

# Supplementary Materials

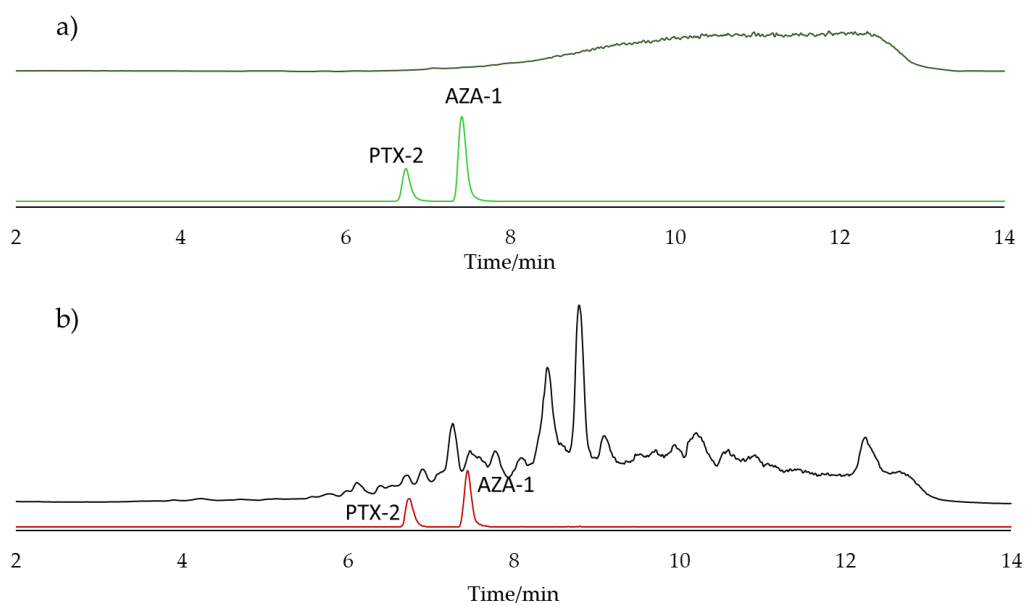
## **Quantitation overcoming matrix effects of lipophilic toxins in *Mytilus galloprovincialis* by liquid chromatography - full scan high resolution mass spectrometry analysis (LC-HR-MS)**

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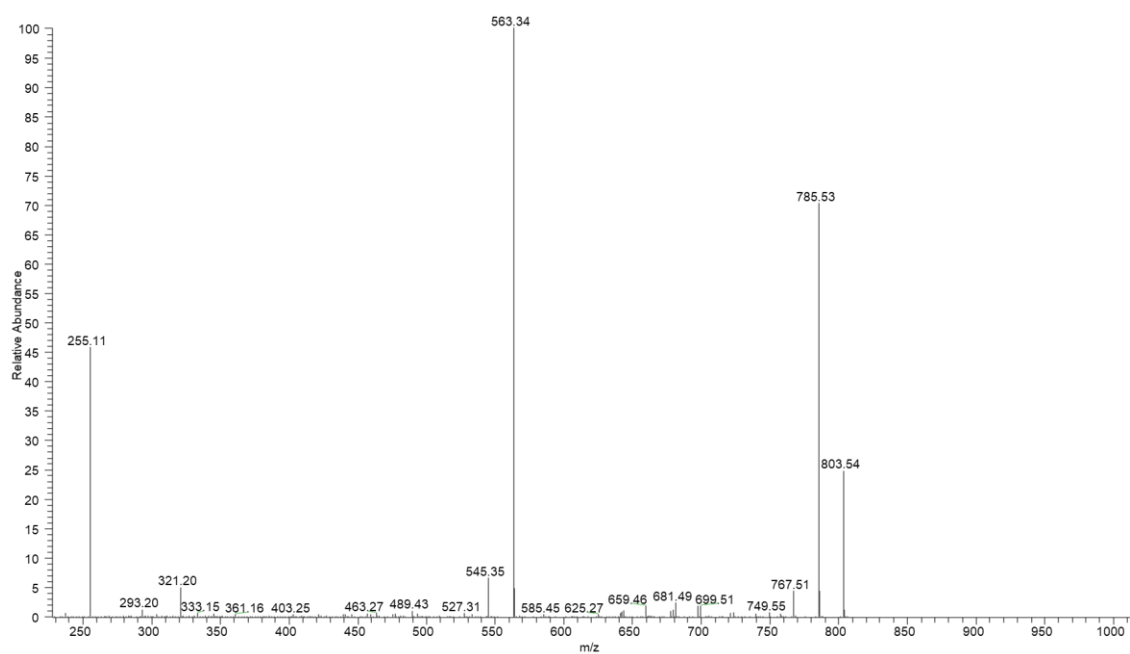
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**Figure S1.** LC-HR-MS full scan profiles and correspondent single ion chromatograms obtained after  $m/z$  extraction of values 842.5049 and 876.5104 with  $\pm 5$  ppm window of a methanol standard (a) containing PTX-2 and AZA-1, and a mussel extract after alkaline hydrolysis (b) fortified with the same compounds.



**Figure S2.** CID MS<sup>2</sup> spectrum of OA.

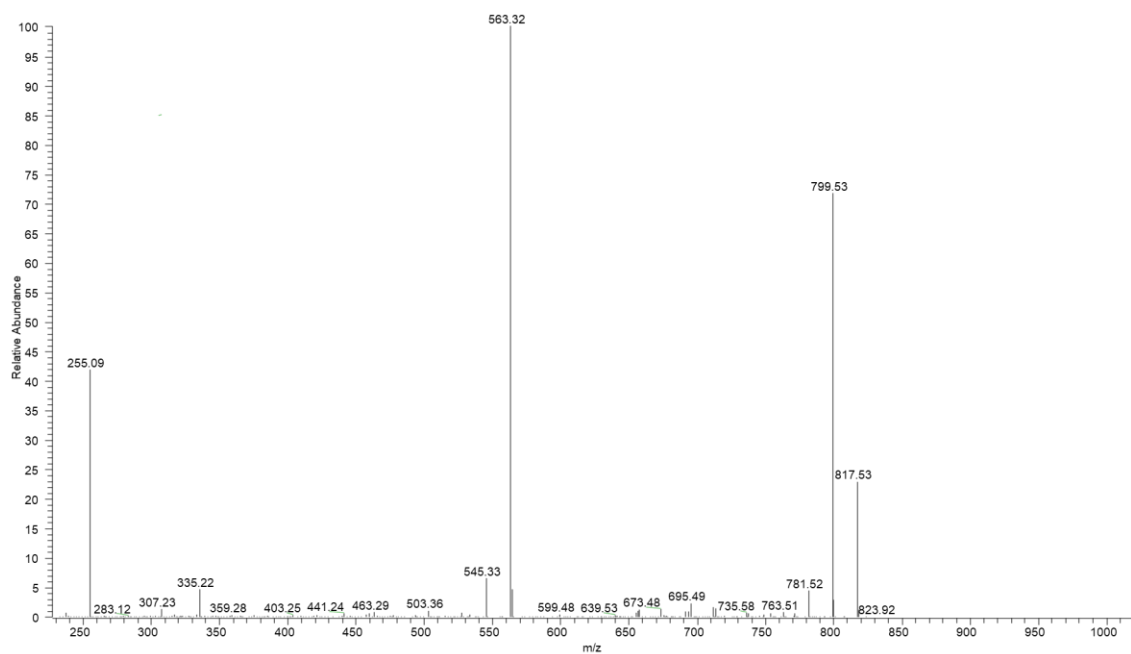


Figure S3. CID MS<sup>2</sup> spectrum of DTX-1.

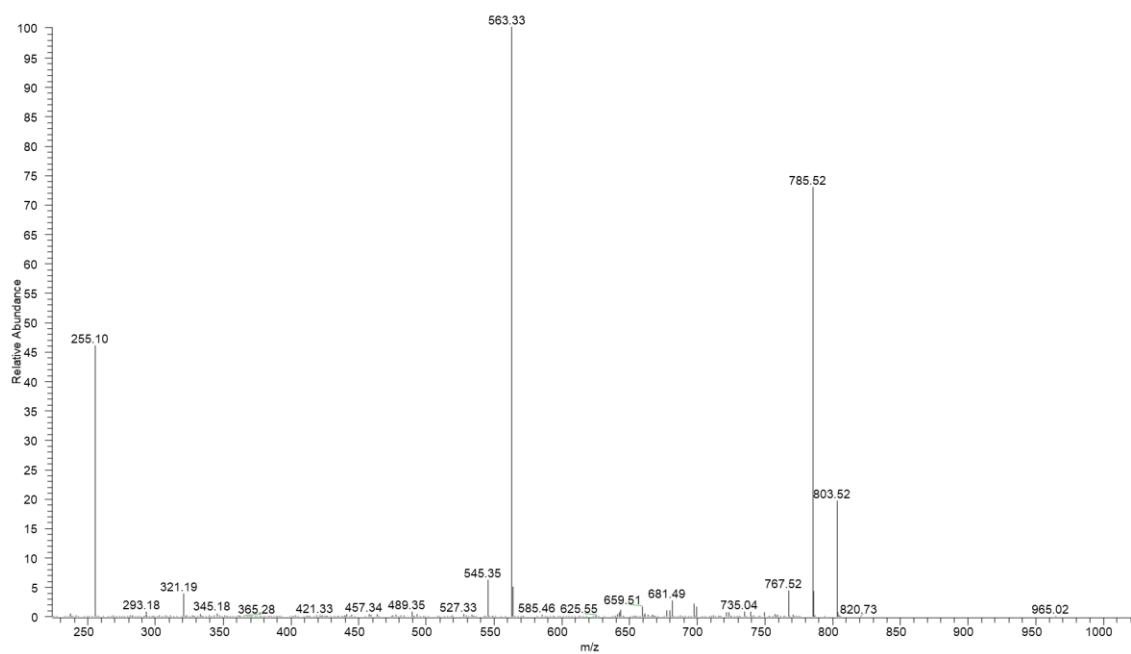


Figure S4. CID MS<sup>2</sup> spectrum of DTX-2.

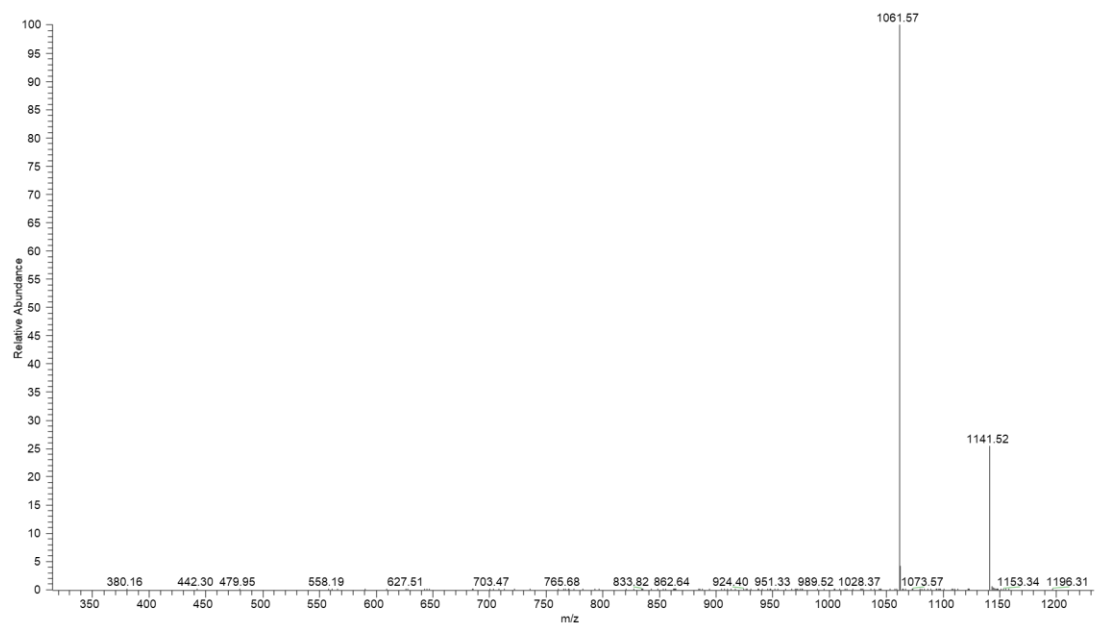


Figure S5. CID MS<sup>2</sup> spectrum of YTX.

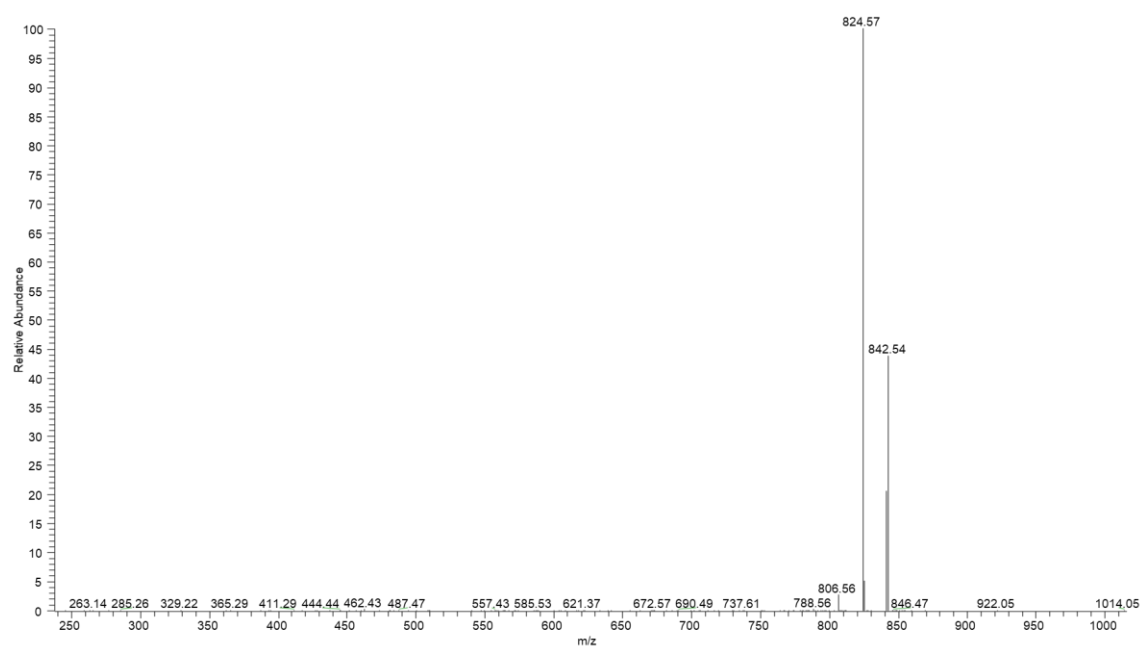
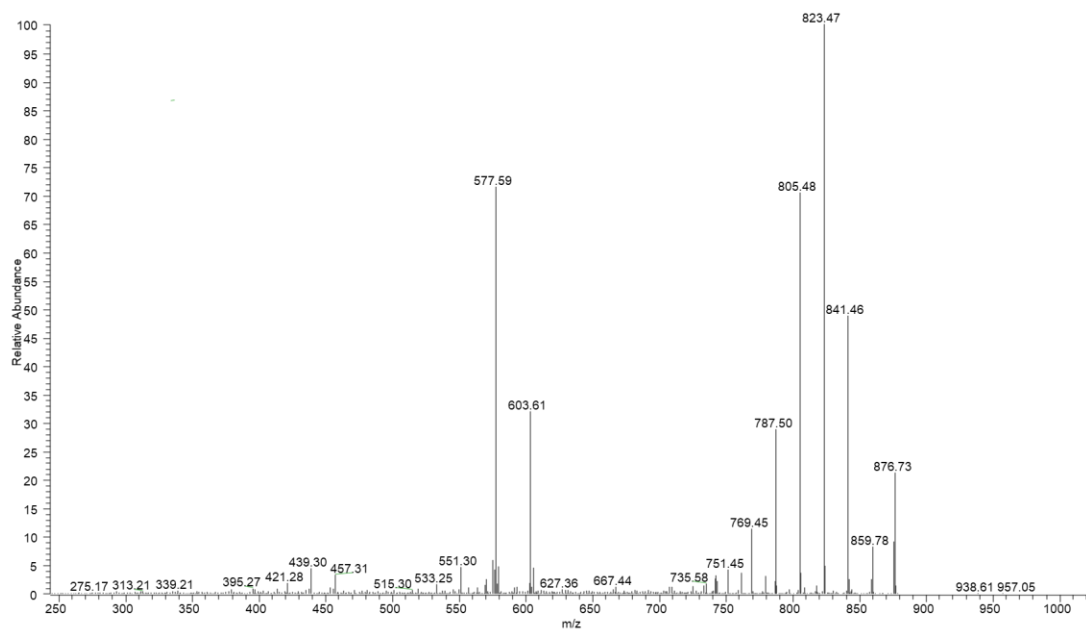


Figure S6. CID MS<sup>2</sup> spectrum of AZA-1.

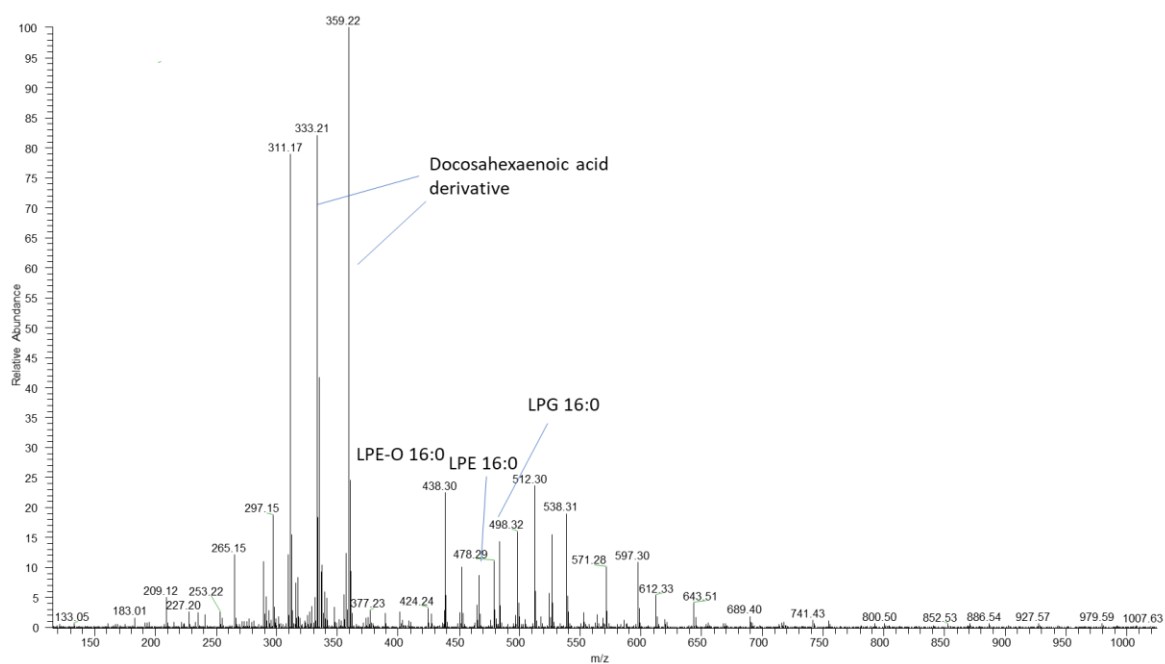


**Figure S7.** CID MS<sup>2</sup> spectrum of PTX-2.

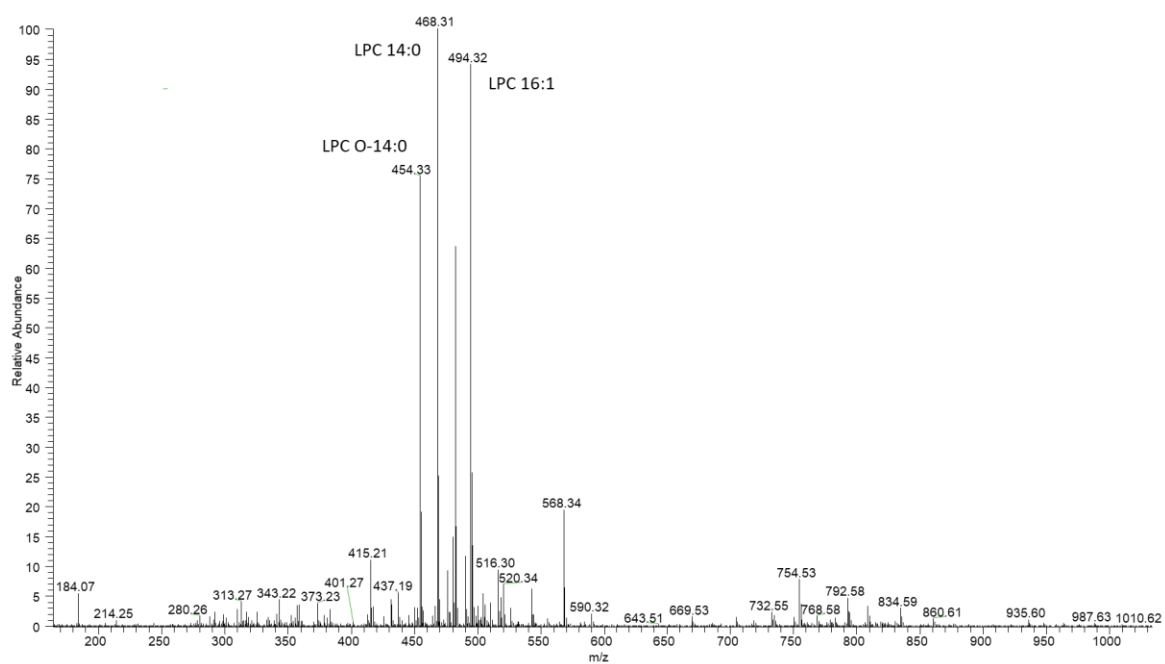
**Table S1.** Ions, retention times, transitions and LOQ of studied toxins.

Toxin	Mass of ion (+/-)	RT (min)	SRM	*LOQ (ng/mL)	**LOQ (ng/mL)
OA	803.46 (-)	6.31	803 → 785; 563; 255	1.6	3.1
DTX-1	817.47 (-)	7.18	817 → 799; 563; 255	3.1	3.1
DTX-2	803.46 (-)	6.57	803 → 785; 563; 255	1.5	3.1
YTX	1141.47 (-)	6.44	1141 → 1061	2.3	1.9
AZA-1	842.50 (+)	7.45	842 → 806; 824	0.7	0.4
PTX-2	876.51 (+)	6.75	876 → 823; 805; 577	1.4	1.6

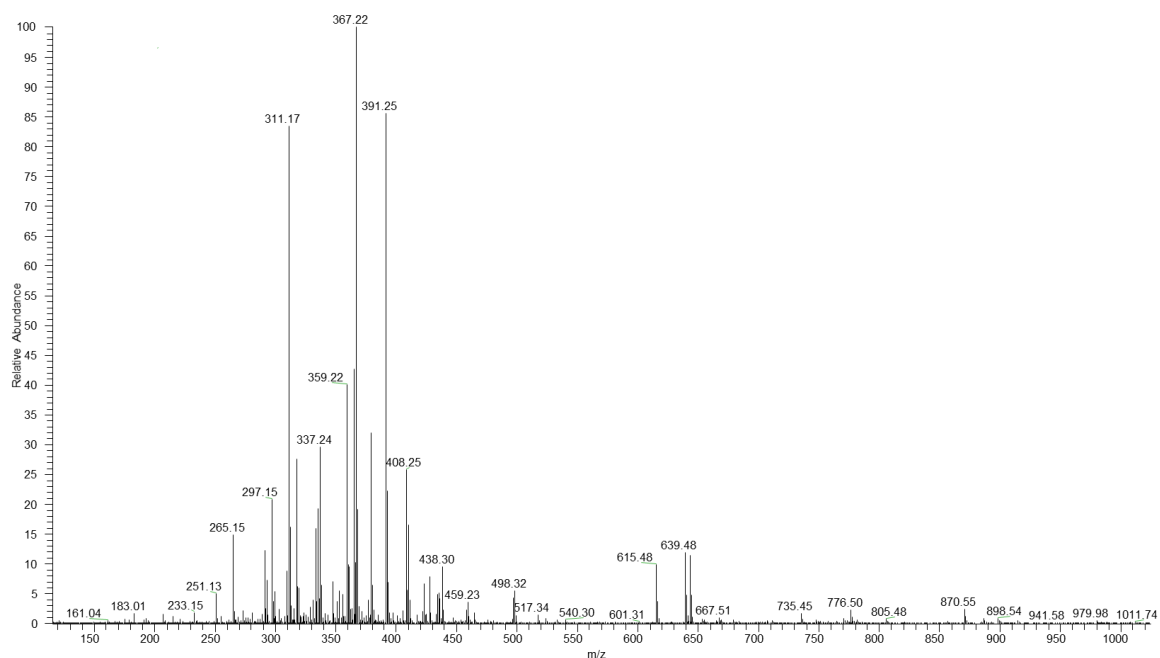
\*Calculated from the standard deviation (SD) of areas obtained after 5 injections of the second lowest concentration (SD\*10). Matrices were extracts of fresh mussels samples, hydrolyzed for OA, DTX-1 and DTX-2 and non-hydrolyzed for the remaining toxins. \*\*Taken from reference Rodriguez et al., Talanta 2018, 189, 622-628.



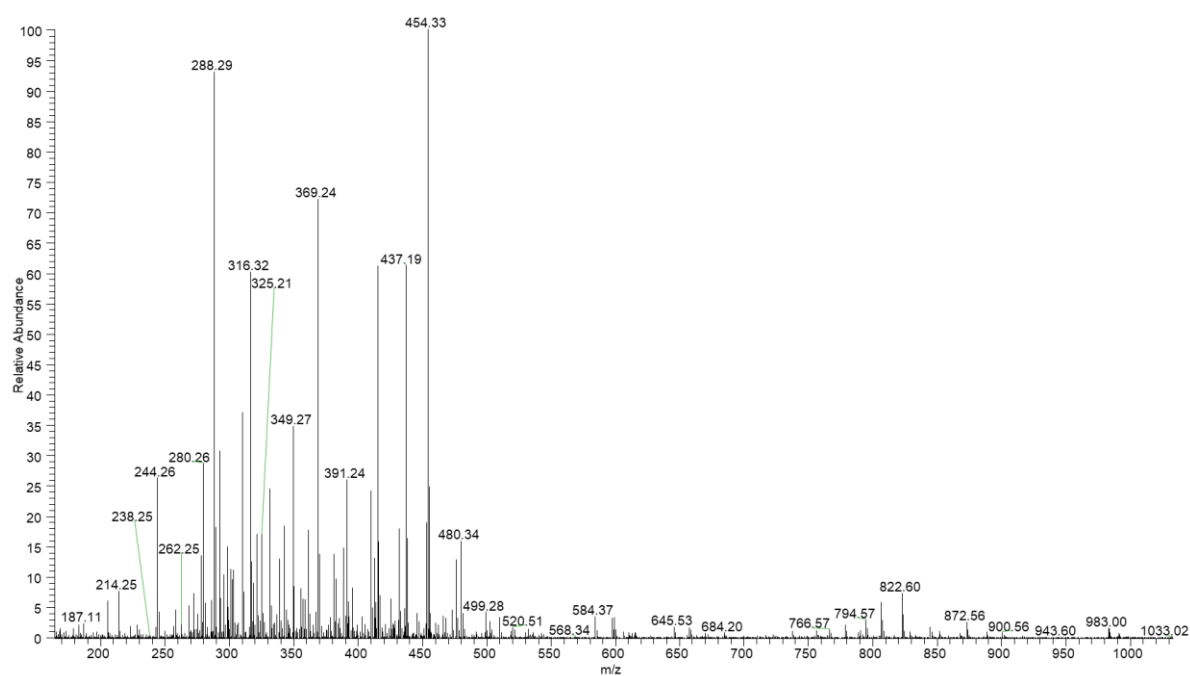
**Figure S8.** Negative polarity average spectrum between 6.1 and 6.7 minutes after injection of a non-hydrolyzed extract of mussel sample.



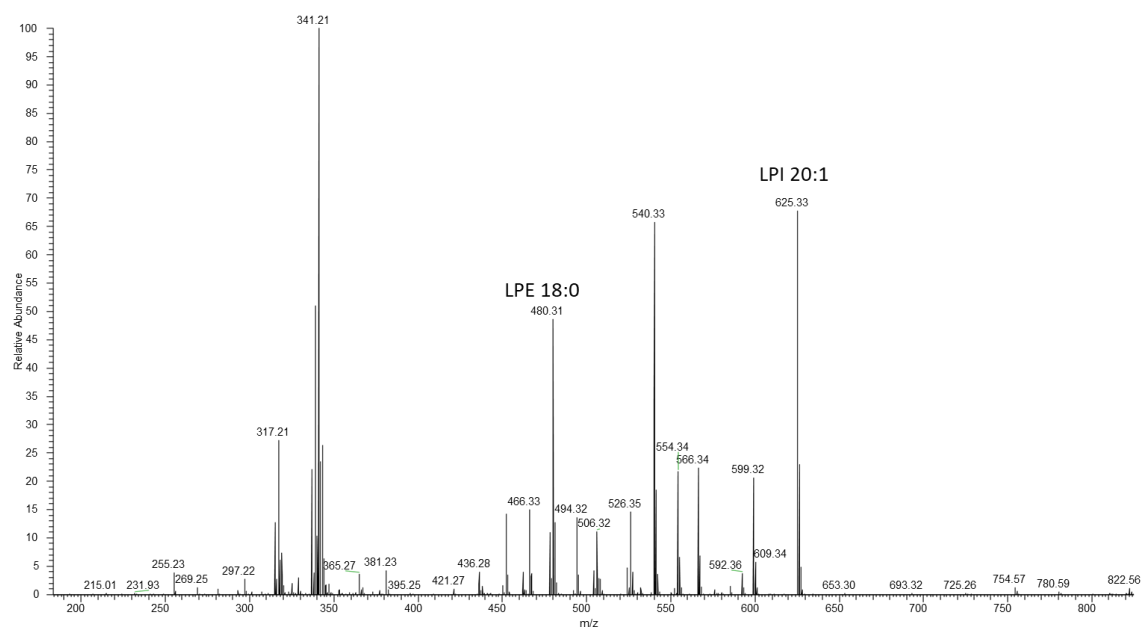
**Figure S9.** Positive polarity average spectrum between 6.1 and 6.7 minutes after injection of a non-hydrolyzed extract of mussel sample.



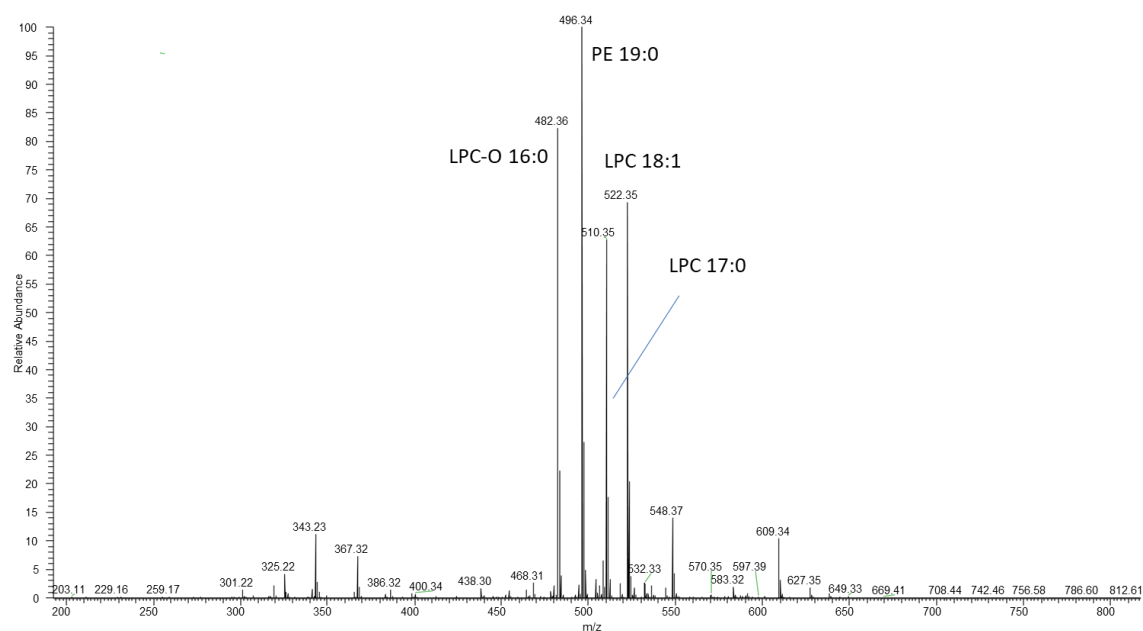
**Figure S10.** Negative polarity average spectrum between 6.1 and 6.7 minutes after injection of a hydrolyzed extract of mussel sample.



**Figure S11.** Positive polarity average spectrum between 6.1 and 6.7 minutes after injection of a hydrolyzed extract of mussel sample.

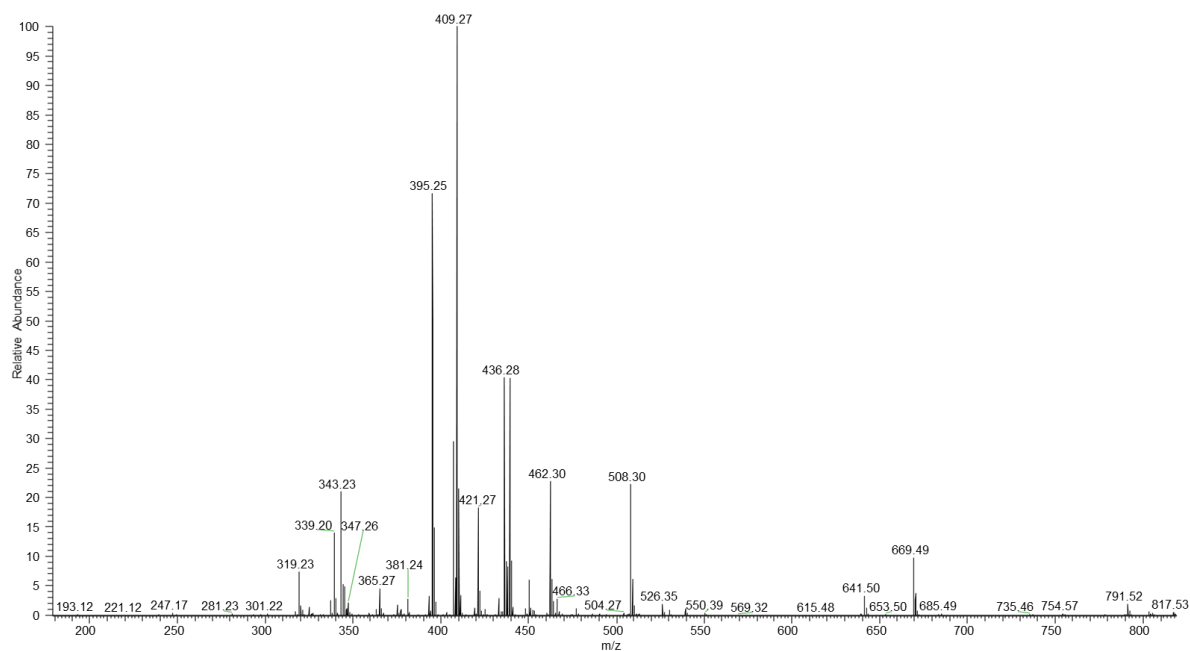


**Figure S12.** Negative polarity average spectrum between 7.1 and 7.6 minutes after injection of a non-hydrolyzed extract of mussel sample.

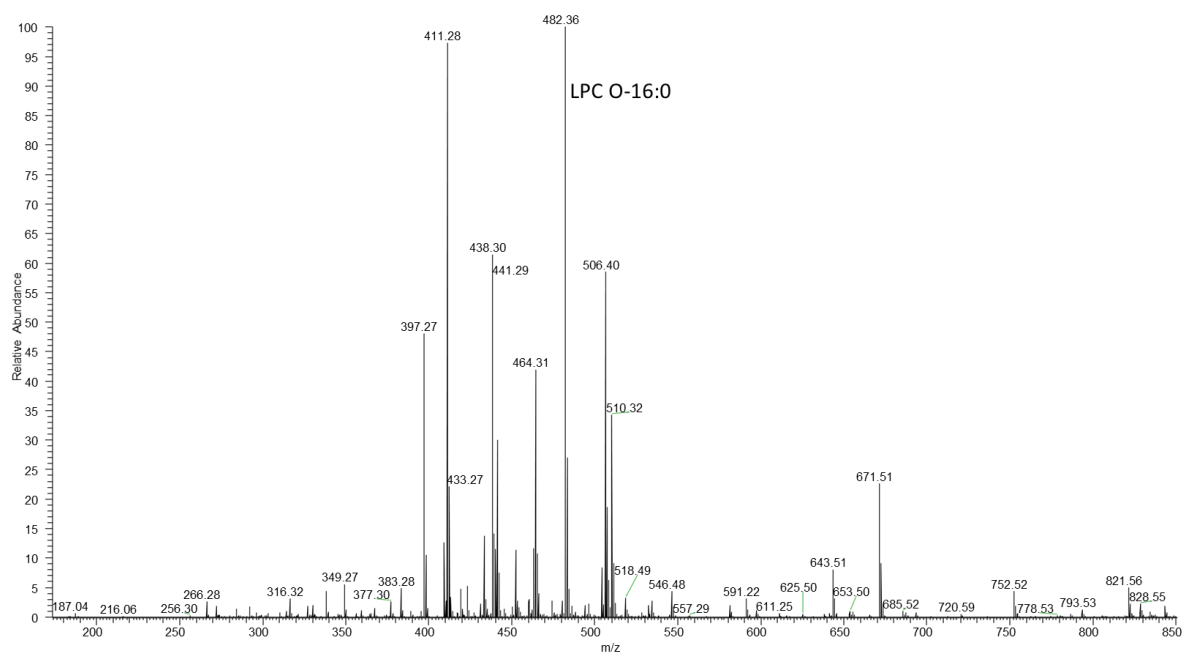


**Figure S13.** Positive polarity average spectrum between 7.1 and 7.6 minutes after injection of a non-hydrolyzed extract of mussel sample.

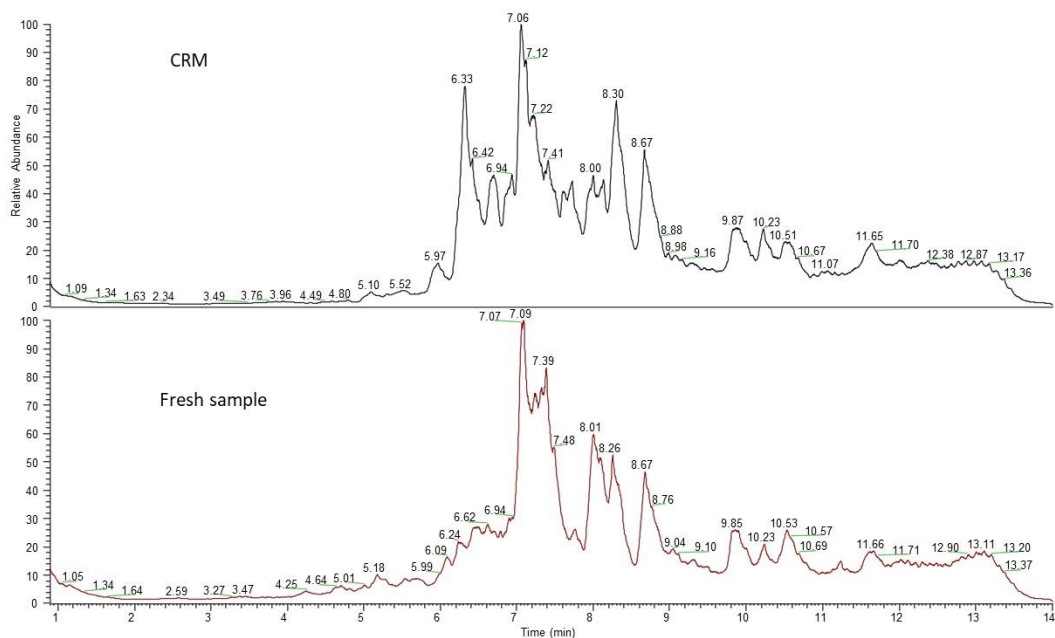




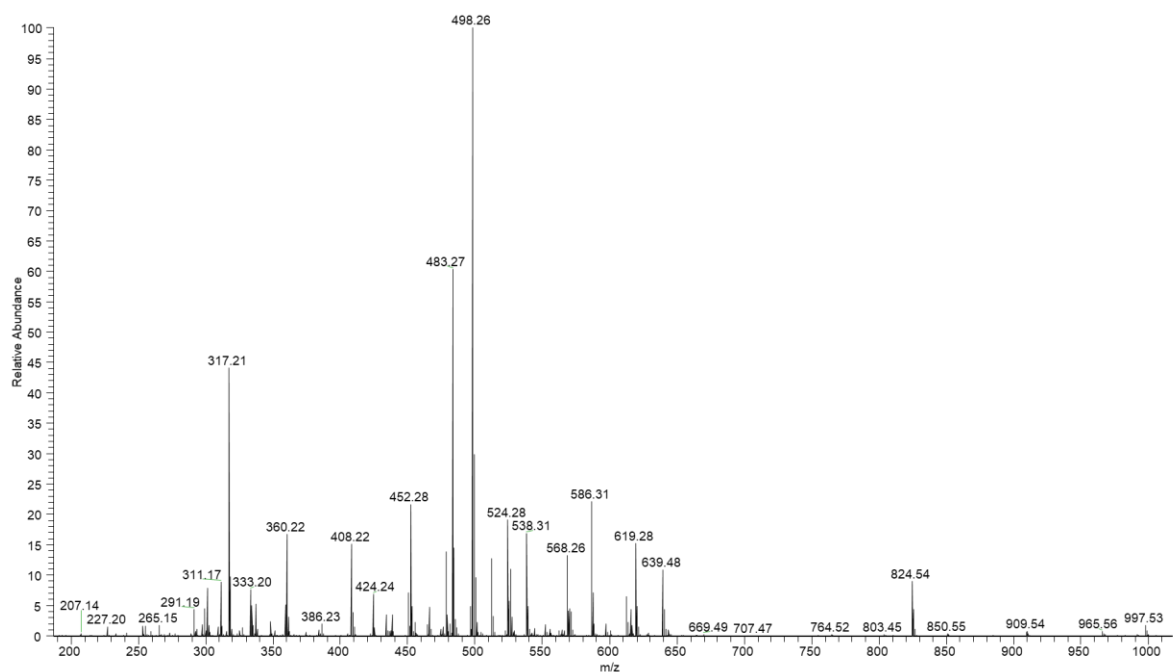
**Figure S14.** Negative polarity average spectrum between 7.1 and 7.6 minutes after injection of a hydrolyzed extract of mussel sample.



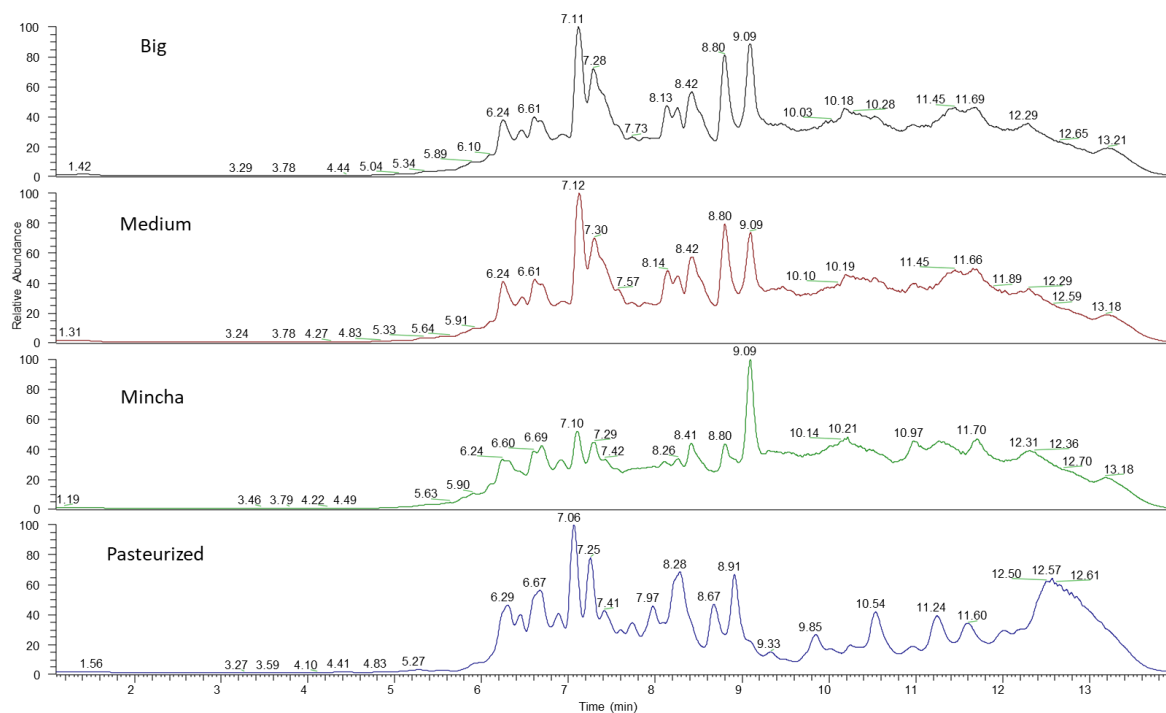
**Figure S15.** Positive polarity average spectrum between 7.1 and 7.6 minutes after injection of a hydrolyzed extract of mussel sample.



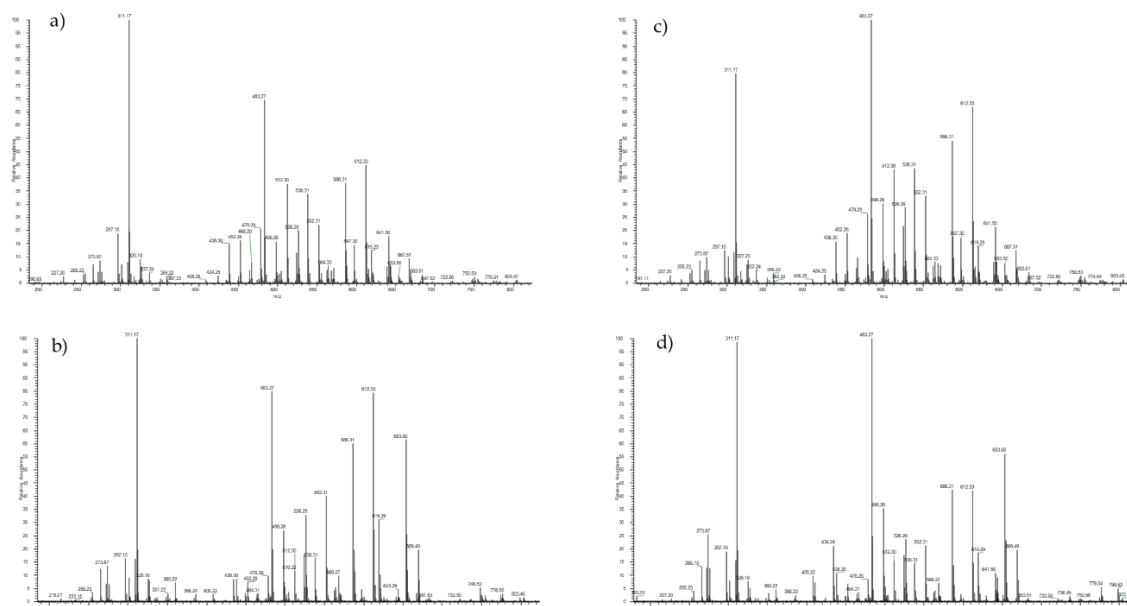
**Figure S16.** LC-HR-MS negative polarity profiles of extracts of a CRM sample and a mussel sample.



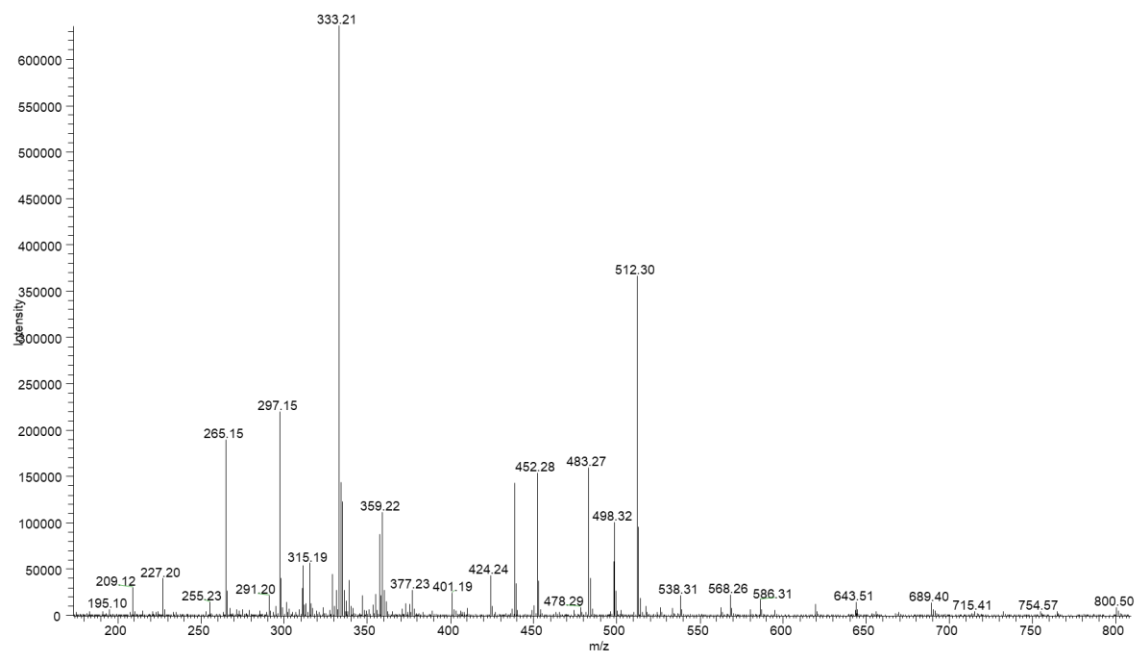
**Figure S17.** Negative polarity average spectrum between 6.1 and 6.7 minutes after injection of a non-hydrolyzed extract of CRM-Zero-Mus sample.



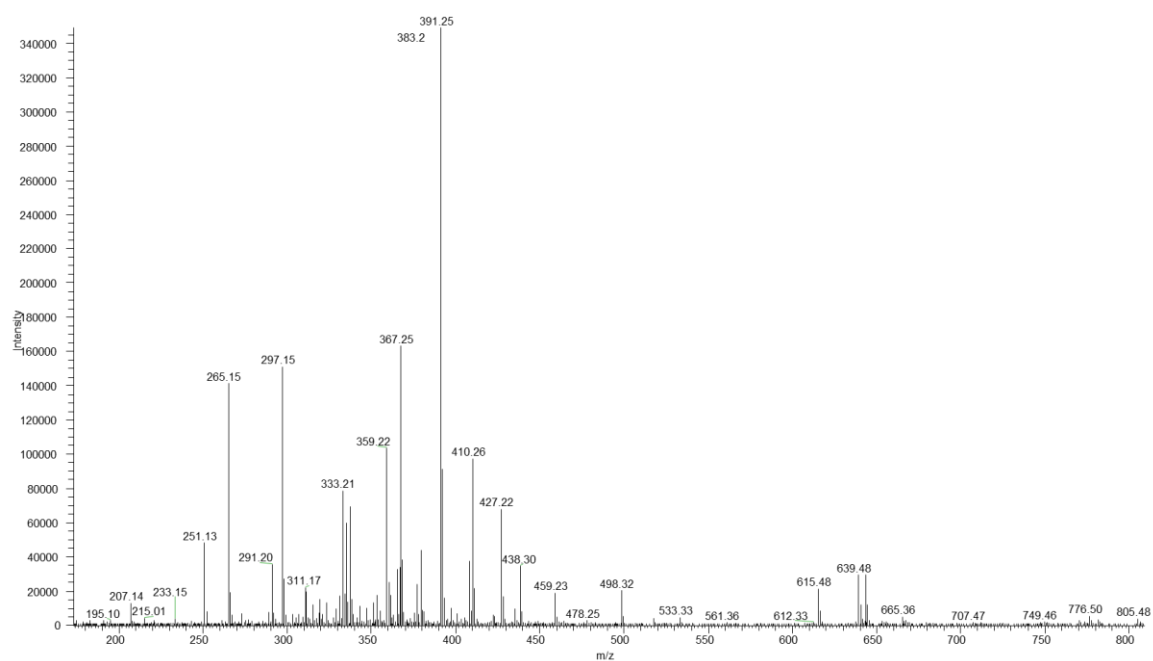
**Figure S18.** LC-HR-MS profiles of extracts of Pasteurized, Mincha, Medium and Large mussels under negative polarity.



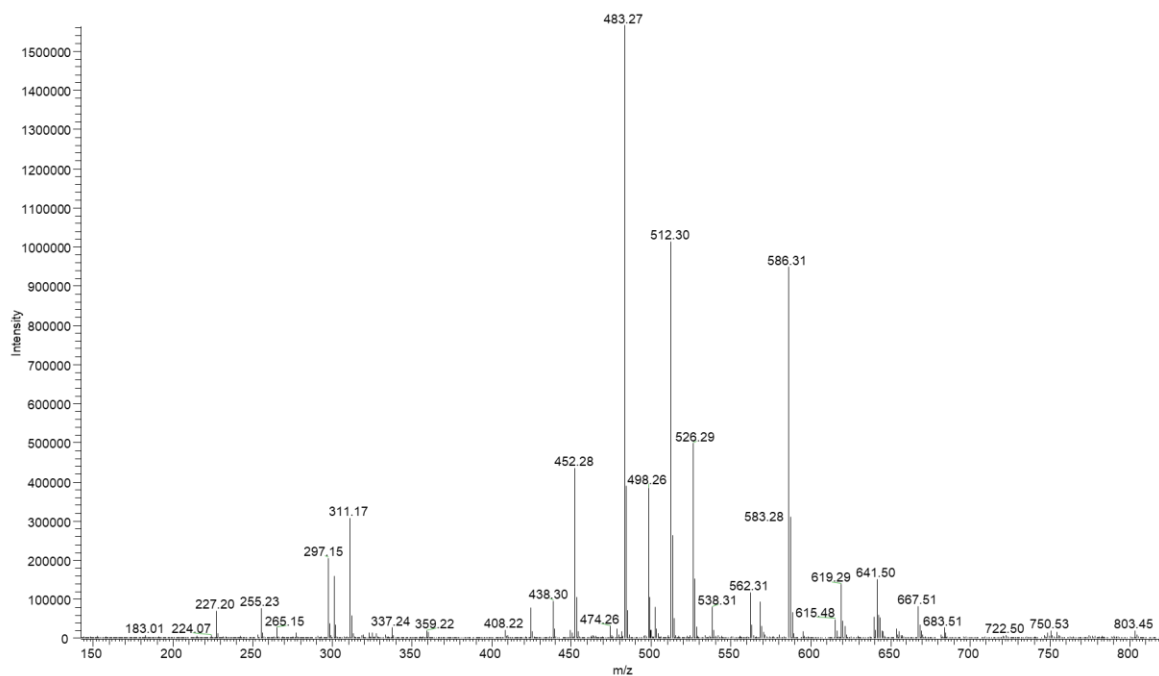
**Figure S19.** Average negative polarity spectra of processed mussel samples between 6.1 and 6.7 min. a) Big; b) Medium; c) Mincha; d) Pasteurized.



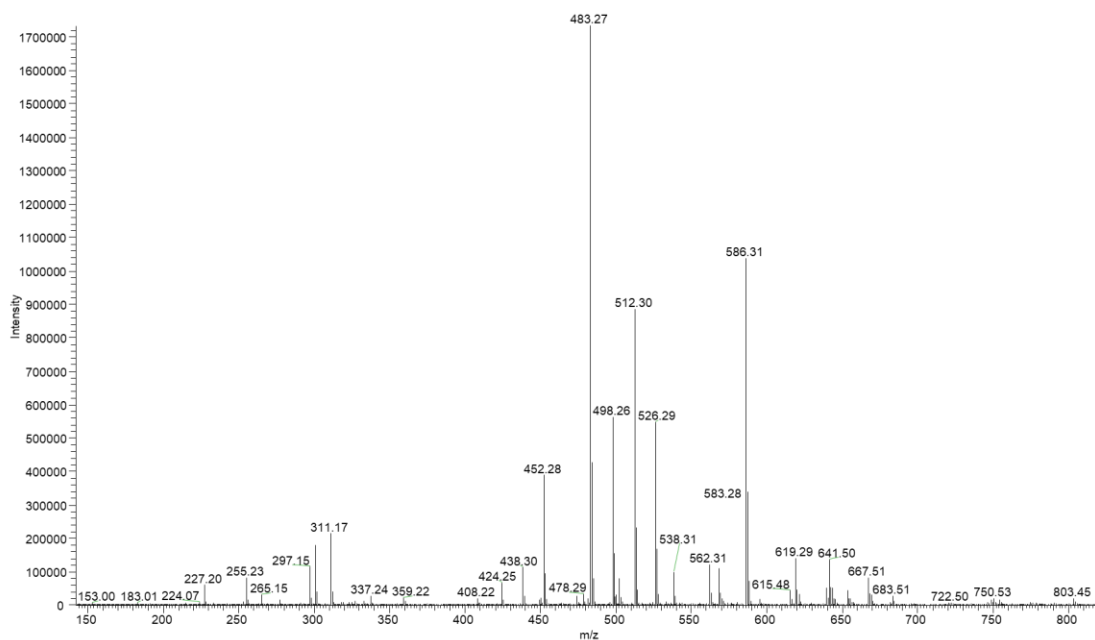
**Figure S20.** Negative polarity average spectrum between 6.2 and 6.4 minutes (OA) after injection of a non-hydrolyzed extract of mussel sample.



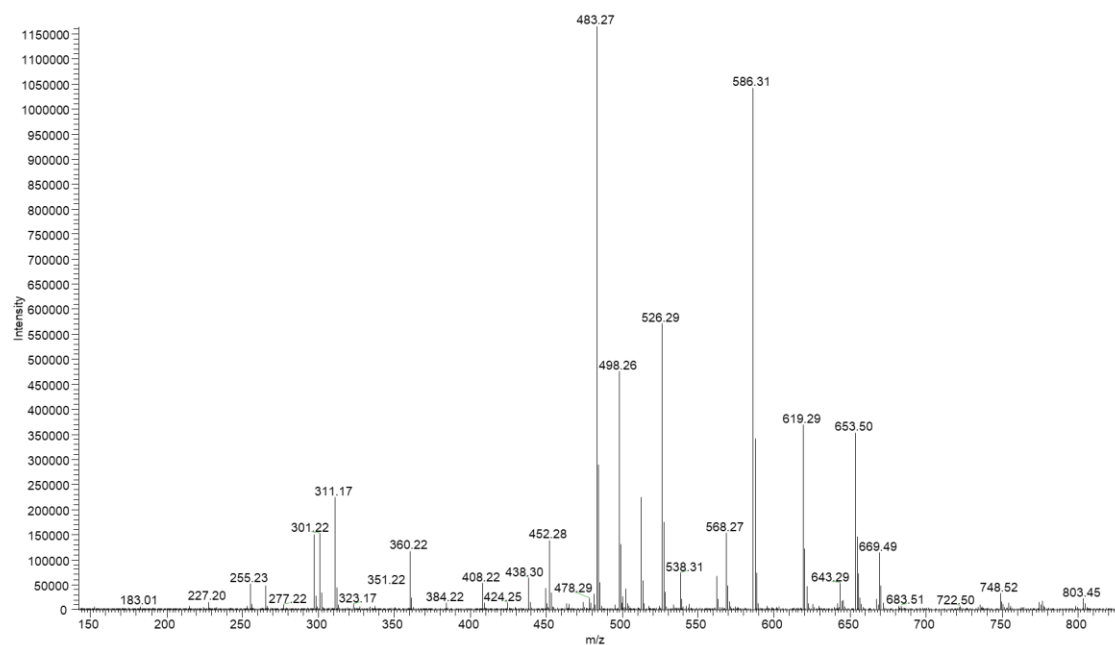
**Figure S21.** Negative polarity average spectrum between 6.2 and 6.4 minutes (OA) after injection of an hydrolyzed extract of mussel sample.



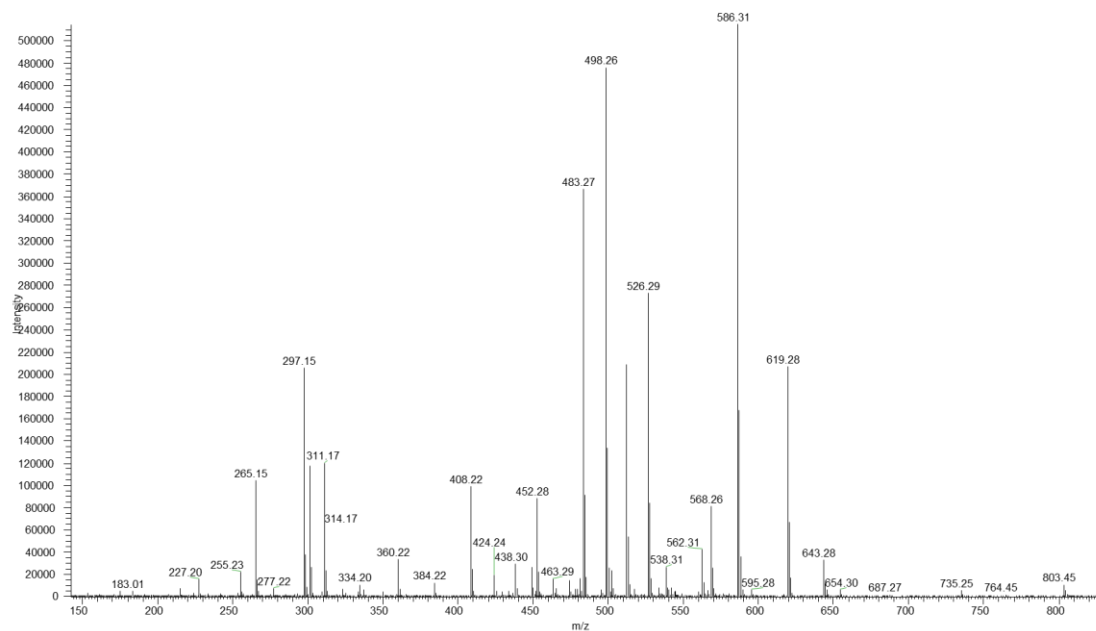
**Figure S22.** Negative polarity average spectrum between 6.2 and 6.4 minutes (OA) after injection of sample Big.



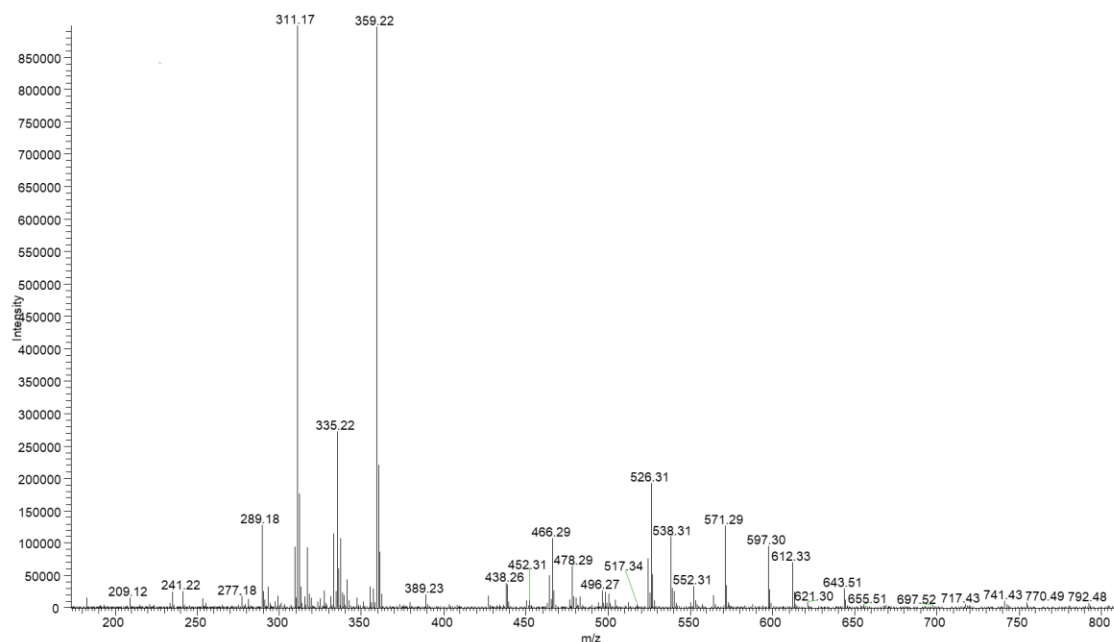
**Figure S23.** Negative polarity average spectrum between 6.2 and 6.4 minutes (OA) after injection of sample Medium.



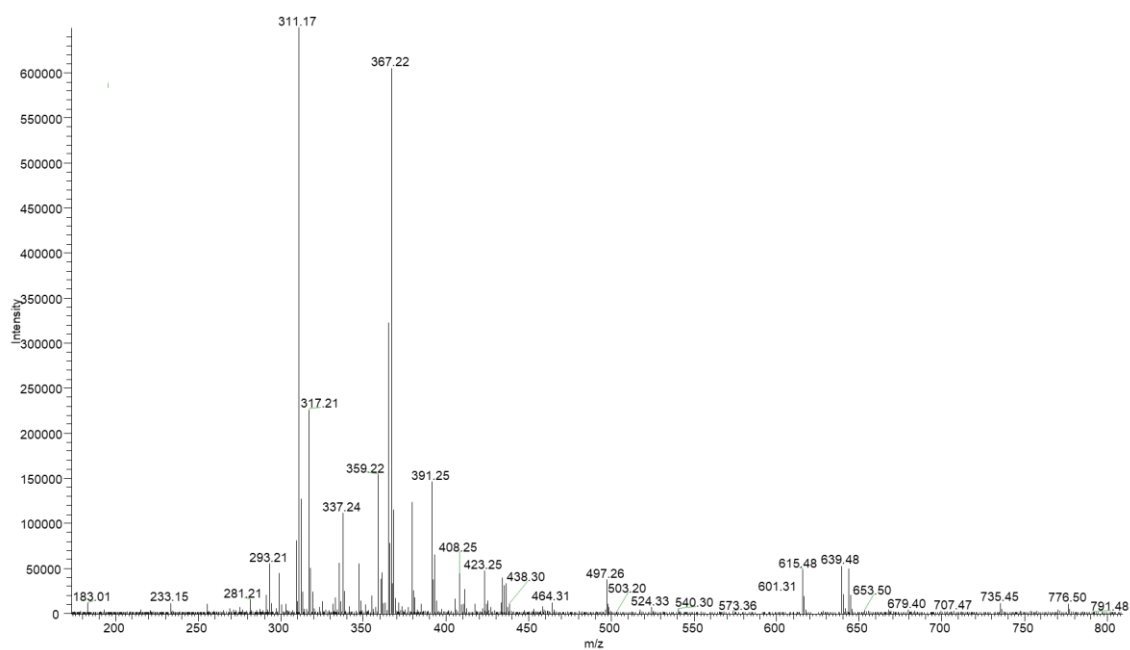
**Figure S24.** Negative polarity average spectrum between 6.2 and 6.4 minutes (OA) after injection of sample Mincha.



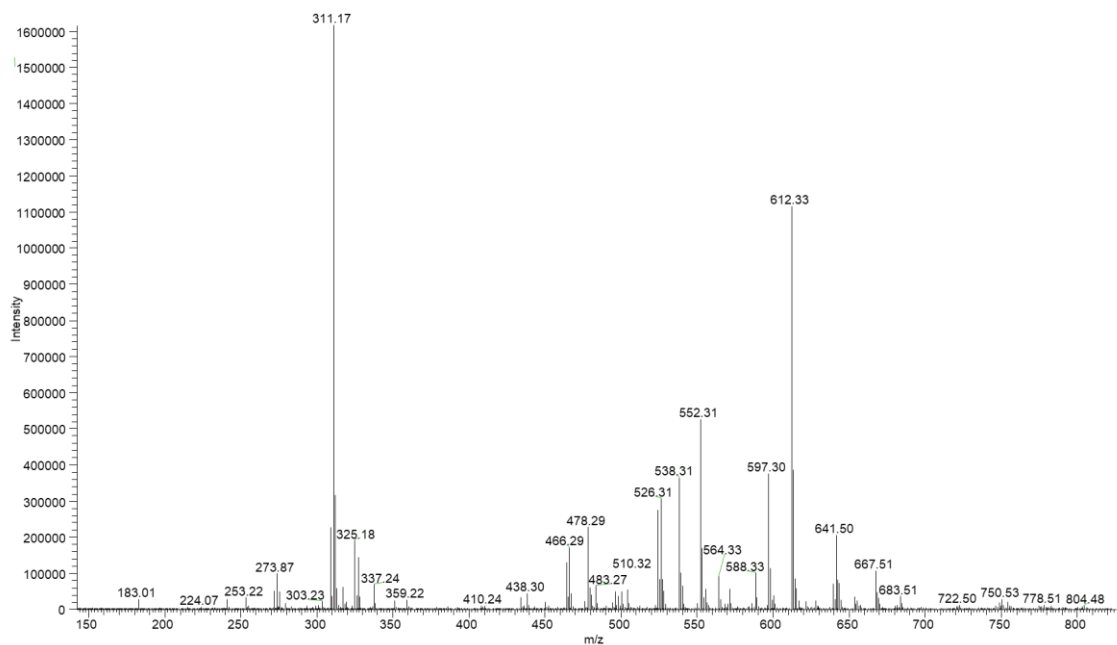
**Figure S25.** Negative polarity average spectrum between 6.2 and 6.4 minutes (OA) after injection of sample Pasteurized.



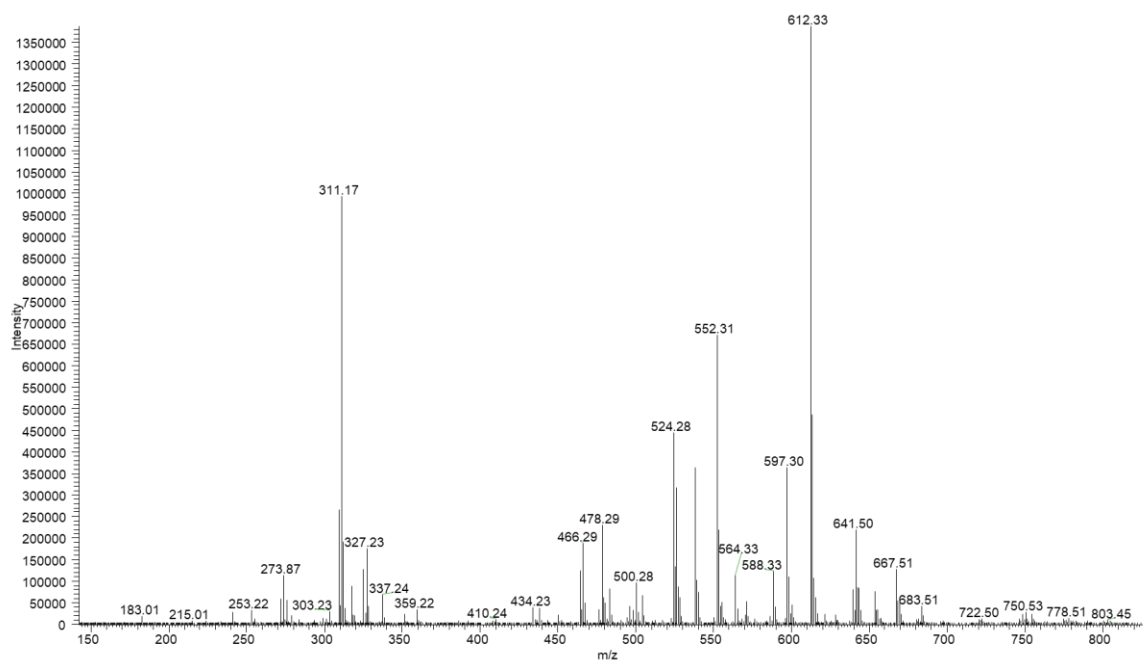
**Figure S26.** Negative polarity average spectrum between 6.5 and 6.7 minutes (OA) after injection of a non-hydrolyzed extract of mussel sample.



**Figure S27.** Negative polarity average spectrum between 6.5 and 6.7 minutes (OA) after injection of hydrolyzed extract of mussel sample.

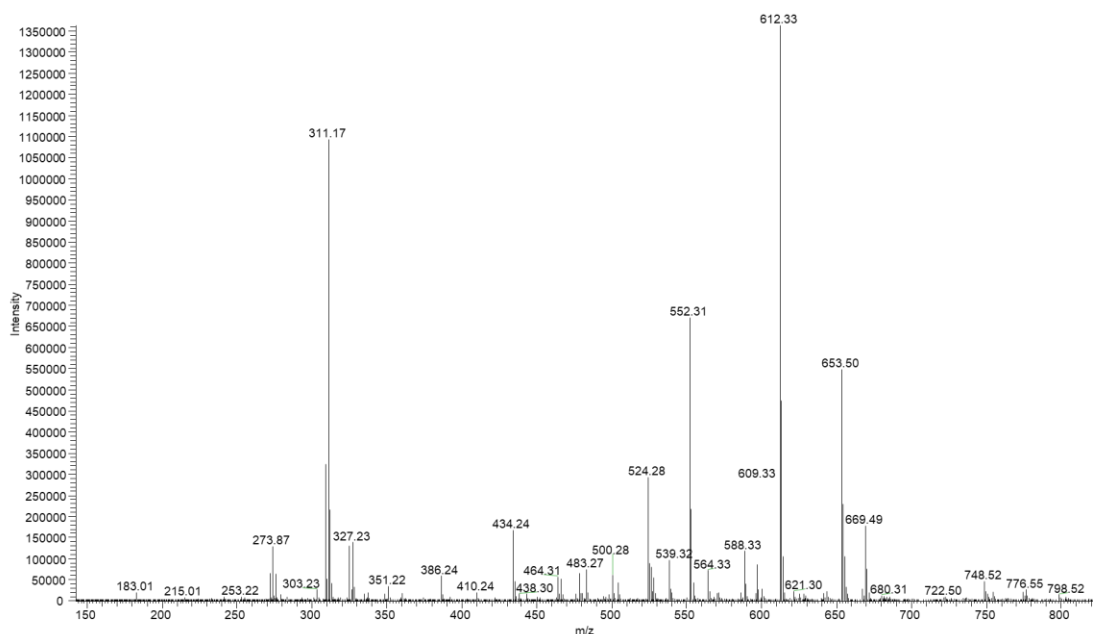


**Figure S28.** Negative polarity average spectrum between 6.5 and 6.7 minutes (OA) after injection of sample Big.

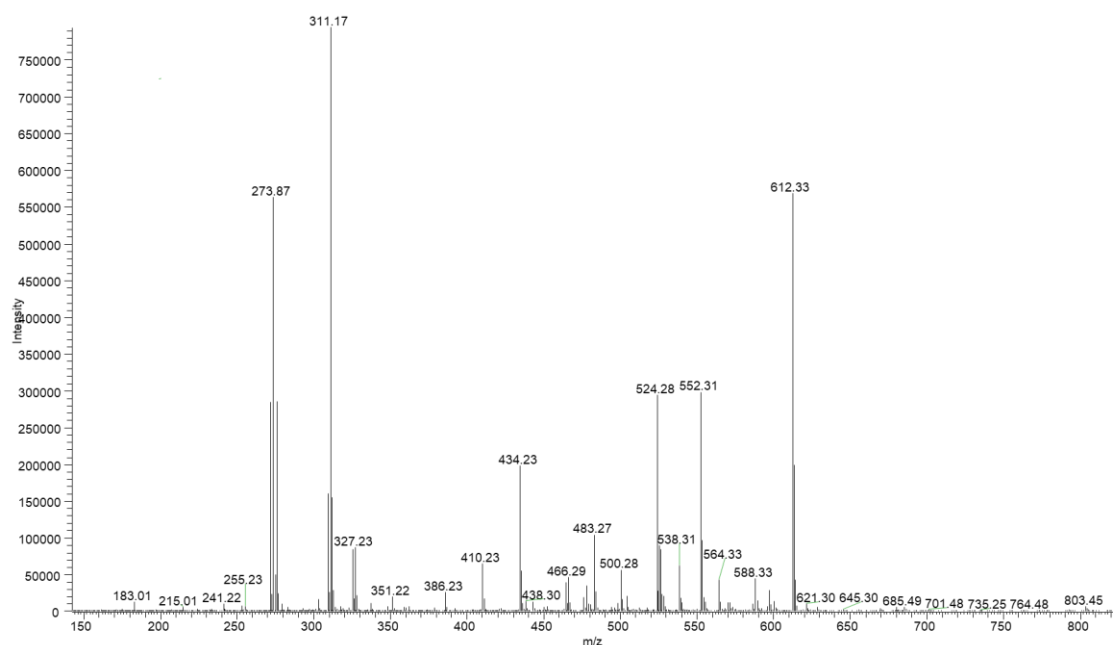


**Figure S29.** Negative polarity average spectrum between 6.5 and 6.7 minutes (OA) after injection of sample Medium.





**Figure S30.** Negative polarity average spectrum between 6.5 and 6.7minutes (OA) after injection of sample Mincha.



**Figure S31.** Negative polarity average spectrum between 6.5 and 6.7minutes (OA) after injection of sample Pasteurized.

**Table S2.** Matrix effects and correspondent % RSD (in-batch) on OA and DTX-2 in contaminated processed samples (n=3).

Toxin/sample	Big (% RSD)	Medium (% RSD)	Mincha (% RSD)	Pasteurized (% RSD)
OA	162 (5.4)	114 (4.1)	104 (7.0)	54 (7.2)
DTX-2	160 (6.2)	122 (4.8)	166 (5.4)	55 (4.9)