

**Untargeted Identification of Organo-bromine Compounds in Lake Sediments by Ultra-High Resolution Mass Spectrometry with Data-Independent Precursor Isolation and Characteristic Fragment (DIPIC-Frag) Method**

Hui PENG, Chunli CHEN, David M.V. Saunders, Jianxian SUN, Song TANG, Garry Codling, Markus Hecker, Steve Wiseman, Paul D. Jones, An Li, Karl J. Rockne, John. P. Giesy

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This supporting information provides text, figures and tables addressing (1) Calculation of isotopic peaks intensities distribution; (2) Information on 25 model NSBCs; (3) Sampling map; (4) Product ion spectra of OH-TBB; (5) Scanning scheme of the DIPIC-Frag method; (6) workflow to identify precursor ions of NSBCs compounds; (7) distribution of isotopic peaks of NSBCs; (8) isotopic peaks of bromine fragment would help to identify bromine number and precursor ions; (9) Validation of bromophenol; (10) Validation of bromoindole; (11) validation of tetrabromocarbazole.

**Isotopic Peaks Intensities Distribution.** Intensities of isotopic peaks for precursor ions and Br fragments were compared semi-quantitatively to further confirm the identities of precursor ions. Since the relative abundances of chlorine and bromine isotopes were much greater than those of other elements, only isotopic peaks of bromine and chlorine were considered for semi-quantitative calculation of isotopic peak. For a given NSOBC with formula  $C_xH_yO_zN_iCl_nBr_m$ , ( $x$ ,  $y$ ,  $z$ , and  $i$  are usually not available), isotopic peaks of the compound were assumed to have a known distribution which follow Pascal's triangle (Equation 1).

$$(a + b)^m (c + d)^n \quad (1)$$

Where:  $a = 0.51$  and  $b = 0.49$  are the relative abundances of Br isotopes  $^{79}\text{Br}$  ( $m/z=78.9183$ ) and  $^{81}\text{Br}$  ( $m/z=80.9163$ ), respectively.  $m$  indicates the number of bromine contained in the compound.  $c = 0.76$  and  $d = 0.24$  are the relative abundances of Cl isotopes  $^{35}\text{Cl}$  ( $m/z=34.9689$ ) and  $^{37}\text{Cl}$  ( $m/z=36.9659$ ), respectively.  $n$  indicates the number of chlorine contained in the compound.

Based on the binomial distribution of the isotopic peaks from Equation 1, the relative abundance of each isotopic peak (with  $k$   $^{79}\text{Br}$  and  $j$   $^{35}\text{Cl}$ ) to total abundance could be calculated as Equation 2.

$$Abundance_i = \frac{m!}{k!} a^k b^{m-k} \times \frac{n!}{j!} c^j d^{n-j} \quad (2)$$

Where:  $Abundance_i$  indicates the relative abundance of the  $i^{\text{th}}$  isotopic peak of the compound,  $k$  is the number of  $^{79}\text{Br}$  and  $j$  is the number of  $^{35}\text{Cl}$  in the isotopic peak.

Since the isotopic peaks of bromine fragments were also useful information for precursor ion alignment, the relative abundance of the peak for monoisotopic  $^{79}\text{Br}$  at  $m/z=78.9183$  was calculated for each isotopic peak by multiplying the relative abundance of the monoisotopic peak (Equation 2).

$$Br_i = Abundance_i \times (k + 1) \times 2 / m \quad (3)$$

Where  $Br_i$  is the relative abundance of  $^{79}\text{Br}$  from the isotopic peak of the compound,  $(k+1) \times 2/m$  is the relative proportion of  $^{79}\text{Br}$  in the isotopic peak to total  $^{79}\text{Br}$ . Based on Equation 3, the relative abundance of  $^{79}\text{Br}$  from each isotopic peak could be calculated.

**Intensities of precursor ion and bromine fragments ions.** Since the relative abundances of precursor ion candidates and bromine fragments are also important information for precursor ion alignment, the threshold of the ratio between abundances of precursor ions (indicated by peak intensities in the present study) and the  $^{79}\text{Br}$  fragment at  $m/z=78.9183$  was predicted. Intensity of the precursor ions should be greater than that of product ions divided by fragment number (Equation 4).

$$\text{Intensity}_{\text{precursor}} > \text{Intensity}_{\text{product}} / m \quad (4)$$

Where:  $\text{Intensity}_{\text{precursor}}$  is the intensity of precursor ions,  $\text{Intensity}_{\text{product}}$  is the intensity of product ions,  $m$  is the number of product ion fragments contained in the formulae (the number of Br atom in the NSOBC).

Because the bromine fragments were monitored from a  $5-m/z$  precursor isolation window, which might contain up to 3 isotopic precursor ions for the same NSOBC, the summed intensities of isotopic precursor ions and bromine fragment were predicted according to Equation 5.

$$\sum \text{Intensity}_{\text{precursor}} > \sum_{k=1,2,3} \text{Intensity}_i / m = \text{Intensity}_{\text{bromine}} / m \quad (5)$$

Where:  $\sum \text{Intensity}_{\text{precursor}}$  is total intensity of isotopic precursor ions from the same NSOBC in the precursor isolation window.  $\text{Intensity}_i$  is intensity of Br fragment ions from the  $i^{\text{th}}$  isotopic

precursor ion, which was expected to be less than the intensity of the corresponding precursor ion, and  $\text{Intensity}_{\text{bromine}}$  is the summed intensity of total Br fragments detected in the corresponding precursor isolation window. Because the precursor isolation window was  $5\text{-}m/z$ , which allowed at most 3 isotopic peaks for brominated compounds, the intensity of the maximal precursor ion in the window should be greater than one third of the summed intensity of all isotopic peaks of precursor ions (Equation 6).

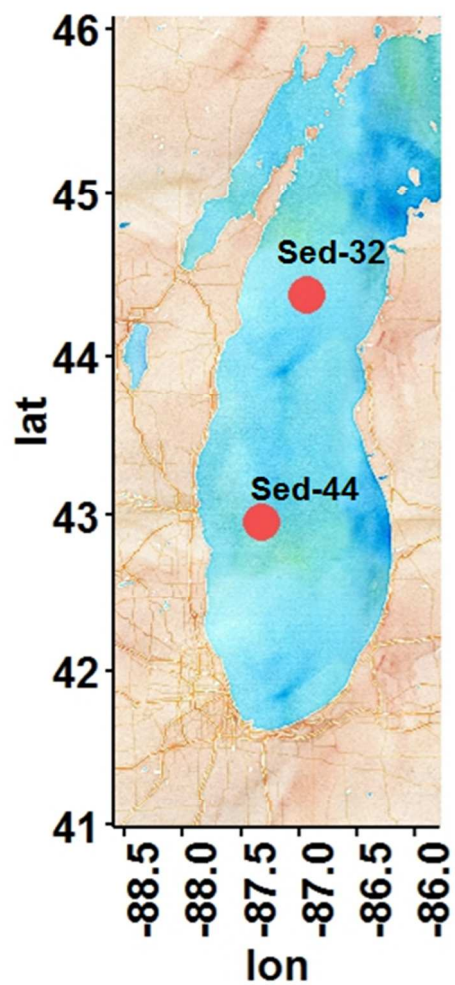
$$\text{Intensity}_{\text{max}} > \text{Intensity}_{\text{bromine}} / (3 \times m) \quad (6)$$

Where:  $\text{Intensity}_{\text{max}}$  is the intensity of the most abundant isotopic precursor ion peak in the isolation window.

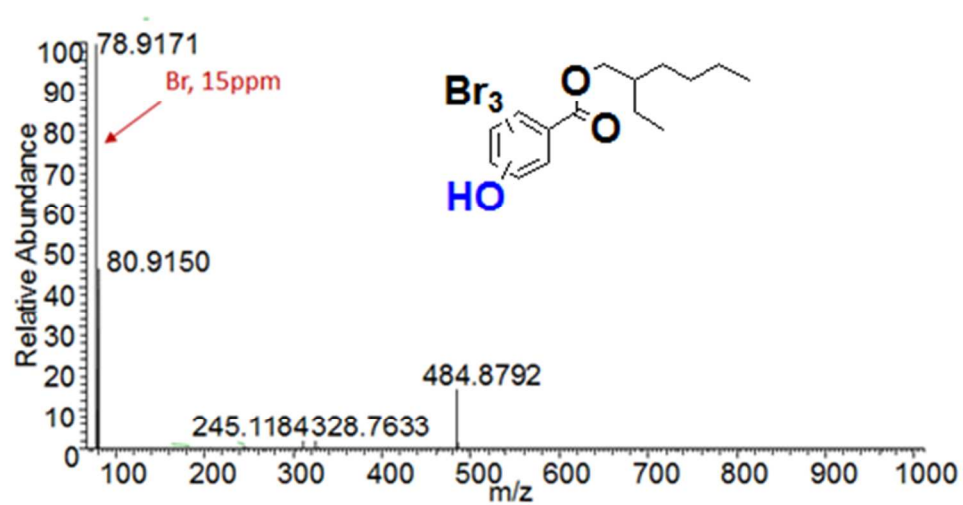
**Table S1.** Name, molecular weight, K<sub>OW</sub> value, and instrument sensitivity of 25 model brominated chemicals.

Compounds	Formula	MW	Log K <sub>OW</sub> <sup>a</sup>	IDLs (µg/L)
HBCD-α	C <sub>12</sub> H <sub>18</sub> Br <sub>6</sub>	635.6509	5.07	0.2
HBCD-β	C <sub>12</sub> H <sub>18</sub> Br <sub>6</sub>	635.6509	5.12	0.3
HBCD-γ	C <sub>12</sub> H <sub>18</sub> Br <sub>6</sub>	635.6509	5.47	0.1
TBBPA	C <sub>15</sub> H <sub>12</sub> Br <sub>4</sub> O <sub>2</sub>	539.7571	4.5	0.05
TBB	C <sub>15</sub> H <sub>18</sub> Br <sub>4</sub> O <sub>2</sub>	545.8040	8.8	2.0
OH-TBB	C <sub>15</sub> H <sub>19</sub> Br <sub>3</sub> O <sub>3</sub>	483.8884	-	0.005
OH-TBPH	C <sub>24</sub> H <sub>35</sub> Br <sub>3</sub> O <sub>5</sub>	640.0035	9.56	0.01
TBPH	C <sub>24</sub> H <sub>34</sub> Br <sub>4</sub> O <sub>4</sub>	701.9191	11.95	5.0
6-OH-BDE-47	C <sub>12</sub> H <sub>6</sub> O <sub>2</sub> Br <sub>4</sub>	497.7101	6.4	0.05
4'-OH-BDE-49	C <sub>12</sub> H <sub>6</sub> O <sub>2</sub> Br <sub>4</sub>	497.7101	6.4	0.05
2-OH-BDE-123	C <sub>12</sub> H <sub>5</sub> O <sub>2</sub> Br <sub>5</sub>	575.6206	7.2	0.05
6-MeO-BDE-47	C <sub>13</sub> H <sub>8</sub> O <sub>2</sub> Br <sub>4</sub>	511.7258	7.3	0.3
4'-MeO-BDE-49	C <sub>13</sub> H <sub>8</sub> O <sub>2</sub> Br <sub>4</sub>	511.7258	7.3	0.3
4'-MeO-BDE-99	C <sub>13</sub> H <sub>7</sub> O <sub>2</sub> Br <sub>5</sub>	589.6363	8.2	0.3
BDE-47	C <sub>12</sub> H <sub>6</sub> Br <sub>4</sub> O	481.7152	6.8	3.0
BDE-49	C <sub>12</sub> H <sub>6</sub> Br <sub>4</sub> O	481.7152	6.8	4.0
BDE-66	C <sub>12</sub> H <sub>6</sub> Br <sub>4</sub> O	481.7152	6.8	2.0
BDE-85	C <sub>12</sub> H <sub>5</sub> Br <sub>5</sub> O	559.6257	7.7	0.8
BDE-99	C <sub>12</sub> H <sub>5</sub> Br <sub>5</sub> O	559.6257	7.7	0.9
BDE-100	C <sub>12</sub> H <sub>5</sub> Br <sub>5</sub> O	559.6257	7.7	2.0
BDE-153	C <sub>12</sub> H <sub>4</sub> Br <sub>6</sub> O	637.5362	8.6	2.0
BDE-154	C <sub>12</sub> H <sub>4</sub> Br <sub>6</sub> O	637.5362	8.6	3.0
BDE-183	C <sub>12</sub> H <sub>3</sub> Br <sub>7</sub> O	715.4467	9.4	5.0
BDE-209	C <sub>12</sub> Br <sub>10</sub> O	949.1783	12.1	20

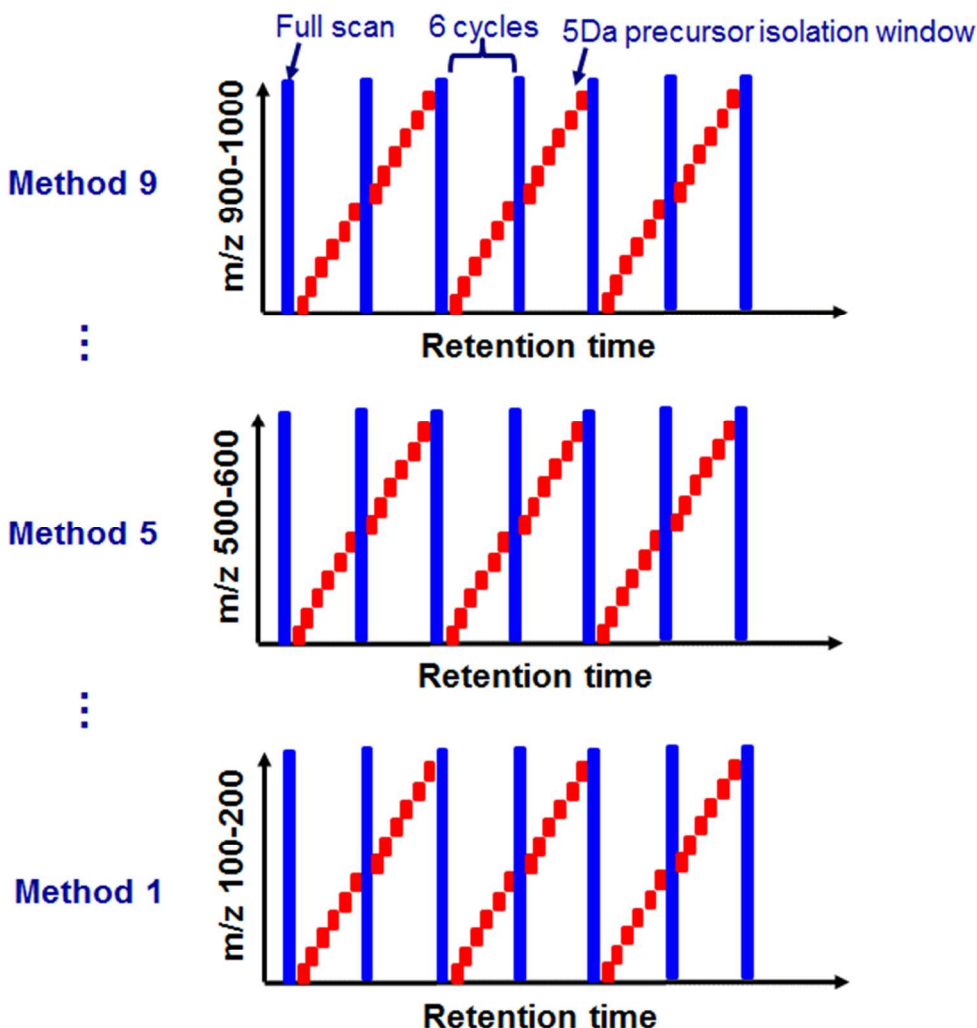
<sup>a</sup> K<sub>OW</sub> values were from references<sup>1-5</sup>



**Figure S1.** Sampling locations of two sediment samples (sed-32 and sed-44) from Lake Michigan.

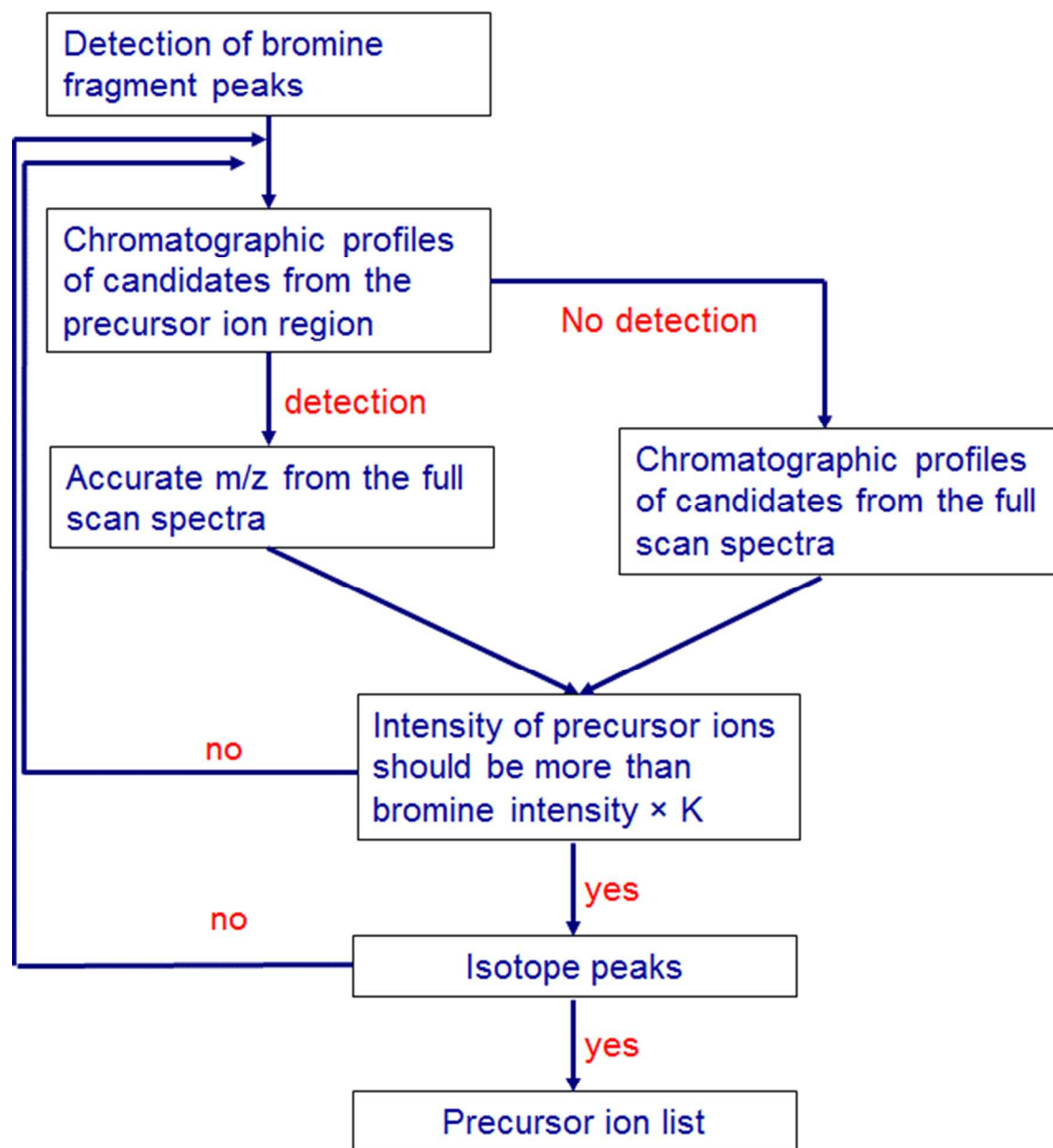


**Figure S2.** Product ion of a bromine fragment from brominated compounds (hydroxylated TBB in this sample) under relatively high collision energy (>30eV).

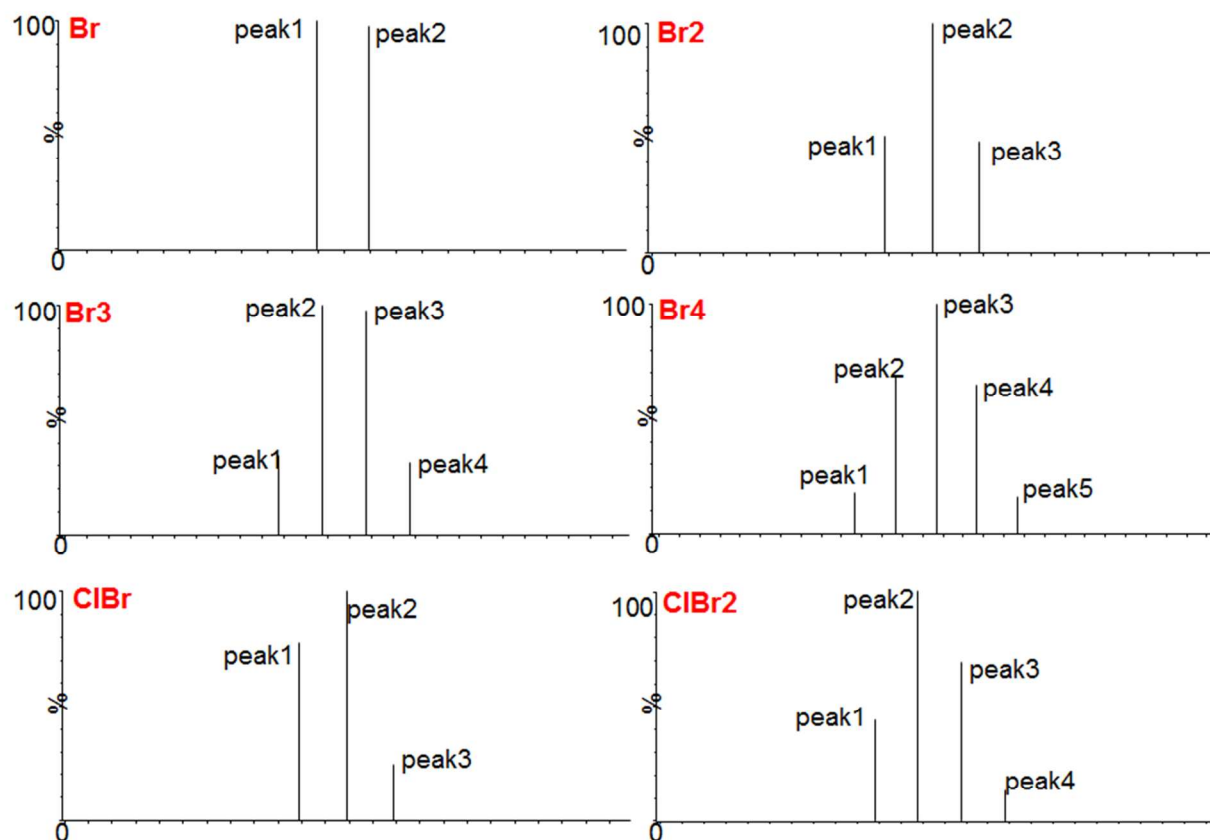


**Figure S3.** Scheme for data independent precursor isolation and characteristic fragment (DIPIC-Frag) method. Nine different methods (Method1 - Method 9) were performed for a single sample, each method covered a mass range of 100 Da. For each method, the full scan was used for each 7 cycles, and then 6 following successive data independent isolation (DIA) windows (5  $m/z$ ) was scanned. 20 DIA window was used for each method to cover the 100 Da mass range. Stepped collision energy at 10, 30 and 60 eV was used for the DIA scanning to simultaneously record information on bromine fragment and precursor ions in the same mass spectra.

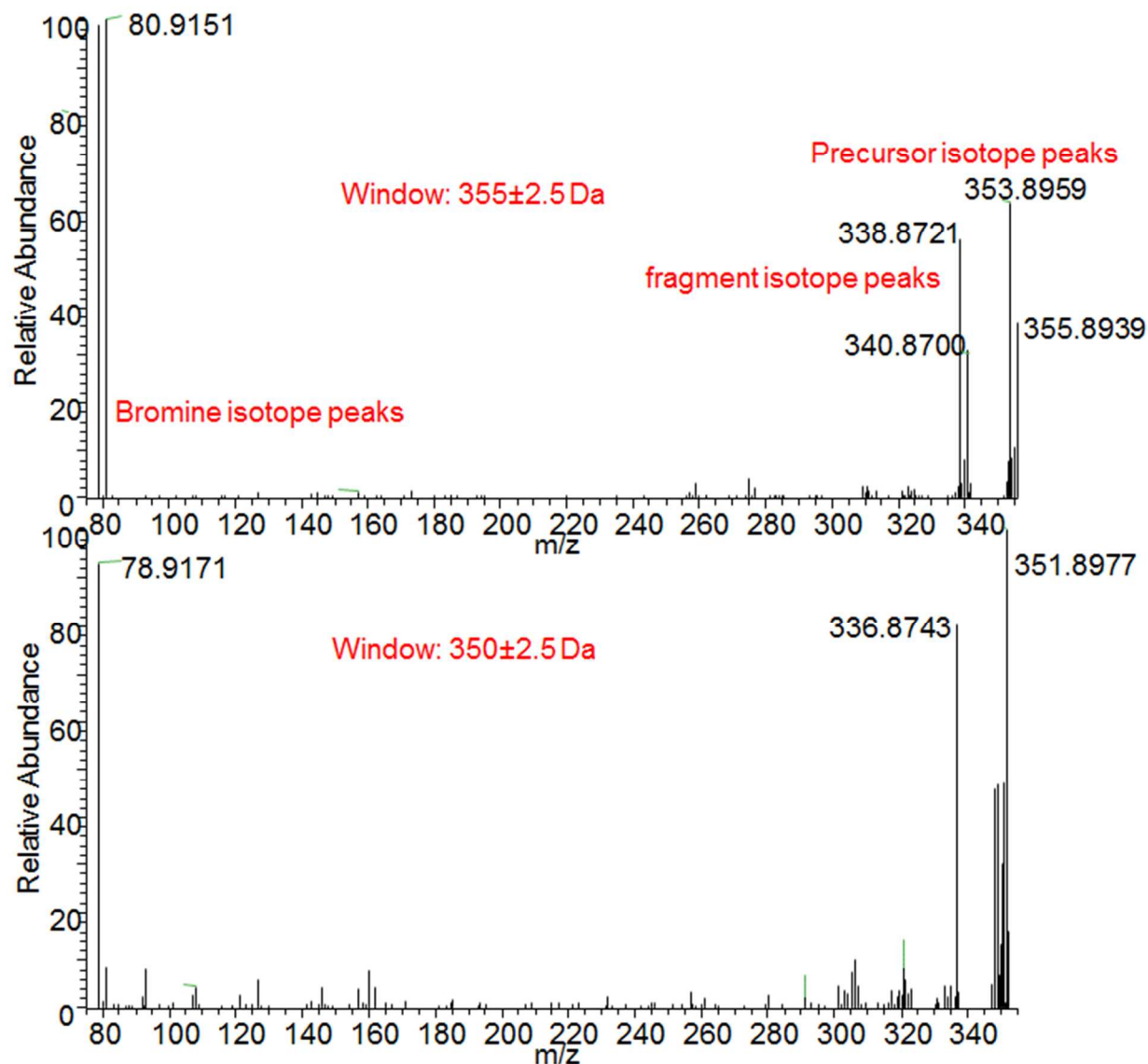




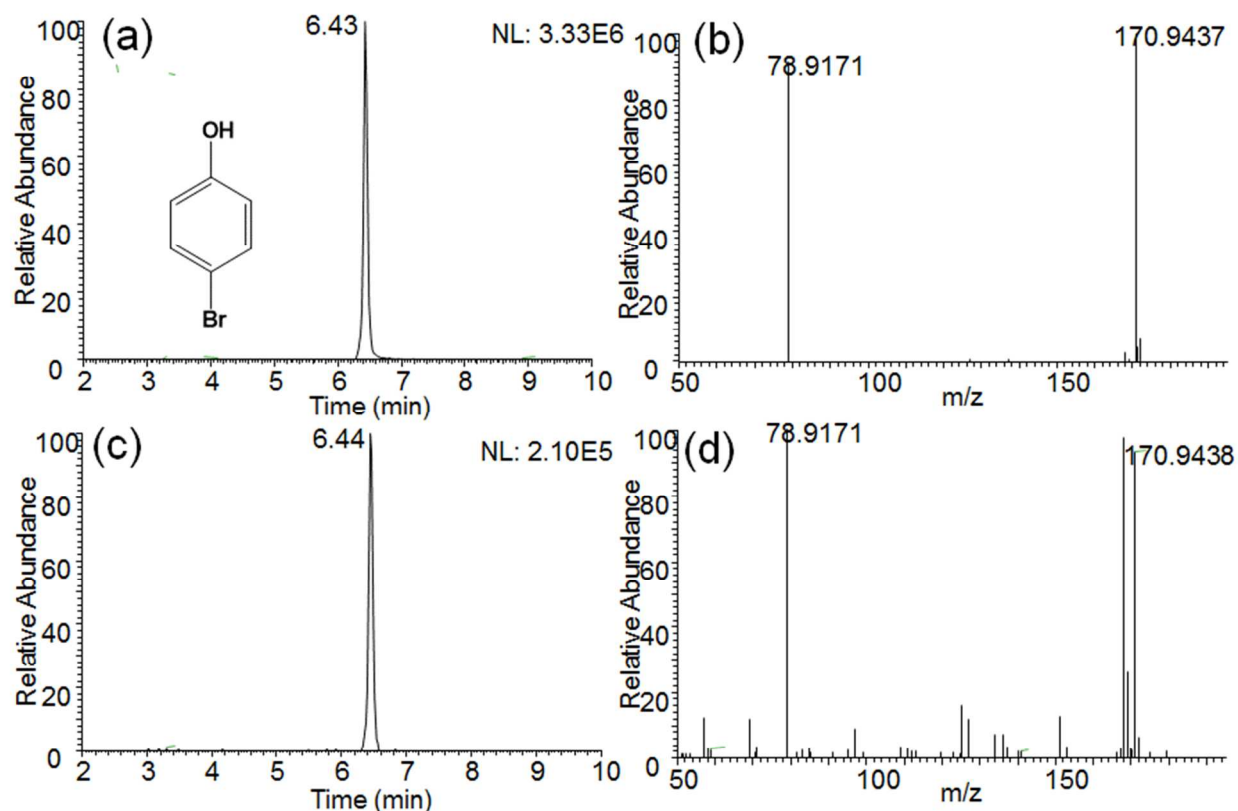
**Figure S4.** Workflow to identify precursor ions of NSOBCs. The first step is to get the chromatographic profiles of candidate ions from precursor ion regions in separated precursor isolation DIA windows (there are typically 20-30 ions). If we detected an ion with the same chromatographic profile with the bromine fragment, we further used full scan spectra to get more accurate  $m/z$  values. If we could not detect precursor ion in the precursor ion region, we searched precursor ion from full scan spectra. For the potential precursor ions detected in full scan or precursor ion region, we calculated the intensity of the ions to further make sure that intensities of precursor ions were greater than that of the fragment divided by number of atom. Then, we also checked the isotopic peaks of the precursor ions. If the potential precursor ions' intensities were low or no isotopic peaks were detected, we moved to the next candidate ions in the precursor ion region for the next round of data analysis. Finally, the list of likely precursor ions was produced.



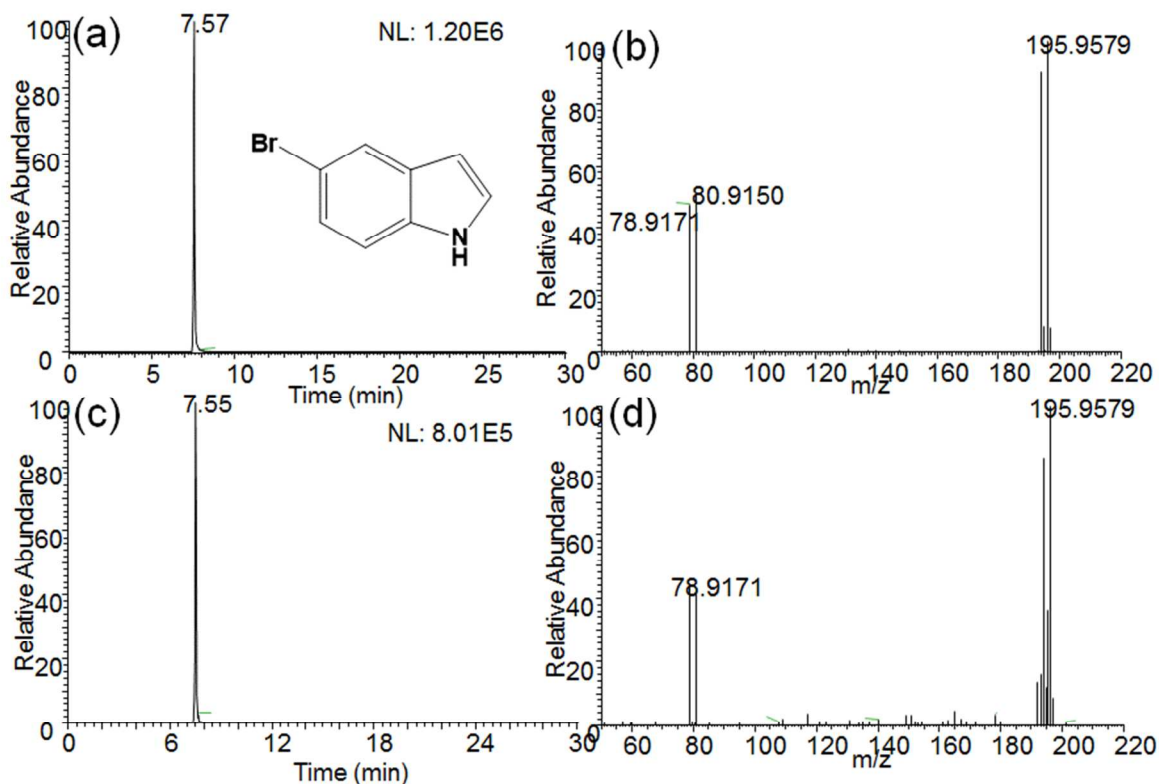
**Figure S5.** Distribution of isotopic peaks of brominated compounds with different compositions of bromine/chlorine. Y-axis indicated the relative intensities of the isotopic peaks to the maximal intensity of the peak.



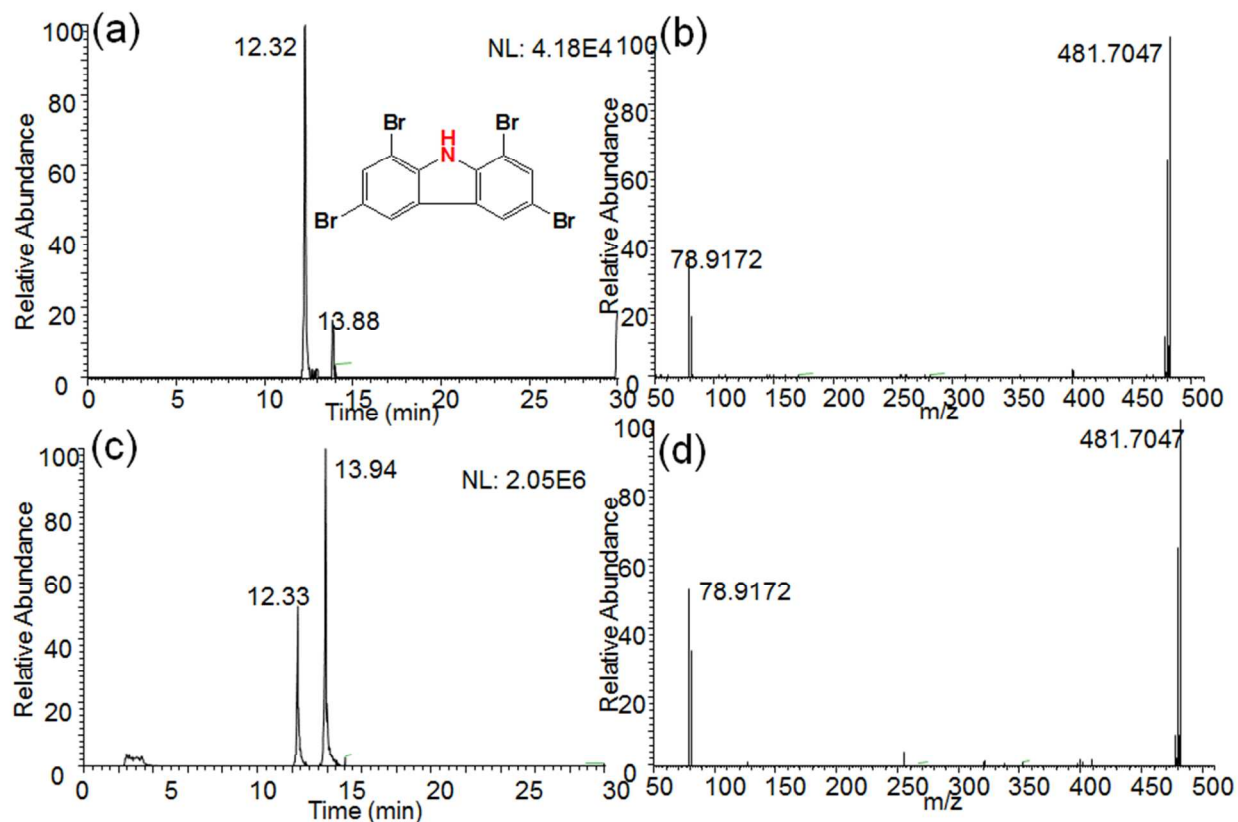
**Figure S6.** Isotopic peaks of bromine fragment in separated DIA windows could help to identify bromine numbers and molecular ions of brominated compounds. As shown in the bottom figure, only the bromine ion at  $m/z=78.9171$  was detected in the window at  $350 \pm 2.5$   $m/z$ , which meant that the primary monoisotopic ion of the compounds should be between 350-352  $m/z$ . If the molecular ion was lower than 350  $m/z$ , we should have observed the isotopic peaks of bromine at  $m/z=80.9151$  because the mass span of brominated compounds is  $\sim 2$   $m/z$ . As shown in the top figure, two isotopic peaks were observed at  $m/z=353.8959$  and  $m/z=355.8939$  respectively. In this figure, isotopic peak of bromine at  $m/z=80.9151$  were detected at similar intensity to the ion at  $m/z=78.9171$ . By combining the isotopic peaks of the bromine fragment in different precursor isolation windows and the distribution of the isotopic peaks of the precursor ions, the compound was identified as  $C_{13}H_8NOBr_2$  with mass error of 1.1 ppm and a monoisotopic molecular ion at  $m/z=351.8977$ .



**Figure S7.** Validation of an identified bromophenol by use of the commercially available standard 4-bromophenol. (a) Extracted ion chromatogram at  $m/z=170.9445$  (10 ppm mass width) for a standard of 4-bromophenol (100  $\mu\text{g/L}$ ). (b)  $\text{MS}^2$  spectra for 4-bromophenol from a  $170 \pm 2.5$  DIA window. (c) Extracted ion chromatogram at  $m/z=170.9445$  (10 ppm mass width) from sediment extract. (d)  $\text{MS}^2$  spectra for a bromophenol peak in sediment extract from a  $170 \pm 2.5$  DIA window.



**Figure S8.** Validation of an identified bromoindole by use of commercially available standard of 5-bromoindole. (a) Extracted ion chromatogram at  $m/z=193.9605$  (10 ppm mass width) for a standard of 5-bromoindole (100 µg/L). (b) MS<sup>2</sup> spectra for 5-bromoindole from a  $195\pm2.5$  DIA window. (c) Extracted ion chromatogram at  $m/z=193.9605$  (10 ppm mass width) from sediment extract. (d) MS<sup>2</sup> spectra for a 5-bromoindole peak in sediment extract from a  $195\pm2.5$  DIA window.



**Figure S9.** Validation of an identified brominated carbazole by use of the commercially available standard of 1,3,6,8-tetrabromocarbazole. (a) Extracted ion chromatogram at  $m/z=477.7077$  (10 ppm mass width) for standard of 1,3,6,8-tetrabromocarbazole (100 µg/L). (b)  $MS^2$  spectra for 1,3,6,8-tetrabromocarbazole from a  $480\pm 2.5$  DIA window. (c) Extracted ion chromatogram at  $m/z=477.7077$  (10 ppm mass width) from sediment extract. NSOBC at 13.94 min was an isomer of 1,3,6,8-tetrabromocarbazole with different bromine positions on the aromatic ring. (d)  $MS^2$  spectra for a tetrabromocarbazole peak in sediment extract from  $480\pm 2.5$  DIA window.

## References

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