons with 3, 4 and 5 rings such as hydroxypyrene and for vitamin A even though it has 1 aromatic ring. Figure 7.

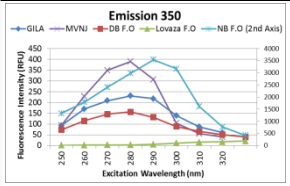


Fig 1. Fluorescent spectra of GILA, MVNJ, DB, Lovaza and NB fish oil for naphthol-like PAHs. Vitamin E is detected in commercial fish oil and very high in NB at Ex290nm. Menhaden fish oil from NJ had more PAHs than that from LA right after the DWH spill.

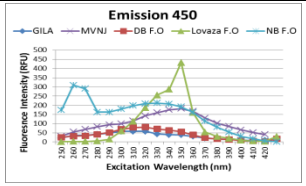


Fig 2. Fluorescent spectra of GILA, MVNJ, DB, Lovaza and NB fish oil for hydroxypyrene-like PAHs. Vitamin A is detected at Em320-330nm and appeared present in wild and commercial fish oils. NB appeared to contain fluoranthrene (Fig 7). Menhaden fish oil from NJ had more PAHs than LA.

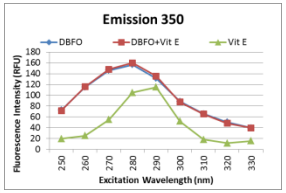


Fig 3. Fluorescent spectra of vitamin E spiked fish oil. For naphthol-like spectra, Vit E fluoresced at Ex290nm. DB spiked with Vit E showed a similar spectra but shifted a little to the left.

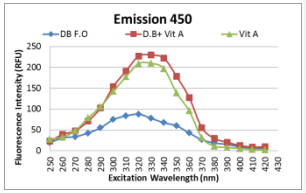


Fig 4. Fluorescent spectra of vitamin A spiked fish oil. For hydroxypyrene-like spectra, Vit A fluoresces at Ex320nm. DB menhaden fish oil showed a peak similar to Vit A.

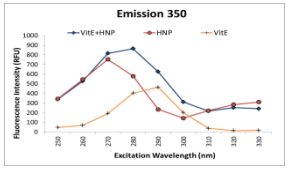


Fig 5. Fluorescent spectra for vitamin E spiked with naphthol (HNP). Separate peaks for VitE and HNP emerged into one peak, Em350.Ex280, also seen in menhaden fish oils. Vit E =5,000ng/mL, HNP=100ng/mL.

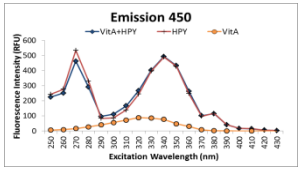


Fig 6. Fluorescence spectra for Vitamin A spiked with hydroxypyrene (HPY). The combination of HPY and Vit A modestly alters their individual spectra with a slight increase at Ex310-320. Vit A = 2500ng/mL, HPY = 100ng/mL.

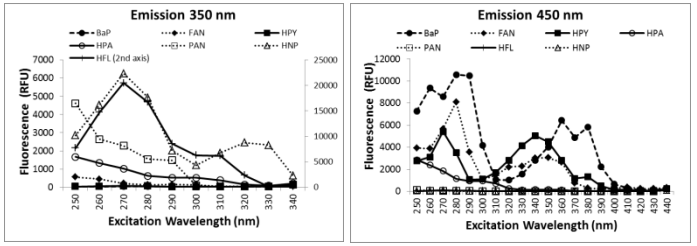


Fig 7. Fluorescent Spectra of PAH standards at two fixed emissions. High molecular weight PAHs fluoresce most strongly at EM450 and low molecular weight PAHs fluoresce most strongly at EM350. PAH =1250 ng/ml.

Table 1. Fluorescent intensities (RFU) for vitamin and PAH standards at fixed excitation (Ex) and emission (Em) wavelengths. Standards were extracted into 48% or 75% EtOH. Fixed wavelengths were based on literature values used for biomonitoring PAHs in bile (Kreitsberg et al, 2010). Gray boxes indicate the fixed wavelengths used to monitor the associated PAH. PAH = 500 ng/ml, Vitamins = 5000 ng/ml. HNP= naphthol, PHE= phenanthrene, HPY= hydroxypyrene, BaP= benzo(a)pyrene, VitA= vitamin A and Vit E= vitamin E. Note that HNP and Vit E both fluoresce at Ex290/Em335.

		RFU values					
%ETOH	Ex/Em	HNP	PHE	HPY	BaP	Vit A	Vit E
48	290/335	491	2	5	8	0	281
48	260/380	1154	5483	9374	686	0	8
48	341/383	81	344	20497	289	0	5
48	380/430	1	2	1536	10043	2	1
75	290/335	467	0	7	14	0	581
75	260/380	1230	7053	7824	682	0	14
75	341/383	112	445	17931	289	0	5
75	380/430	0	0	1451	9698	3	0

Discussion/Conclusion

Based on our data using FACS, vitamins can be a confounding factor when detecting PAHs. Vitamin A and E peaks were detected in wild fish oil and commercial fish oil products especially in Nature's Bounty. In addition, Nature's Bounty showed a fluoranthene-like spectra suggesting the presence of PAHs. The combination of vitamin E and naphthol generated one major peak, similar to that seen in all menhaden fish oils. Interestingly, wild menhaden from Delaware Bay, NJ had higher levels of PAH-like compounds than those from Barataria Bay, LA where large amounts of crude oil came into the estuary in summer 2010. However, the LA fish were from a single catch. Many researchers use FACs for detecting PAHs. Our data demonstrates that vitamins may be detected instead of PAHs. In particular, vitamin E may be detected at the fixed wavelengths used to monitor naphthalene-like compounds.

References

Kreitsberg, Krandel, et al. Responses of metabolic pathways to polycyclic aromatic compounds in flounder following oil spill in the Baltic Sea near the Estonian Coast, Aquatic Toxicology 99 (2010) 473-478 doi: 10.1016/j.aquatox.2010.06.005

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