Analysis of Persistent Organic Pollutants in Complex Matrices by Gas Chromatography—High Resolution Time-of-Flight Mass Spectrometry

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1. Introduction

Persistent Organic Pollutants (POPs) are halogenated organic compounds used for decades as pesticides, flame retardants, and for the manufacture of a variety of commercial goods.[1] They include, but are not limited to, polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) (Figure 1). Unfortunately, these compounds bioaccumulate, and their unfavorable health and environmental effects have led to their regulation in many countries.[2] Analysis of POPs in environmental or biological samples is challenging due to a wide range of concentrations in nature and because of signal reduction and inadequate chromatographic baselines induced by major components in complex environmental matrices.[3] In addition, analysis can be further complicated by POP decomposition products, metabolites or miscellaneous halogenated materials present in samples.[4-7] Many of these interfering compounds produce similar mass spectral data and can be present in higher concentrations than targeted compounds.[8] The development of instrumental techniques that can provide a comprehensive analysis of environmental samples in a minimal amount of time would significantly improve analysis of POPs.
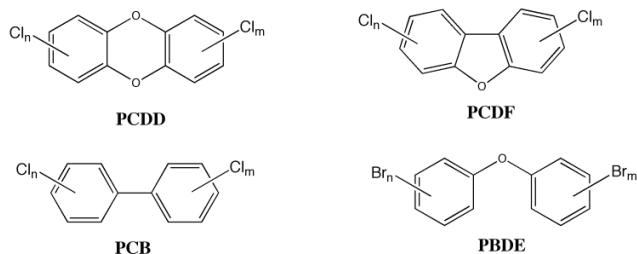

Figure 1. Structures of four different POP classes.

In this study, polychlorinated biphenyls (PCBs) were targets in the analysis of sediment and fish tissue samples; however, several untargeted classes of POPs were discovered and characterized. LECO's Pegasus® GC-HRT with Folded Flight Path™ (FFP™) technology provided the required resolving power for accurate mass measurements and robust molecular formula determinations (Figure 2).
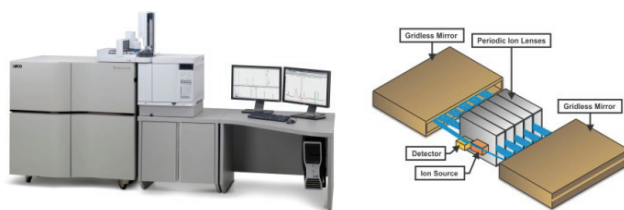

Figure 2. LECO Pegasus GC-HRT (left) and its FFP™ Mass Analyzer (Right).

2. Experimental Conditions

Samples

Environmental samples were prepared[9] by extraction using dichloromethane:hexane (1:4 v/v), clean up with silica pre-packed cartridges (1g) and elution with 15 mL of dichloromethane:hexane (1:4 v/v). Extracts were evaporated to 1 mL final volume in isooctane.

Experimental

Samples were analyzed on a LECO Pegasus GC-HRT using an Agilent 7890 GC and 7693 auto sampler. Mass spectral data were collected using two of the three possible GC-HRT operating modes (Figure 3): High Resolution (R = 25,000, FWHM at 218.985080) and Ultra-High Resolution mode (R = 50,000, FWHM at 218.985080).
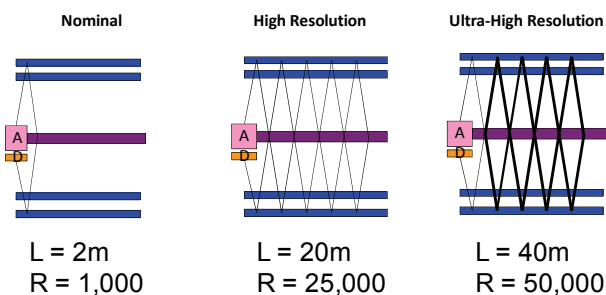

Figure 3. Pegasus GC-HRT operating modes.

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Instrument Parameters

GC

Instrument:	Agilent 7890
Column Type:	Restek Rxi-5Sil MS (60 m x 0.18 mm x 0.10 μm)
Injection:	Splitless, 1 μL
Inj. Temp.:	300°C
Oven:	80°C (1 min) to 160°C at 30°C/min to 300°C at 3.0°C/min (4 min)
Carrier Gas:	He, 1.00 mL/min constant flow

MS

Spectrometer:	LECO Pegasus GC-HRT
Ion Source:	LECO EI
Polarity:	Positive (70 eV)
Flight Path:	High Resolution Mode (R=25,000) and Ultra-High Resolution Mode (R=50,000)
Acquisition:	3 spectra/second
m/z Range:	50 to 1000 High Resolution Mode, 200 to 600 Ultra-High Resolution Mode
Calibration:	PFTBA

3. Results

Instrument Performance

High resolution (HR) mode analysis of a sediment sample produced the analytical ion chromatogram (AIC) and extracted ion chromatogram (XIC) shown in Figure 4. The XIC highlights three PCBs (CB 47, CB 162 and CB 209) congeners, and Table 1 lists formulas, retention times and areas for all PCBs in the sample. Peak True (deconvoluted) and NIST library mass spectral data for CB 47, CB 162 and CB 209, are shown in Figure 5. Library matches for these PCBs were 953, 826 and 865 respectively. Expansions of the molecular ion regions, as well as, calculated molecular ion, observed molecular ion and mass accuracy values are shown in Figure 6. Further evidence of the high-quality spectral data produced by the GC-HRT can be seen in excellent correlations between expected and observed relative abundance ratios of molecular cluster ions for the PCBs (Figure 7).

Table 1. PCBs (tri- to decasubstituted congeners) in sediment sample.

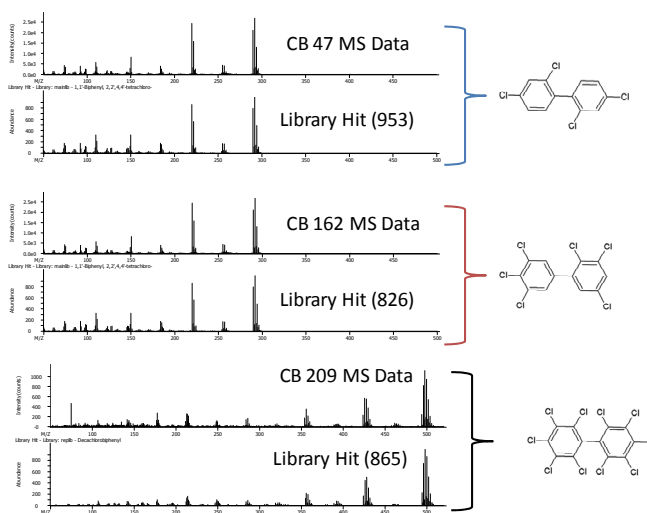| Name	Formula	R.T. (s)	Area	Name	Formula	R.T. (s)	Area	Name	Formula	R.T. (s)	Area
TriCB	$C_{12}H_7Cl_3$	1052.503	90036	PenCB	$C_{12}H_5Cl_5$	1525.263	80451	HepCB	$C_{12}H_3Cl_7$	1748.87	129908
TetCB	$C_{12}H_6Cl_4$	1075.019	3499	PenCB		1544.015	666924	HepCB		1774.702	35378
TetCB		1111.079	7052	PenCB		1577.974	39075	HepCB		1820.115	62586
TetCB		1154.306	123226	PenCB		1620.693	103680	HepCB		1838.886	14415
TetCB		1165.816	87021	PenCB		1636.637	466732	HepCB		1849.893	474501
TetCB		1173.737	61107	PenCB		1668.432	14225	HepCB		1868.133	217316
TetCB		1222.568	79640	PenCB		1719.663	170999	HepCB		1904.287	29288
TetCB		1252.307	81059	PenCB		1798.772	50147	HepCB		1928.108	180423
TetCB		1272.978	2909	PenCB		2106.842	1244	HepCB		1943.651	123691
TetCB		1315.938	11301	HexCB	$C_{12}H_4Cl_6$	1462.554	1555	HepCB		1960.329	64174
TetCB		1330.218	105425	HexCB		1533.09	42658	HepCB		2002.919	53361
TetCB		1345.08	123560	HexCB		1544.607	23796	HepCB		2027.053	795586
TetCB		1352.366	179842	HexCB		1594.599	113417	HepCB		2042.186	14472
TetCB		1405.504	43067	HexCB		1622.51	590073	HepCB		2118.13	302923
PenCB	$C_{12}H_5Cl_5$	1274.859	4809	HexCB		1653.083	22929	HepCB		2219.442	12527
PenCB		1293.439	4094	HexCB		1665.672	23518	OctCB	$C_{12}H_2Cl_8$	1952.966	49055
PenCB		1314.825	1034	HexCB		1687.938	235128	OctCB		1980.925	34026
PenCB		1338.765	16872	HexCB		1710.936	1584764	OctCB		2008.085	16090
PenCB		1349.821	155315	HexCB		1751.473	178022	OctCB		2060.381	16416
PenCB		1370.019	59071	HexCB		1769.057	32412	OctCB		2133.701	7644
PenCB		1409.384	100037	HexCB		1777.395	58051	OctCB		2144.343	161838
PenCB		1415.549	36038	HexCB		1793.046	585510	OctCB		2164.017	192765
PenCB		1429.539	431313	HexCB		1798.483	1049767	OctCB		2258.697	36586
PenCB		1443.644	262592	HexCB		1840.387	5725	OctCB		2331.503	116409
PenCB		1461.734	21851	HexCB		1877.914	10971	OctCB		2344.345	9165
PenCB		1474.569	21212	HexCB		1884.896	119773	NonCB	$C_{12}HCl_9$	2253.951	23453
PenCB		1492.987	60267	HexCB		1898.028	49684	NonCB		2282.695	14128
PenCB		1505.834	25077	HexCB		1970.659	67528	NonCB		2447.722	35026
PenCB		1514.069	139005	HexCB		1986.006	21501	DecCB	$C_{12}Cl_{10}$	2545.513	227994

Figure 5. Peak True and NIST library mass spectra for CB 47 (A), CB 162 (B) and CB 209 (C).
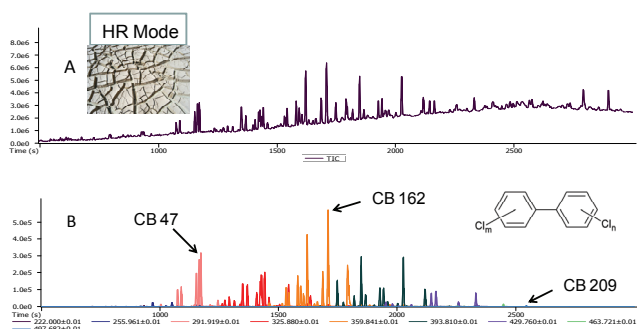

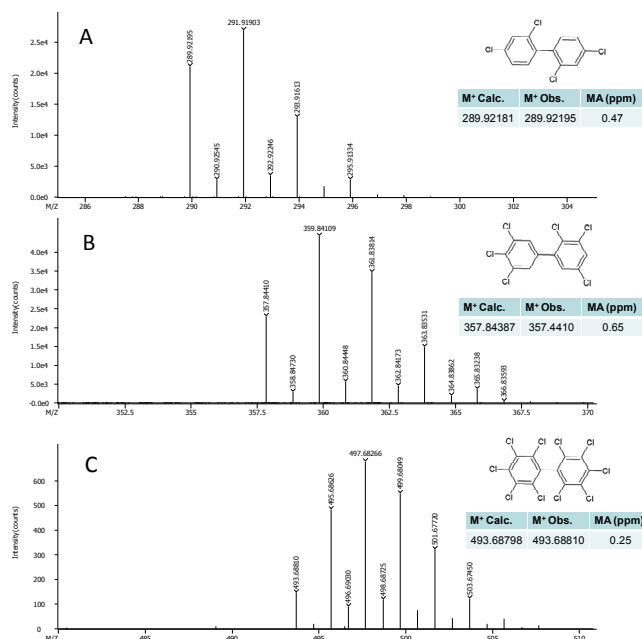**Figure 4. AIC (A) and XIC (B) showing PCBs in sediment sample.**

Figure 6. Calculated molecular ion, observed molecular ion and mass accuracy values for CB 47 (A), CB 162 (B) and CB 209 (C).
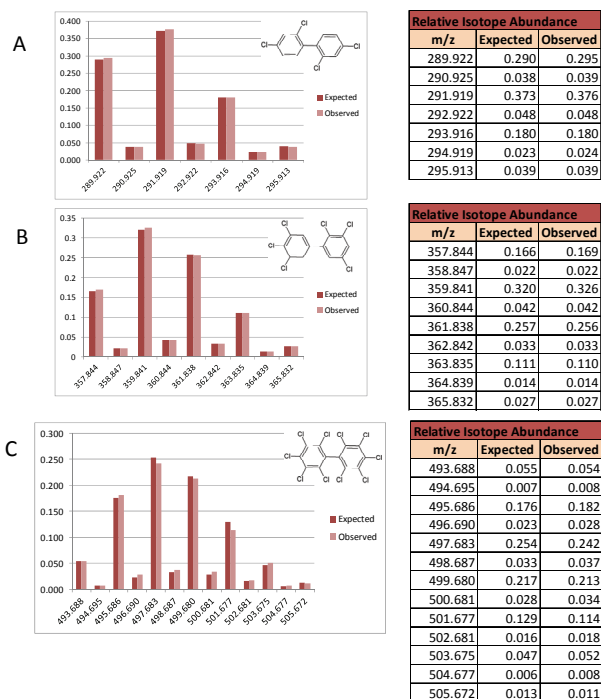

Figure 7. Expected and observed molecular ion cluster relative isotope abundance ratios for CB 47 (A), CB 162 (B) and CB 209 (C).

An example of comprehensive POP analysis is illustrated in Figure 9 where different classes of compounds were extracted from the fish tissue data: Hexachlorobenzene (HCB), octachlorostyrene (OCS), 1-chloro-2,3-*bis*(*p*-chlorophenyl)ethylene (DDMU), 2,2-*bis*(4-chlorophenyl)-1,1-dichloroethene (*p,p'*-DDE), dichlorodiphenyl-trichloroethane (DDT), dibromophenyl ether (BDE 47), and 2,2',3,4,4',5,5'-heptachloro-1,1-biphenyl (CB 180). Table 3 lists molecular ion and mass accuracy values for each of these compounds.
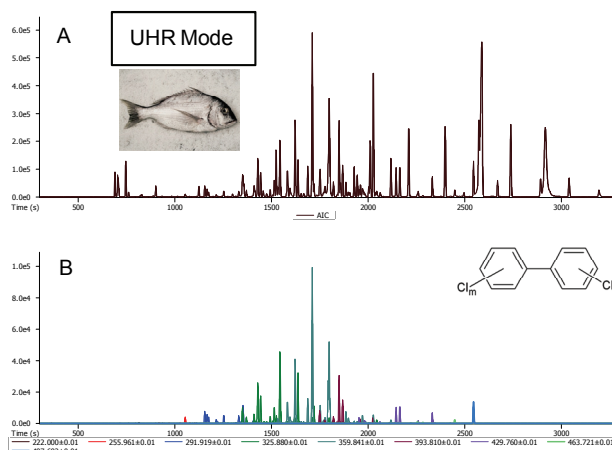

Figure 8. AIC (A) and XIC (B) showing different PCB congeners in a fish tissue sample.

Table 2. Mass accuracy values for PCBs in fish tissue sample.

Name	M⁺	MA (ppm)	Name	M⁺	MA (ppm)
1,1'-Biphenyl, 2,4',5-trichloro-	255.9607	-0.33	1,1'-Biphenyl, 2,2',3,4,5,6'-Hexachloro-	357.8441	0.54
1,1'-Biphenyl, 2,2',3,3'-tetrachloro-	289.92179	-0.08	2,3,3',4',5,6-Hexachloro-1,1'-biphenyl	357.8441	0.70
2,2',3,6-Tetrachloro-1,1'-biphenyl	289.92152	-1.01	3,3',4,5,5'-Pentachloro-1,1'-biphenyl	323.8834	1.85
1,1'-Biphenyl, 2,2',6,6'-tetrachloro-	289.92206	-0.85	1,1'-Biphenyl, 2,3,3',4,4',5,6-Heptachloro-	391.804	-2.41
2,2',4,5-Tetrachloro-1,1'-biphenyl	289.92295	0.90	1,1'-Biphenyl, 2,2',3,4,4',6-heptachloro-	357.8436	-0.78
1,1'-Biphenyl, 2,2',3,4-tetrachloro-	289.92101	-2.77	2,2',3,4,5,6-Hexachloro-1,1'-biphenyl	357.8442	0.84
1,1'-Biphenyl, 3,3',4,4'-tetrachloro-	289.92114	-2.32	2,2',3,3',4,5',6-Heptachlorobiphenyl	391.8048	-0.32
2,3,3',5-Tetrachloro-1,1'-biphenyl	289.92201	0.68	2,2',3,4,5,6'-Hexachloro-1,1'-biphenyl	357.8446	1.99
1,1'-Biphenyl, 3,3',4,5'-tetrachloro-	289.92127	-1.87	2,3,3',4',5,5'-Hexachloro-1,1'-biphenyl	357.8438	-0.11
1,1'-Biphenyl, 2,4,4',6-tetrachloro-	289.92124	-1.97	2,2',3,4,4',6'-Hexachloro-1,1'-biphenyl	357.8438	-0.27
2,2',3,5,6'-Pentachloro-1,1'-biphenyl	323.88311	0.83	1,1'-Biphenyl, 2,2',3,4,5',6-heptachloro-	391.8056	1.85
1,1'-Biphenyl, 2,3',4',5-tetrachloro-	289.92201	0.68	1,1'-Biphenyl, 2,2',3,3',5,5',6-heptachloro-	391.8037	-2.97
1,1'-Biphenyl, 2,2',3,5'-pentachloro-	323.88289	-0.09	1,1'-Biphenyl, 2,2',3,3',4,4',6-heptachloro-	391.8046	-0.70
1,1'-Biphenyl, 2,3',5,5'-tetrachloro-	289.92198	0.58	1,1'-Biphenyl, 2,2',3,4,4',5',6-heptachloro-	391.8049	-0.12
1,1'-Biphenyl, 2,2',3',4,5-Pentachloro-	323.88281	0.15	1,1'-Biphenyl, 2,3,3',4,4'-hexachloro-	357.844	0.23
1,1'-Biphenyl, 2,2',3,5,5'-Pentachloro-	323.88293	0.28	1,1'-Biphenyl, 2,3,4,4',5,5'-hexachloro-	357.8436	-0.78
1,1'-Biphenyl, 2,2',4,5,6'-pentachloro-	323.88287	0.09	1,1'-Biphenyl, 2,2',3,3',4,4',5-heptachloro-	391.8052	0.73
1,1'-Biphenyl, 2,2',4,5,5'-pentachloro-	323.99311	0.83	1,1'-Biphenyl, 2,2',3,3',5,5',6-heptachloro-	391.8051	0.57
1,1'-Biphenyl, 2,2',3,3'-6-pentachloro-	323.99238	-1.42	1,1'-Biphenyl, 2,2',3,3',4,5',6'-heptachloro-	391.8042	-1.70
1,1'-Biphenyl, 2',3,4,5,5'-Pentachloro-	323.88289	0.15	1,1'-Biphenyl, 2,2',3,3',4,5,6,6'-octachloro-	425.766	0.27
2,2',3,4,4'-Pentachloro-1,1'-biphenyl	323.88297	0.40	1,1'-Biphenyl, 2,2',3,3',4,4',5-heptachloro-	391.8046	-0.68
1,1'-Biphenyl, 2,2',3,4,6-Pentachloro-	323.88253	-0.96	1,1'-Biphenyl, 3,3',4,4',5,5'-hexachloro-	357.8446	2.07
2,3,4,4',5,6-Hexachloro-1,1'-biphenyl	357.84444	-0.85	1,1'-Biphenyl, 2,2',3,4,4',5,6,6'-octachloro-	425.766	0.13
1,1'-Biphenyl, 2,3,3',4',6-pentachloro-	323.88314	0.93	1,1'-Biphenyl, 2,2',3,4',5,5',6-heptachloro-	391.8055	1.42
1,1'-Biphenyl, 2,3,3',4,4'-pentachloro-	357.84255	2.45	1,1'-Biphenyl, 2,2',3,3',4,4',6,6'-octachloro-	425.7666	1.68
1,1'-Biphenyl, 2,3',4,4',5-pentachloro-	323.88208	-2.35	2,3,3',4,4',5,5',6-Octachloro-1,1'-biphenyl	425.7657	-0.85
2,2',3,4',5,6-Hexachloro-1,1'-biphenyl	357.84415	0.79	2,2',3,3',4,5,6-Heptachloro-1,1'-biphenyl	391.8004	0.01
1,1'-Biphenyl, 2,2',3,3',4,5-hexachloro-	357.8441	0.65	2,2',3,4,4',5,5',6-Octachloro-1,1'-biphenyl	425.7662	0.72
1,1'-Biphenyl, 2,2',3,3',4,4'-hexachloro-	357.8441	0.65	1,1'-Biphenyl, 2,2',3,3',4,4',5,5'-octachloro-	425.7655	-1.02
1,1'-Biphenyl, 2,3,3',4',6-hexachloro-	323.88256	-0.86	1,1'-Biphenyl, 2,2',3,3',4,4',5,5',6-nonachloro-	459.7262	-1.65
1,1'-Biphenyl, 2,2',3,4',5',6-hexachloro-	357.84405	0.51	1,1'-Biphenyl, 2,2',3,3',4,5,5',6'-octachloro-	425.7654	-1.18
1,1'-Biphenyl, 2,3,4',5,6-Pentachloro-	323.88301	0.52	1,1'-Biphenyl, 2,2',3,3',4,5',6'-octachloro-	425.766	0.09
1,1'-Biphenyl, 2,2',3,4',5,5'-hexachloro-	357.84278	-3.04	1,1'-Biphenyl, 2,2',3,3',4,5,5',6,6'-nonachloro-	459.7284	3.22
1,1'-Biphenyl, 2,2',3,3',5,6-hexachloro-	357.84436	1.38	Decachlorobiphenyl	493.6879	-0.22

Analysis of a fish tissue sample containing elevated levels of PCBs produced the AIC and XIC shown in Figure 8. An average mass accuracy of 1.01 ppm was obtained for 70 PCBs discovered in the sample (Table 2). The enhanced resolving power of the GC-HRT in Ultra-High Resolution (UHR) mode (R = 50,000) facilitates selective extraction of POPs from the "forest of ions" that constitute the total ion chromatogram.
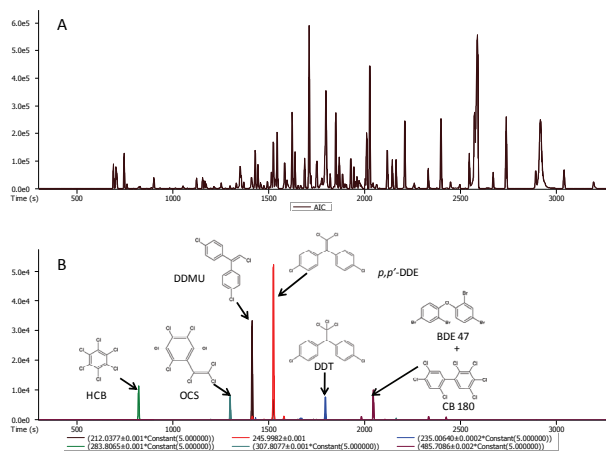
Figure 9. AIC (A) and XIC (B) showing different classes of POPs in fish tissue sample.

Table 3. Mass accuracies for different POP classes in fish tissue sample.

POP	Name	Formula	R.T.(s)	M⁺ Obs	Mass Accuracy (PPM)
HCB	Hexachlorobenzene	C_6Cl_6	821	281.81262	0.19
OCS	Octachlorostyrene	C_8Cl_8	1298	375.75071	1.16
DDMU	1-Chloro-2,3-*bis(p*-chlorophenyl)Ethylene	$C_{14}H_9Cl_3$	1410	281.97669	0.90
p,p'-DDE	2,2-*Bis*(4-chlorophenyl)-1,1-dichloroethene	$C_{14}H_8Cl_4$	1524	315.93736	-0.33
DDT	Dichlorodiphenyltrichloroethane	$C_{14}H_9Cl_5$	1795	235.00784*	0.73
BDE-47	Dibromophenyl ether	$C_{12}H_6Br_4O$	2044	481.71430	-0.55
CB-180	2,2',3,4,4',5,5'-Heptachloro-1,1-biphenyl	$C_{12}H_3Cl_7$	2044	391.80481	-0.38
				*M-CCl_3	

A mass spectrum for coeluting POPs BDE 47 and CB 180 is shown in Figure 6. Mass accuracy values for the molecular ions of CB 180 and BDE 47 were -0.38 and -0.55 ppm. Robust analysis of this data would be difficult without an instrument with high resolving power due to interfering mass fragments with nominal mass of 324: $[M-Cl_2+2]^{+\bullet}$ for CB 180 and $[M-Br_2]^{+\bullet}$ for BDE 47. According to Alaee[10], resolution of these isobaric fragments would require an MS instrument with a resolving power $\geq$ 25,000 (m/Δm). Operation of LECO's Pegasus GC-HRT in ultra-high resolution mode results in baseline resolution for the fragment ions.
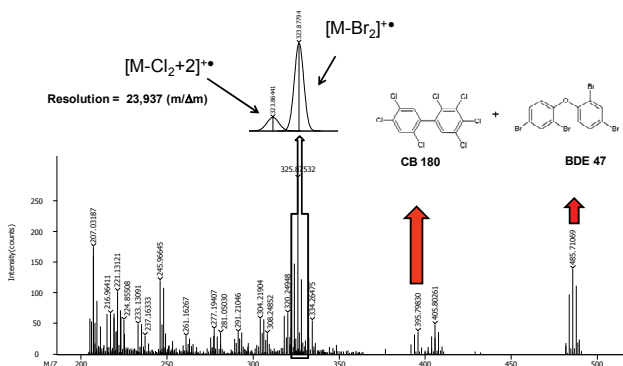

Figure 10. Mass spectrum of BDE 47/CB 180 in fish tissue sample.

4. Conclusions

LECO Corporation's Pegasus GC-HRT allows for acquisition of spectral data across a wide mass range, screens for different POP classes in a single run and produces high quality data that can be searched against nominal mass libraries (e.g., NIST, Wiley). The selectivity and exceptional mass accuracy values provided by the high resolution mass spectrometer facilitate robust determination of elemental composition. Operation of the instrument in ultra-high resolution mode resulted in clean separation of isobaric fragments for BDE 47 and CB 180. The Pegasus GC-HRT is an indispensable tool for targeted and non-targeted analysis of persistent organic pollutants in complex environmental matrices.

5. References

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