

# Comprehensive Analysis of the Great Lakes Top Predator Fish for Novel Halogenated Organic Contaminants by GCxGC-HR-ToF Mass Spectrometry

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## Supporting Information

### Experimental-Chemicals and Methods

#### Halogenated script and Figure S1

**Table S1.** Comparison of concentrations determined using GCxGC-HRT semi-quantitative method and our target method.

**Table S2.** Halogenated organics detected in fish from the Great Lakes from the current study that are also part of the routine monitoring program. Note that multiple isomers were detected for DDE even though the *p,p'*-DDE isomer was the most abundant. Concentrations are derived from our GCxGC-HRT semi-quantitative method. Also, the concentration of PCBs and PBDEs are presented as the total of all congeners detected.

**Table S3.** Halogenated organics detected in fish from the Great Lakes from the current study that have been reported previously. Concentrations are derived from our GCxGC-HRT semi-quantitative method.

#### Mass Spectra of Unknown Chemicals from Table 3

### **Experimental-Chemicals**

A PCB standard mix (68B-PAR) containing 27 congeners was purchased from Wellington Laboratories (Guelph, Ontario, Canada). Toxaphene Parlar 26, 50 and 62 were purchased from Cambridge Isotopes Laboratories (Tewksbury, MA). 2-Bromo-4-methoxyphenol was purchased from TCI. Sodium polyacrylate (cross linked), PSA bonded silica SPE cartridges (500mg, 6mL), phenanthrene-d<sub>10</sub> (98%), pyrene-d<sub>10</sub> (98%), 2-chloro-4-methoxyphenol and pesticide standard mix (Pesticide 8081) which contained *cis*- and *trans*-Chlordane, *p,p*-DDE, *p,p*-DDD and Dieldrin were purchased from Sigma Aldrich (St. Louis, Missouri). Bio-Beads (S-X3) were purchased from Bio-Rad Laboratories (Hercules, California) for the preparation of a gel permeation chromatography column. Dichloromethane (GC purity), acetone (Optima), hexane (GC purity) were purchased from Fisher Scientific (Pittsburgh, PA).

### **Experimental-Methods**

An aliquot of fish mega composite (10g) from each lake was combined with 15g of drying agent (sodium polyacrylate) in a 66mL ASE cell and extracted with DCM using an Accelerated Solvent Extractor (ASE, Dionex350). The ASE was programmed to perform 3 extraction cycles at 125°C with a static time of 5 mins which resulted in an approximately 120mL extract. Prior to extraction 100 ng of phenanthrene-d<sub>10</sub> (in DCM) was added as a recovery standard to each sample. Lipids were isolated via gel-permeation chromatography (GPC) using two stainless steel columns in series. The primary column (30cm x30mm ID) was packed with biobeads (Bio-Rad) while the secondary column (30cm x 21.2mm) was packed with Phenogel (Phenomenex). The 120mL extract was blown down to 8 mL and 4 equal volume injections (4 x 2 mL) were made. DCM was used as the mobile phase at a flow rate of 5 mL/min. The first 210 ml of the eluate, which

contained lipids, was discarded and the following 50 ml fraction was collected. The extract was blown down to dryness and reconstituted in 2 mL of acetone/hexane (1:1, v:v). The 2 mL sample was loaded onto a Primary Secondary Amine (PSA) bonded silica SPE cartridge for fatty acid removal. After loading, the SPE was flushed with an additional 6mL of acetone/hexane (1:1, v:v) to elute all non-polar components of the sample. The non-polar fraction was then concentrated to approximately 100  $\mu$ L at 40 °C under a gentle stream of nitrogen (TurboVap), followed by the addition of 100 ng of pyrene-d<sub>10</sub> (in DCM) to serve as an injection standard. A sample blank consisting of 15 g of drying agent spiked with 100 ng of phenanthrene-d<sub>10</sub> (recovery standard) was extracted and treated (GPC and SPE) in the same manner as the samples.

Extracts were analyzed using a Pegasus 4D GC $\times$ GC-HRT (LECO, St. Joseph, MI). Helium was used as the carrier gas in constant flow mode at 1 mL/min. A 5  $\mu$ L aliquot was injected into a multimode inlet at an initial temperature of 50 °C for 6 seconds, then ramped to 350 °C at 500 °C /second. Solvent vent conditions were utilized whereby the inlet solvent vent time was set to 6 seconds at a flow of 100 mL/min. The GC $\times$ GC column pair consisted of a 30 m SLB-IL60 (0.25 mm i.d., 0.2  $\mu$ m film thickness; SUPELCO) and 2m Rtx-200 column (0.25 mm i.d., 0.25  $\mu$ m film thickness; RESTEK), respectively. The primary column oven was set to 50 °C for 2 min, ramped to 300 °C at 5 °C/min, then held for 30 min. A 15 °C offset was used for the secondary oven temperature. The GC $\times$ GC modulator period was 5 seconds with a 15 °C offset. The MS was operated in EI mode (250 °C) at a scan rate of 200 spectra/second and a mass resolution of 25,000.

## Halogenated Script

The script was built in the 'Peak Filter' option using the statements allowed by the vendor software (ChromaTOF v1.90.60).

The relative abundance of the first isotopologue in a Cl/Br containing molecule is seldom the most abundant. Therefore, the script was written referencing the M+2 isotopologue. The script [*script command* (**process description**)] and reference Figure S1 are provided below.

*Abundance of Any Mass is >100* (**Selects peaks containing masses > 100 m/z, denoted here M<sub>+2</sub> and A in Figure S1 below**)

AND

*Abundance of any Monoisotope is = abundance of Any Mass - m/z 1.997 +/- 0.001 Da* (**Reduces the number of peaks to include those with masses > 100 m/z and masses = M<sub>+2</sub> - 1.997 m/z with a precision of 0.001 Da. This step identifies the M<sub>+2</sub>, M<sub>+2</sub>-2 halogen isotopologue, B in the Figure below**)

AND

*Abundance of Any mass - m/z 1 +/- 0.001 Da is <20% of abundance of Any Monoisotope* (**Reduces the peaks further to contain those where M<sub>+2</sub> - 1 m/z is less than 20% of intensity of M<sub>+2</sub>. The mass precision is also 0.001 Da. This step identifies the <sup>13</sup>C isotopologue, C < 20% of A below**)

AND

*Abundance of Any Mass - m/z 1.997 +/- 0.001 Da is >40% of abundance of Any Monoisotope* (**This step reduces the peaks to include those that have the M<sub>+2</sub>, M<sub>+2</sub>-2, where the intensity of M<sub>+2</sub>-2 is at least 40% of M. This step is meant to qualify the Cl/Br containing compound spectra. B is > 40% of A below**)

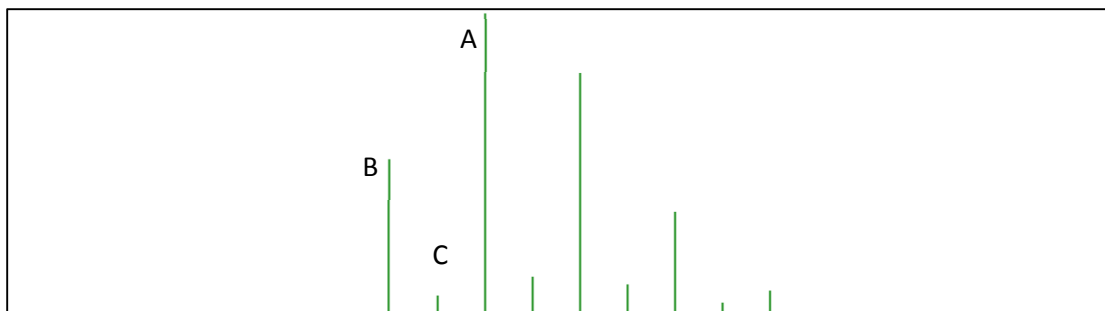


Table S1. Comparison of concentrations determined using GCxGC-HRT semi-quantitative method and our target method.

	<b>Concentration (ng/g ww)</b>				
<b>Compound Class</b>	<b>Superior</b>	<b>Huron</b>	<b>Michigan</b>	<b>Erie</b>	<b>Ontario</b>
PCBs (GCxGC-HRT)	236	366	939	586	461
PCBs (Target method)	264	431	1101	531	558
% difference	-10	-15	-15	10	-17
PBDEs (GCxGC-HRT)	59	46	96	13	47
PBDEs (Target method)	65	45	89	12	48
% difference	-9	4	8	7	-4

Table S2. Halogenated organics detected in fish from the Great Lakes from the current study that are also part of the routine monitoring program. Note that multiple isomers were detected for DDE even though the *p,p'*-DDE isomer was the most abundant. Concentrations are derived from our GCxGC-HRT semi-quantitative method. Also, the concentration of PCBs and PBDEs are presented as the total of all congeners detected.

Tentative ID	1D RT(s)	2D RT(s)	ID Confidence Level	Concentration (ng/g ww)				
				Superior	Huron	Michigan	Erie	Ontario
Nonachlor isomer 1	2106.0	3.2	2	2.1	0.7	2.7	ND	0.2
Oxychlordane	2121.0	3.1	2	2	ND	ND	ND	ND
DDMU isomer 1	2251.0	2.6	2	0.5	ND	ND	ND	0.6
Nonachlor isomer 2	2251.0	2.9	2	22.6	ND	30.8	1.9	11.1
<i>trans</i> -Chlordane	2301.1	2.8	1	3.3	2.7	7.3	1.2	2.3
DDE isomer 1	2306.1	2.6	2	ND	ND	1.6	1.7	2.2
DDMU	2326.1	2.5	2	2.6	5.3	21.2	10	10.7
<i>cis</i> -Chlordane	2351.1	2.6	1	1.5	0.7	1.4	0.6	0.8
<i>p,p'</i> -DDE	2361.1	2.6	1	79.4	107.4	261.9	37.9	111.5
DDE isomer 2	2386.1	2.7	2	2.6	2.7	9.8	1.3	2.9
DDE isomer 3	2426.1	2.6	2	ND	1.9	3.2	ND	2.3
DDE isomer 4	2441.1	2.5	2	ND	ND	ND	ND	2.8
Dieldrin	2461.2	2.7	1	15.9	11.3	29.1	4.8	12.1
Toxaphene (P50)	2486.2	3.2	1	59.2	ND	ND	ND	ND
Nonachlor isomer 3	2546.2	2.6	2	6.2	2.5	16.5	0.5	1.2
DDMU isomer 2	2626.2	2.4	2	ND	2.3	0.8	ND	5.6
Toxaphene (P62)	2636.3	2.7	1	6.2	ND	ND	ND	ND
<i>p,p'</i> -DDD	2691.3	2.3	1	2.2	6	25.1	6	21
Toxaphene (P26)	2696.3	2.8	1	2.7	ND	ND	ND	ND
Total PBDEs				59	46.5	96	12.6	46.5
Total PCBs				236.5	366.3	939	586.1	460.7

ND denotes samples for which the compound of interest was not detected.

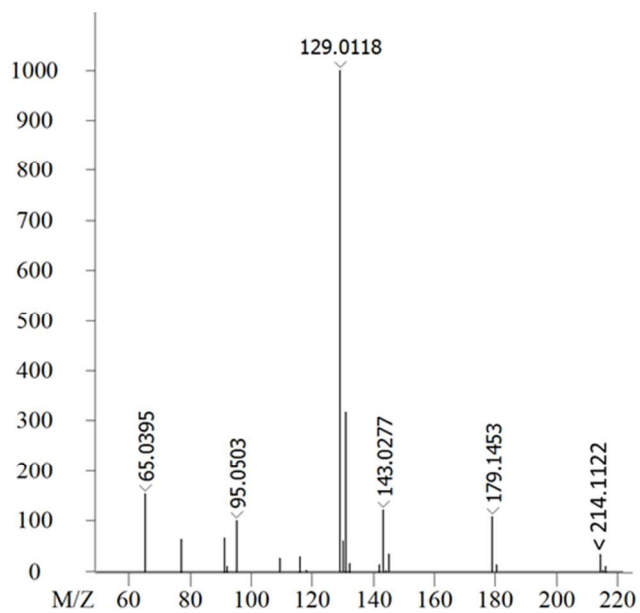
Table S3. Halogenated organics detected in fish from the Great Lakes from the current study that have been reported previously. Concentrations are derived from our GCxGC-HRT semi-quantitative method.

Tentative ID	1D RT(s)	2D RT(s)	ID Confidence Level	Monoisotopic Mass	Formula	Concentration (ng/g ww)				
						Superior	Huron	Michigan	Erie	Ontario
Pentachlorobenzene	1380.6	3.1	2	247.852	C <sub>6</sub> HCl <sub>5</sub>	0.5	0.3	ND	ND	ND
Hexachlorobenzene	1605.7	3.2	2	281.813	C <sub>6</sub> Cl <sub>6</sub>	7.7	12.6	8.7	2.3	ND
Heptachlorostyrene	1755.8	3.1	3	341.789	C <sub>8</sub> HCl <sub>7</sub>	ND	ND	ND	ND	5.9
	1955.9	3.0				ND	ND	ND	1.2	ND
Octachlorostyrene	1935.9	3.3	2	375.751	C <sub>8</sub> Cl <sub>8</sub>	ND	ND	ND	1.4	ND
Pentachloroanisole	1765.8	2.9	2	277.862	C <sub>7</sub> H <sub>3</sub> Cl <sub>5</sub> O	ND	ND	ND	ND	1.0
Tetrachloronaphthalene	1855.8	3.1	2	263.906	C <sub>10</sub> H <sub>4</sub> Cl <sub>4</sub>	ND	ND	ND	1.9	ND
Pentachloronaphthalene	2131.0	3.0	3	297.868	C <sub>10</sub> H <sub>3</sub> Cl <sub>5</sub>	ND	ND	ND	1.5	ND
	2186.0	3.0	3			ND	ND	ND	0.7	ND
Hexachloro diphenylether	2346.1	3.1	3	373.838	C <sub>12</sub> H <sub>4</sub> Cl <sub>6</sub> O	0.4	1.2	1.2	1.7	0.5
	2431.1	3.0	3			2.0	ND	ND	ND	ND
	2501.2	2.8	3			ND	ND	ND	0.3	ND
	2516.2	2.8	3			ND	ND	ND	2.3	0.7
	2581.2	2.9	3			ND	1.9	ND	ND	ND
	2636.3	2.8	3			ND	ND	1.5	ND	ND
Heptachloro diphenylether	2456.2	3.1	3	407.801	C <sub>12</sub> H <sub>3</sub> Cl <sub>7</sub> O	ND	ND	ND	0.5	2.7
Trichloroterphenyl	2706.3	2.6	2	331.992	C <sub>18</sub> H <sub>11</sub> Cl <sub>3</sub>	ND	ND	1.8	1.4	1.8
Tetrachloroterphenyl	2836.4	2.6	2	365.954	C <sub>18</sub> H <sub>10</sub> Cl <sub>4</sub>	ND	ND	ND	0.7	ND
	2981.4	2.7	2			ND	4.5	2.9	2.4	ND

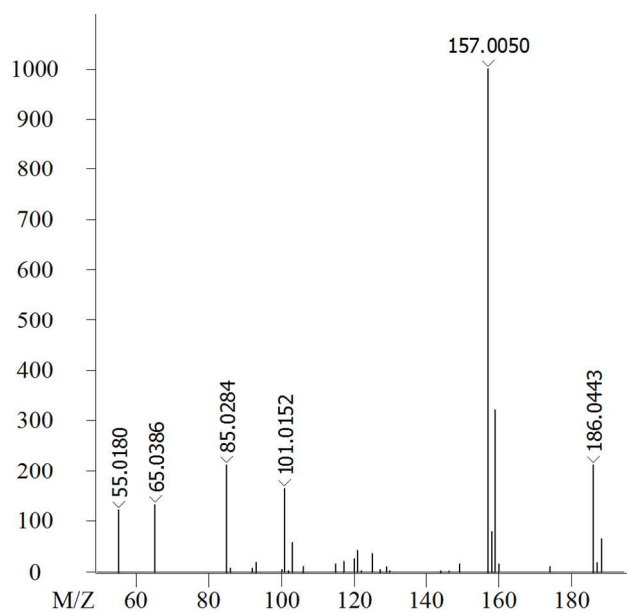
ND denotes samples for which the compound of interest was not detected.

## Mass Spectra of Unknown chemicals from Table 3

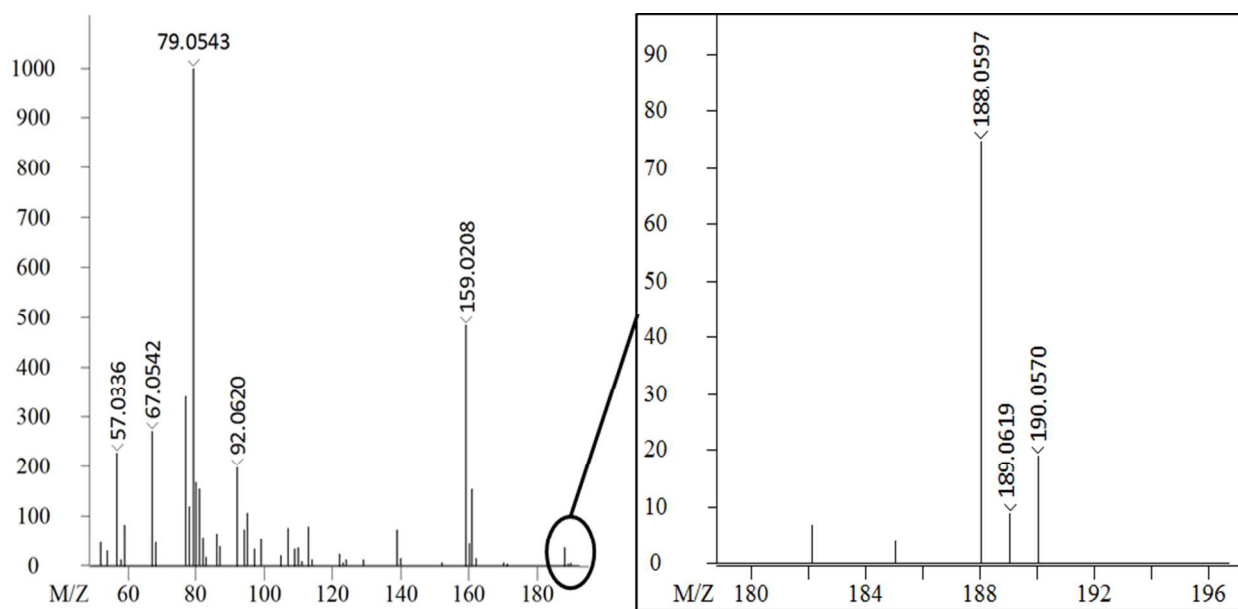
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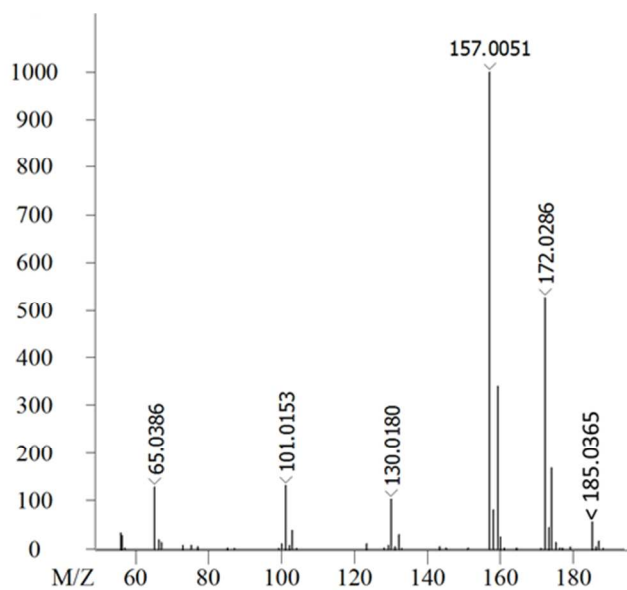
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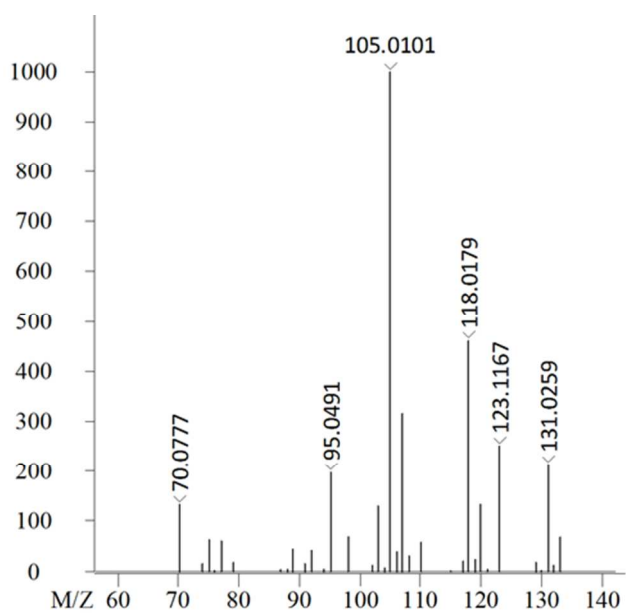
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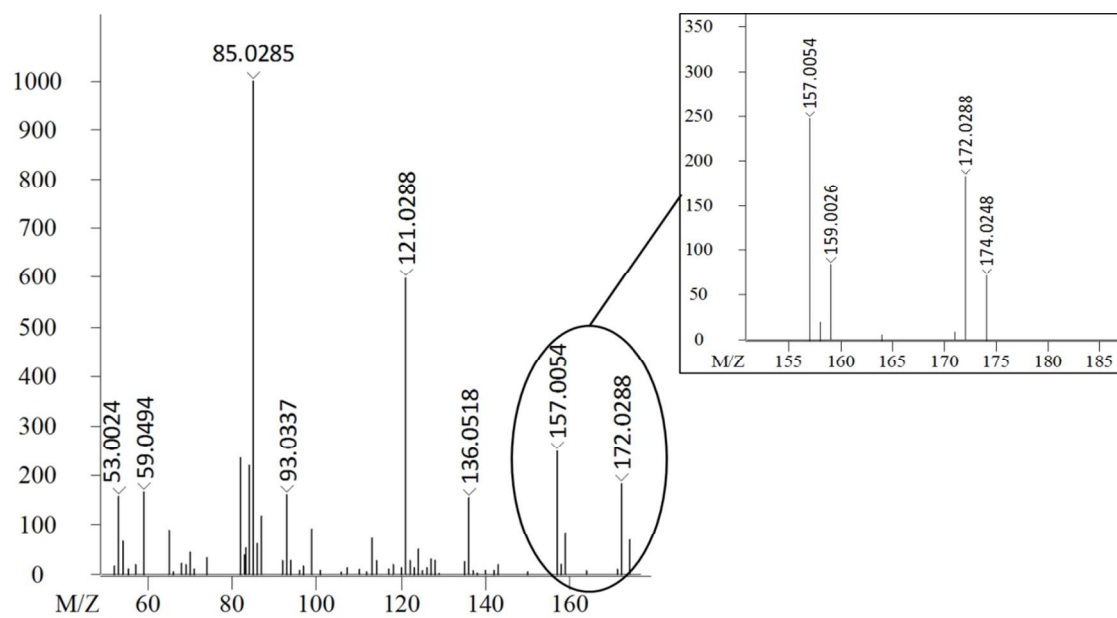
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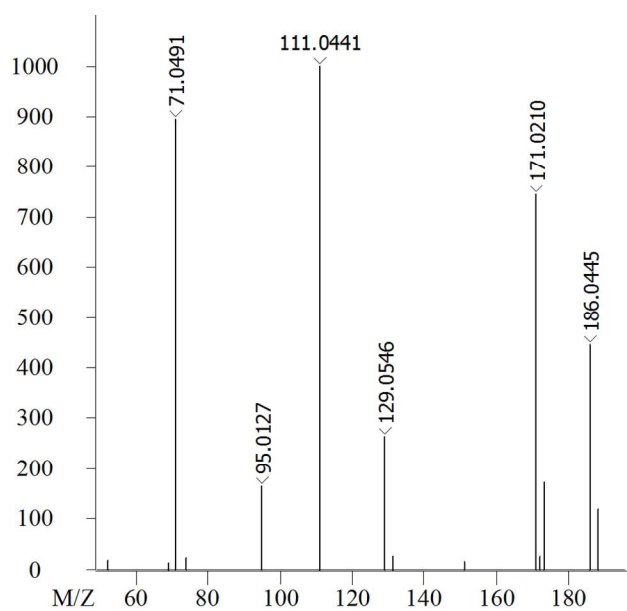
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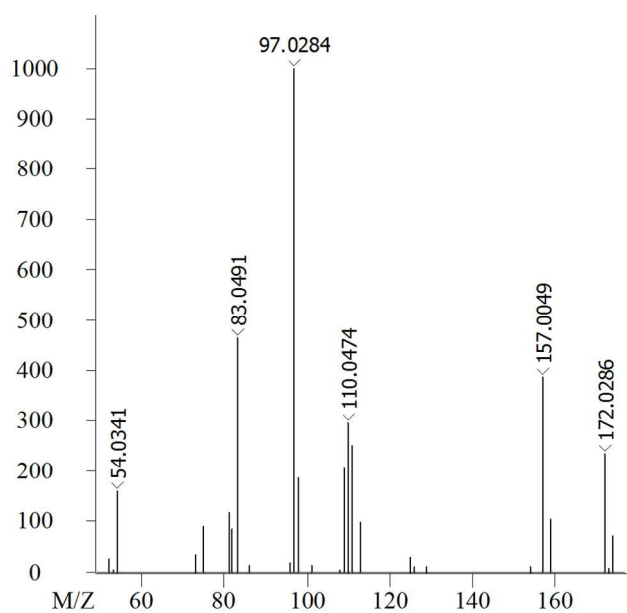
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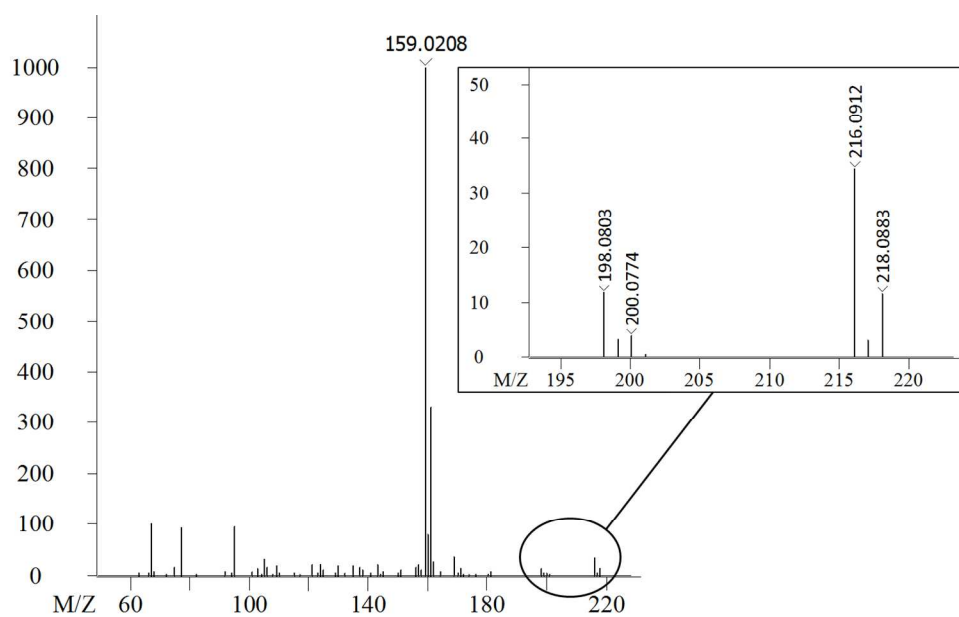
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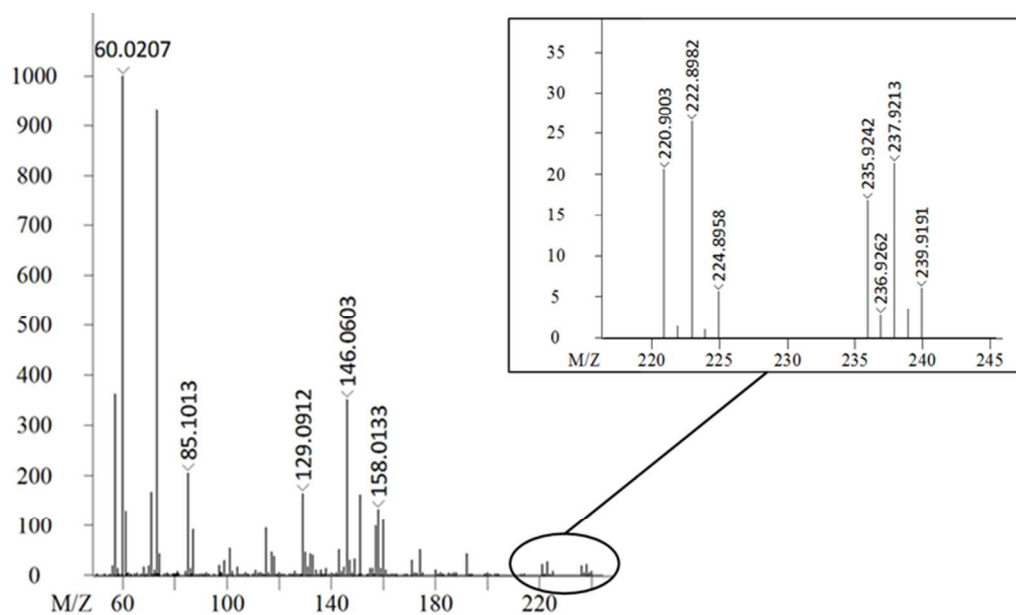
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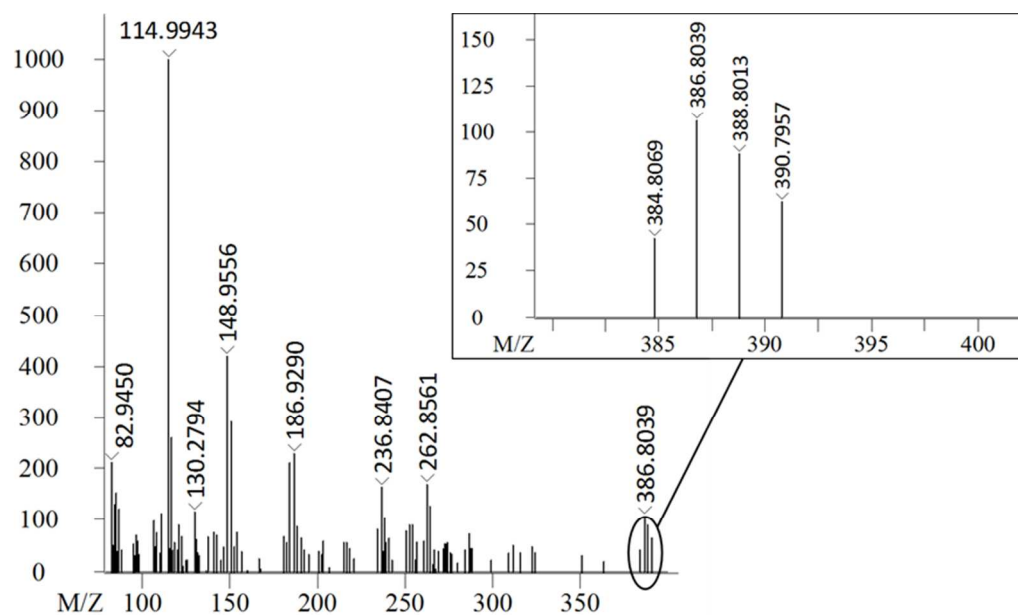
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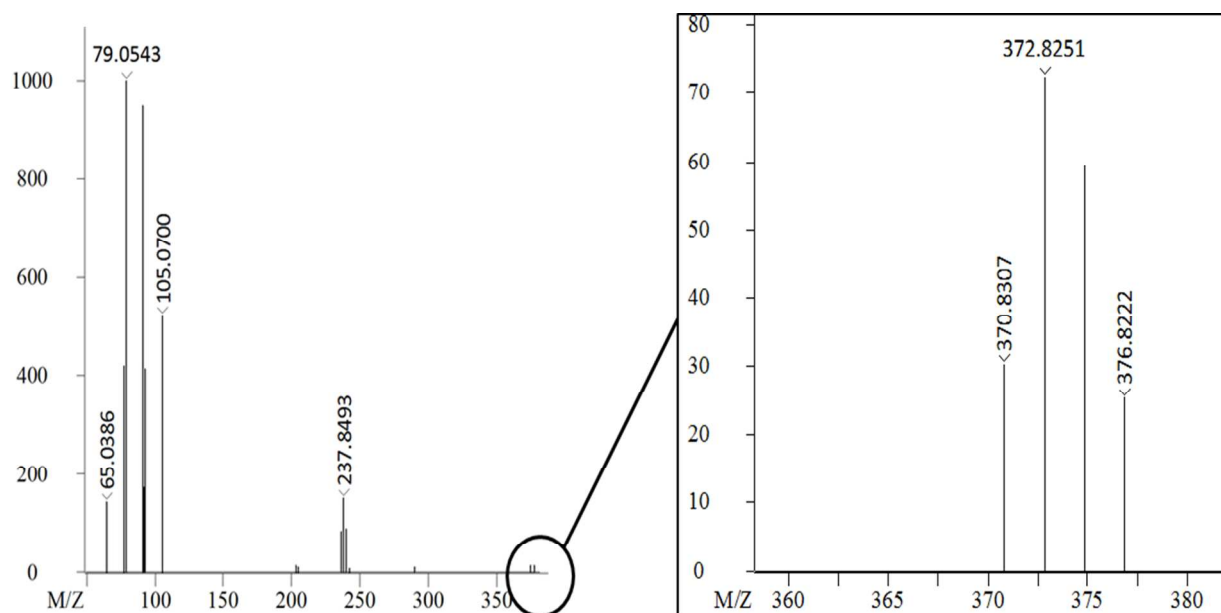
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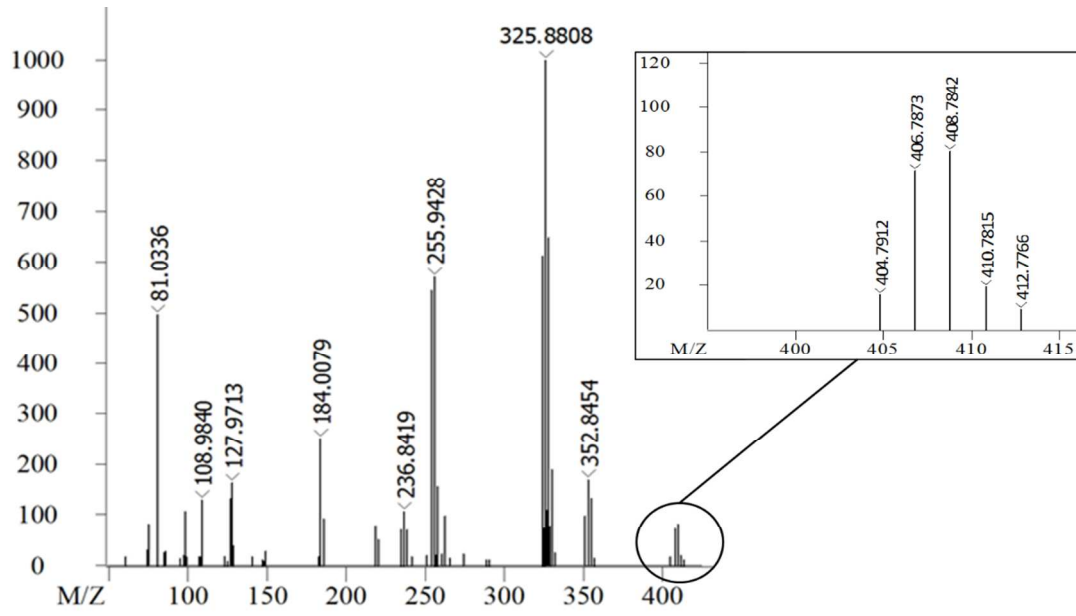
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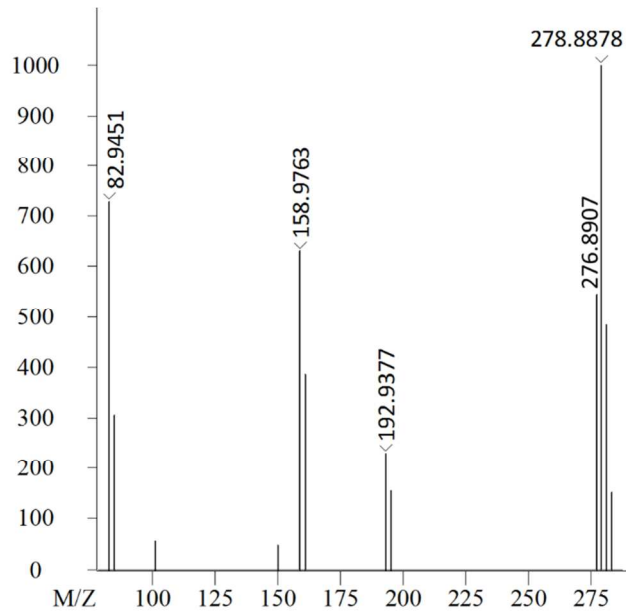
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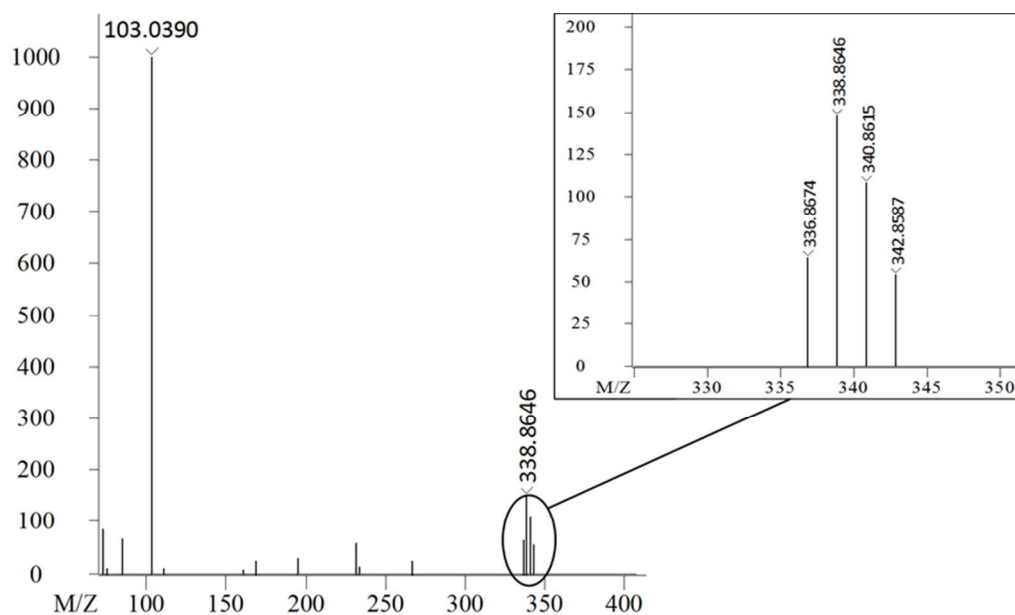
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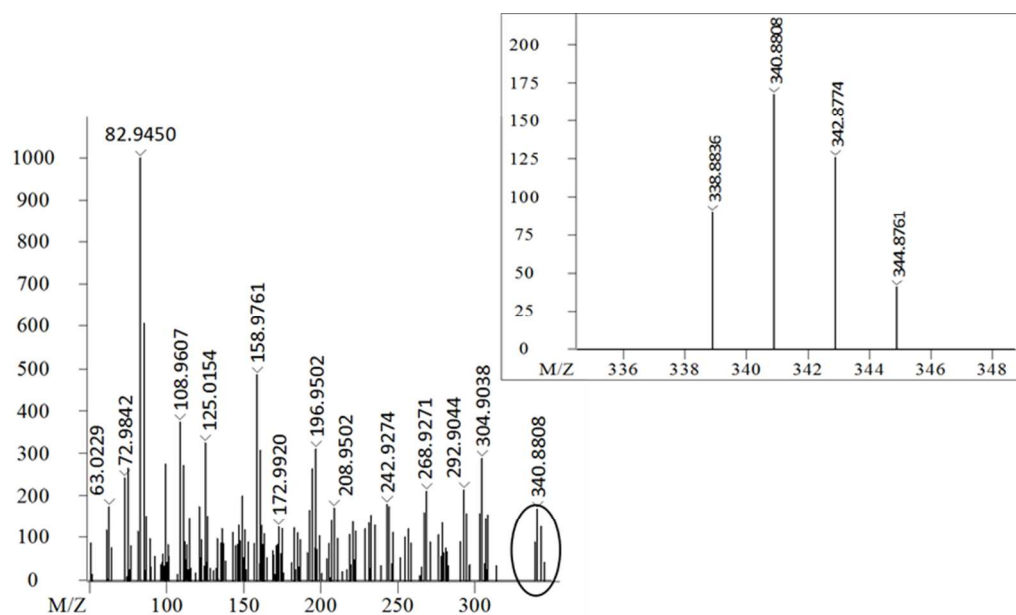
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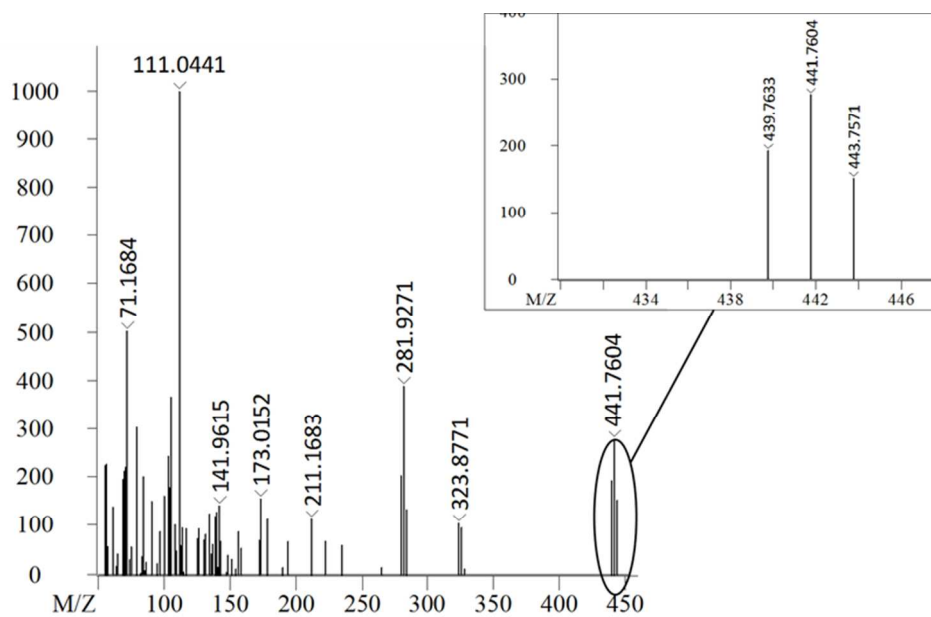
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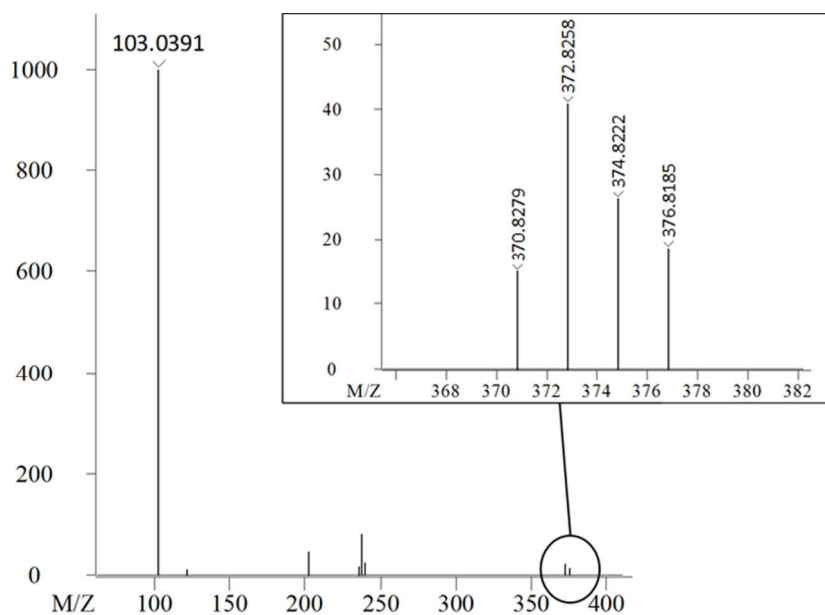
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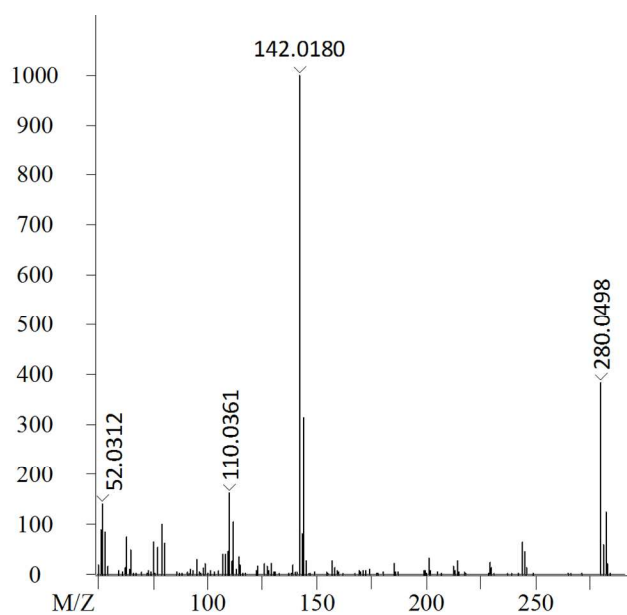
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