Analysis of Polychlorinated Biphenyls (PCBs) in Fish Oil Supplements by Gas Chromatography with High Performance-Time of Flight Mass Spectrometry

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Keywords: Gas Chromatography; High Resolution Time-of-Flight Mass Spectrometry; Persistent Organic Pollutants; Fish Oil

1. Introduction

The American Heart Association recommends eating two servings of fish per week, specifically fish with high fat content such as tuna and salmon.[1] This recommendation stems from the fact that they contain relatively high quantities of omega fatty acids (Figure 1), such as the omega-3 acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Decades of research have linked consumption of these polyunsaturated fats to numerous health benefits such as lowering of triglyceride levels in blood and a decrease in coronary disease. Additional studies have suggested that fish oil also helps ease depression, and promotes brain development in children. Fish oil can be purchased as over-the-counter supplements or as a prescription medication (GSK's Lovaza). Fish oil brands differ significantly in their EPA and DHA content. In addition, fish oils are typically highly processed—which can lead to partial elimination and/or conversion of good omegas to less efficiently absorbed chemical derivatives. A common feature of fish oils is their complexity—they are mixtures of fatty acids, fats, oils, and sterols.

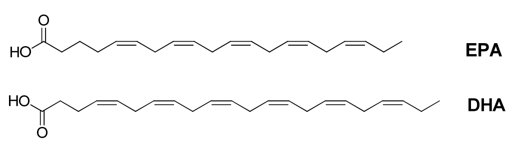

Figure 1. Omega-3 Fatty Acids EPA and DHA.

There is major debate as to the advantages of one brand of fish oil over another, as well as major concerns over the accumulation of fat-soluble, persistent organic pollutants (POPs) in the oil. An example of POPs in fish oil has recently come to the public's attention in the form of a lawsuit by an environmental group claiming these supplements contain elevated levels of polychlorinated biphenyls (PCBs). The World Health Organization has assigned toxicity equivalency factors (TEFs) to estimate the human health risks associated with exposure to PCBs and other toxins.[2] Figure 2 shows the structure of the most toxic PCB 3,3',4,4',5-pentachlorobiphenyl (PCB 126). PCB 126 has a TEF of 0.1 relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In this study, the advantages of using LECO's new Pegasus® GC-HRT to analyze complex matrices such as fish oil are highlighted. The resolving power and sensitivity of the high resolution time of flight mass spectrometer with Folded Flight Path™ (FFP™) technology are critical for targeting specific analysis of trace components in complex mixtures.

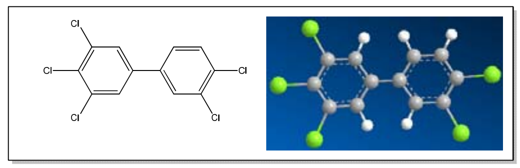

Figure 2. 3,3',4,4'5-pentachlorobiphenyl (PCB 126).

2. Experimental Conditions

General

The contents of several brands of over-the-counter fish oil capsules were spiked with Accustandard's PCB congener standard WHO/NIST/NOAA (28 congener mixture in isooctane) and extracted into acetonitrile using a QuEChERS extraction approach.

Sample Preparation

A 1.0 gram sample of fish oil was placed in a 50 mL centrifuge tube, spiked with PCB congener standard (10 ng or 100 ng standard/g of fish oil sample) and vortexed at 2200 rpm for 20 seconds. Water (9 grams) and acetonitrile (10 mL) were added to the oil and the mixture was homogenized by vortexing at 2200 rpm for 1 min. Extraction salts were added, mixed vigorously for 1 min., vortexed at 2100 rpm for an additional min., and then centrifuged at 3000 rpm for 5 mins. A 1.5 mL aliquot of acetonitrile layer was transferred to an MS vial for analysis.

GC Parameters

GC:	Agilent 7890
Column Type:	Restek Rtx-PCB (20 m, 0.18 µm ID, 0.18 mm df)
Injection:	1 µL, Splitless @ 250°C
Oven:	70°C (1 min) to 320°C @ 20°C/min (1.5 min)
Carrier Gas:	He, 1.00 mL/min

MS Parameters

Spectrometer:	LECO Pegasus GC-HRT
Ion Source:	LECO EI
Polarity:	Positive
Flight Path:	High Resolution (R = 25,000)
Acquisition Rate:	25 spectra/second
m/z Range:	50 – 510
m/z Calibration:	PFTBA

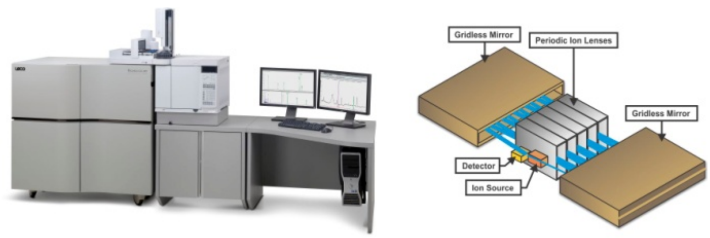

Figure 3. LECO Pegasus GC-HRT with Folded Flight Path (FFP) Technology.

3. Results

Instrument Performance

The Pegasus GC-HRT incorporates FFP technology to achieve high resolving power (Figure 3). An EI source produces ions that are directed between two gridless mirrors along the length of periodic lenses positioned at the center of the mass analyzer. Ions are reflected through the analyzer and returned to the detector located next to the ion source. The instrument can be operated in three modes: Nominal Mode (R = 1000 at m/z = 219 FWHM), High Resolution Mode (R = 25,000 at m/z = 218.985080) and Ultra-High Resolution Mode (R = 50,000 at m/z = 218.985080). It provided the mass accuracy and high resolving power necessary for robust identification of trace components in complex fish oil matrices. An extracted ion chromatogram (XIC) of Accustandard's WHO/NIST/NOAA PCB standard, as well as the mass spectrum for decachlorobiphenyl (PCB 209), are shown below (Figure 4). Mass accuracies for the chromatographically separated components averaged -0.28 ppm and ranged between 0.64 and -1.03 ppm (Table 1). Some of the major components in the sample are shown in Figure 5. Chromatographic peaks for PCB 206 and glycerol tricaprylate coelute under conditions used in this study (Figure 5). The high performance mass spectrometer and LECO's ChromaTOF-HRT™ software allowed for easy extraction and deconvolution of PCB 206 from the oil matrix.

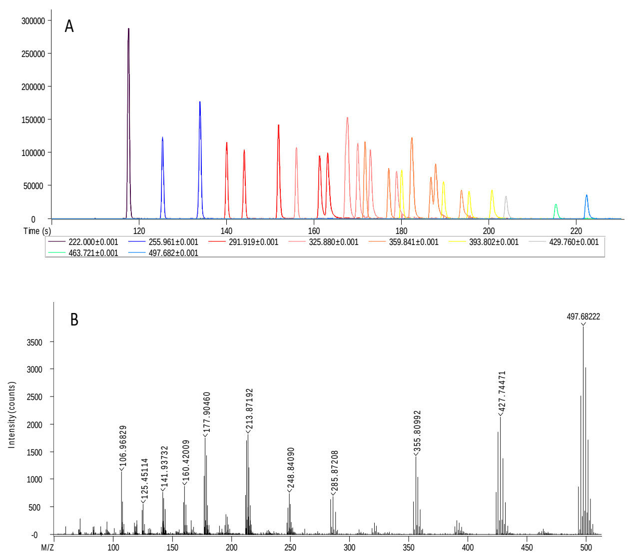

Figure 4. XIC for WHO/NIST/NOAA PCB Standard (A) Mass Spectrum for Decachlorobiphenyl (B).

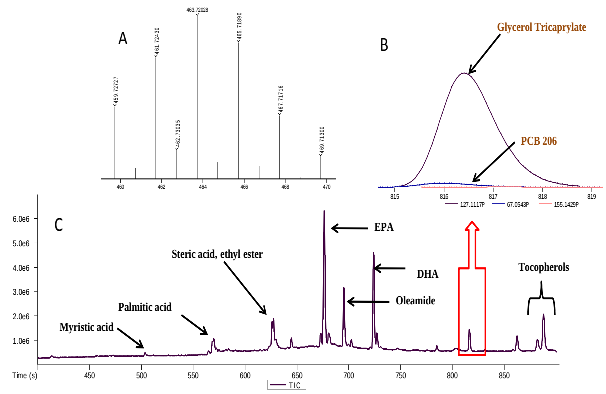

Figure 5. TIC of Fish Oil (C) Showing Coeluting Compounds Glycerol Tricaprylate and PCB 206 (B) and the molecular ion region of the mass spectrum for PCB 206 (A).

Table 1. Mass Accuracy Values for WHO/NIST/NOAA PCB Standard.

PCB	Name	Exact Mass	Measured Mass	Mass Accuracy(ppm)
8	2,4'-Dichlorobiphenyl	221.99976	221.99973	0.12
18	2,2',5-Trichlorobiphenyl	255.96078	255.96069	0.37
28	2,4,4'-Trichlorobiphenyl	255.96078	255.96072	0.25
52	2,2',5,5'-Tetrachlorobiphenyl	289.92181	289.92186	-0.16
44	2,2',3,5'-Tetrachlorobiphenyl	289.92181	289.92191	-0.34
66	2,3',4,4'-Tetrachlorobiphenyl	289.92181	Not Resolved	------
101	2,2',4,5,5'-Pentachlorobiphenyl	323.88284	323.88267	0.53
81	3,4,4',5-Tetrachlorobiphenyl	289.92181	289.92192	-0.37
77	3,3',4,4'-Tetrachlorobiphenyl	289.92181	289.92183	-0.06
123	2',3,4,4',5-Pentachlorobiphenyl	323.88284	323.88305	-0.65
118	2,3',4,4',5-Pentachlorobiphenyl	323.88284	323.8829	-0.18
114	2,3,4,4',5-Pentachlorobiphenyl	323.88284	323.88284	0.00
153	2,2',4,4',5,5'-Hexachlorobiphenyl	357.84387	357.8441	-0.65
105	2,3,3',4,4'-Pentachlorobiphenyl	323.88284	323.88281	0.09
138	2,2',3,4,4',5'-Hexachlorobiphenyl	357.84387	357.84392	-0.15
187	2,2',3,4',5,5',6-Heptachlorobiphenyl	391.80490	391.80512	-0.57
126	3,3',4,4',5-Pentachlorobiphenyl	323.88284	323.88288	-0.12
128	2,2',3,3',4,4'-Hexachlorobiphenyl	357.84387	357.84395	-0.23
167	2,3',4,4',5,5'-Hexachlorobiphenyl	357.84387	357.84364	0.64
156	2,3,3',4,4',5-Hexachlorobiphenyl	357.84387	357.84396	-0.26
157	2,3,3',4,4',5'-Hexachlorobiphenyl	357.84387	357.84399	-0.34
180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	391.80490	391.80529	-1.01
170	2,2',3,3',4,4',5-Heptachlorobiphenyl	391.80490	391.80508	-0.47
169	3,3',4,4',5,5'-Hexachlorobiphenyl	357.84387	Not Resolved	------
189	2,3,3',4,4',5,5'-Heptachlorobiphenyl	391.80490	391.80518	-0.73
195	2,2',3,3',4,4',5,6-Octachlorobiphenyl	425.76592	425.76634	-0.98
206	2,2'3,3',4,4',5,5',6-Nonachlorobiphenyl	459.72695	459.72742	-1.02
209	Decachlorobiphenyl	493.68798	493.68844	-0.93

Figure 6 displays the TIC (Total Ion Chromatogram), XIC at unit mass resolution, and the XIC at high resolution for a 1.0 g fish oil sample spiked with 100 ng PCB standard. This example clearly illustrates the advantages of LECO's Pegasus GC-HRT for analysis of PCBs in complex matrices. The ability of the high performance mass spectrometer to selectively extract accurate masses at high resolution virtually eliminated background interferences even at low analyte concentrations (Figure 7).

Delivering the Right Results

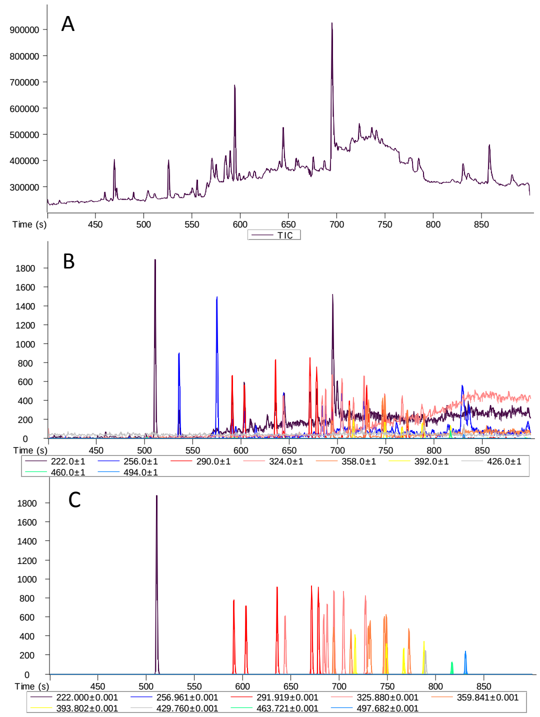

Figure 6. Fish Oil +100 ng/g PCB Standard – TIC (A), XIC at Unit Resolution (B) , XIC at R = 25,000 (C).

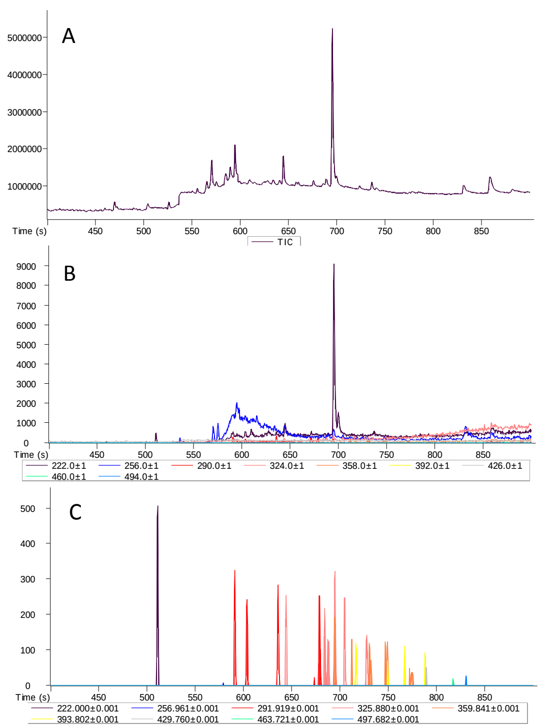

Figure 7. Fish Oil +10 ng/g PCB Standard – TIC (A), XIC at Unit Resolution (B), XIC at R = 25,000 (C).

4. Conclusion

LECO's Pegasus GC-HRT is a powerful and selective tool for the separation and characterization of targeted compounds in complex matrices. The instrument minimizes background contamination and calibration problems typically associated with PCB congener analysis. While complete elimination of background interferences may be challenging, selective targeting of PCBs can be significantly improved with the use of FFP-TOFMS technology. The GC-HRT's state-of-the-art mass analyzer, sensitivity, and fast acquisition capabilities make it ideal for environmental, petrochemical, metabolomics, and forensic applications.

5. References

(1) Kris-Etherton PM, Harris WS and Appel LJ *Circulation*, 2002, *106*:2747-2757.

(2) Ahlborg UG, Becking GC, Birnbaum LS, Brouwer A, Derks HJGM, Feeley M, Golor G, Hanberg A, Larsen JC, Liem AKD, Safe SH, Schlatter C, Waern F, Younes M and Yrjanheikki E *Chemosphere*, 1994, *28*, 6:1049-1067.

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