Supplementary Material

LC–HRMS and Chemical Derivatization Strategies for the Structure Elucidation of Caribbean Ciguatoxins: Identification of C-CTX-3 and -4

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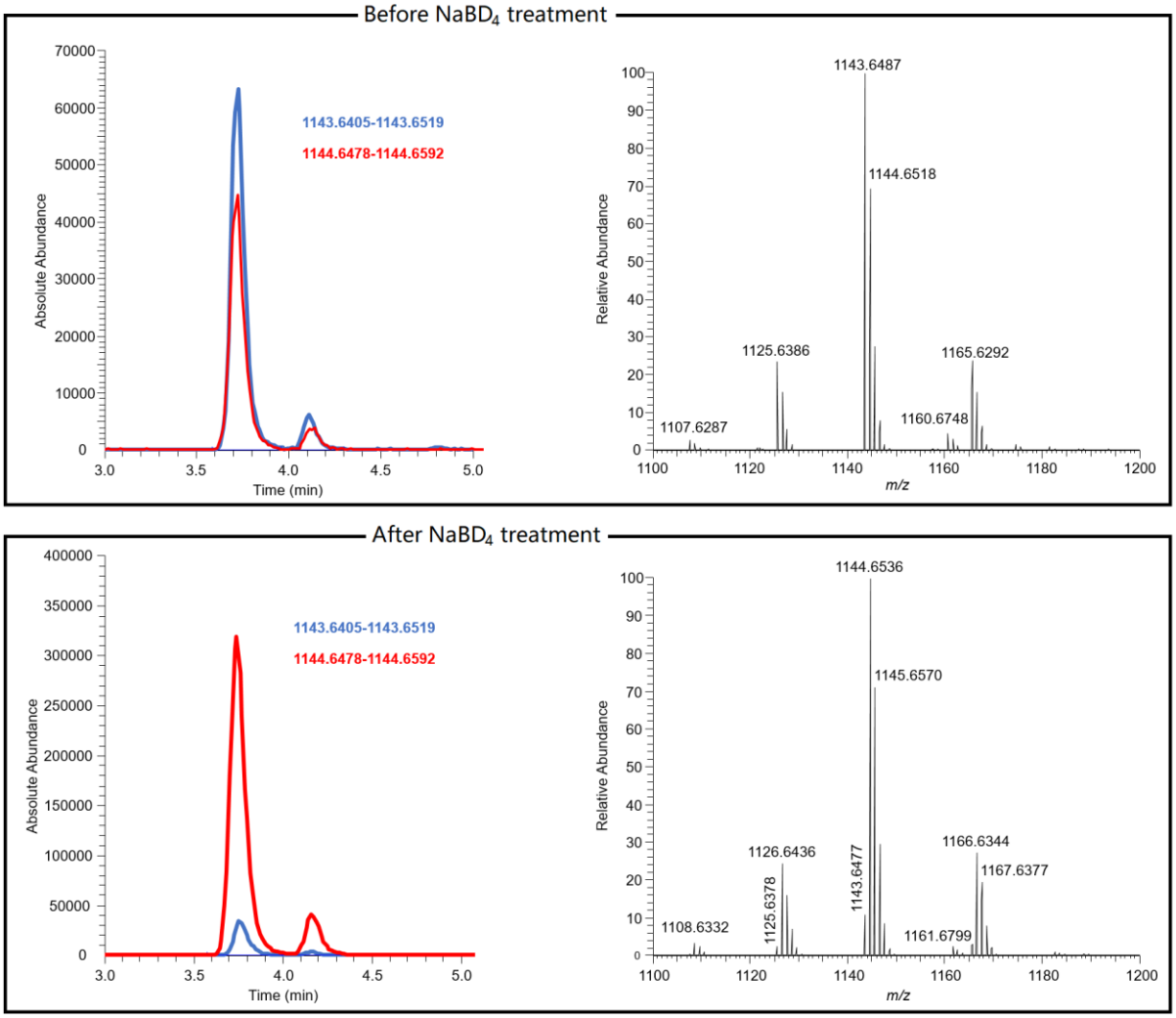

Figure S1. Extracted ion chromatograms (EIC, ± 5 ppm) and HRMS spectra of ciguatoxic fish (*S. barracuda*) extract before (top) and after (bottom) treatment with sodium borodeuteride. The upper trace shows the EIC for $[M+H]^+$ of native C-CTX-3/-4 (**3**/**4**, blue line, *m*/*z* 1143.6412) together with its ^{13}C isotopomer (*m*/*z* 1144.6535, red line), while the lower trace shows the increase in the EIC for *m*/*z* 1144.6535 (red line) due to [56-D]-C-CTX-3/-4 (**5**/**6**) from reduction of C-CTX-1/-2 (**1**/**2**) after treatment with sodium borodeuteride. The ^{13}C isotopomer of **3**/**4** cannot be resolved from $[M+H]^+$ of **5**/**6**.

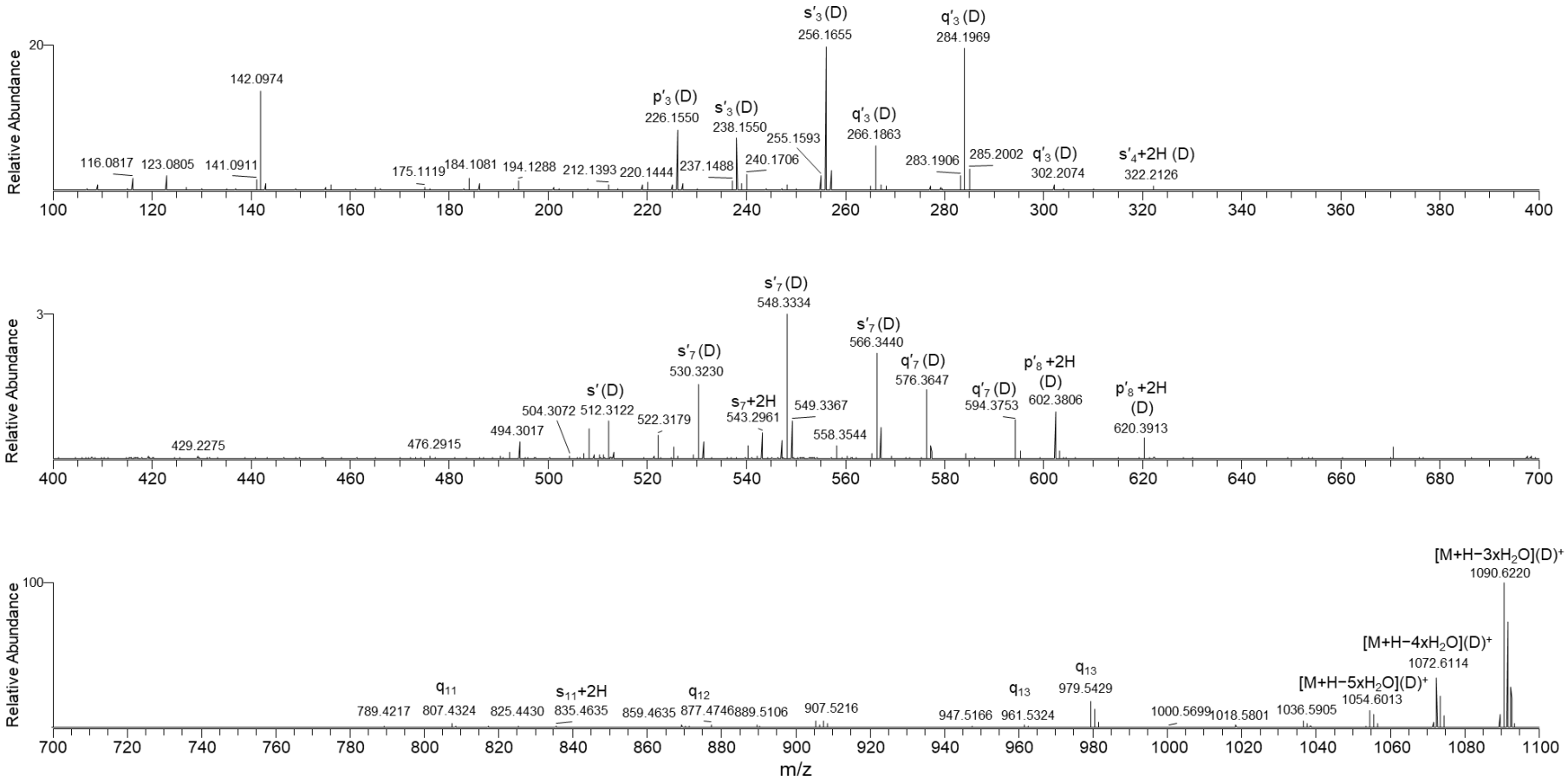

Figure S2. LC–HRMS/MS spectrum from HCD of the $[M+H]^+$ of the major isomer of [56-D]C-CTX-3/-4 (**5/6**) produced via reduction of **1/2** with $NaBD_4$.

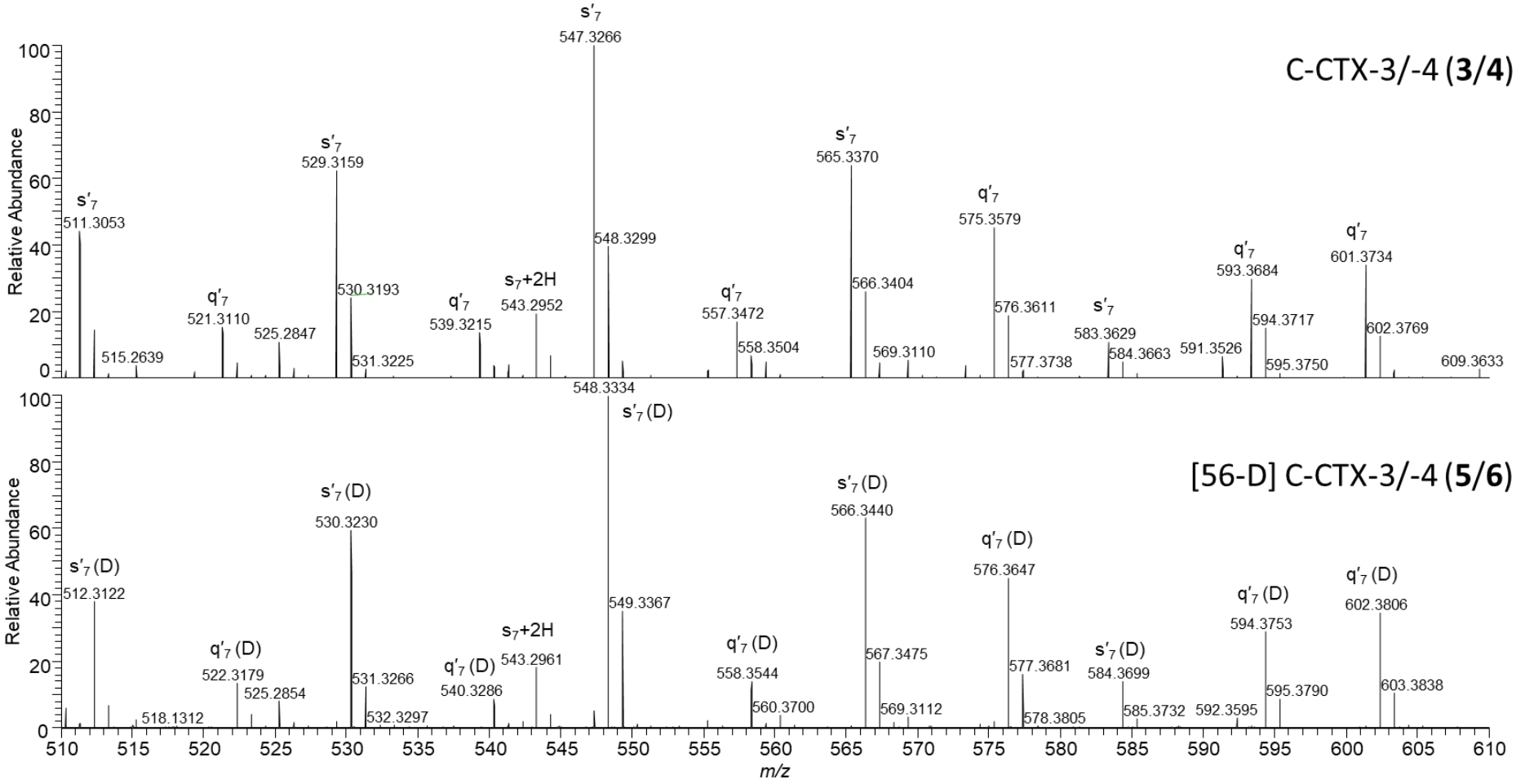

Figure S3. Comparison of *m*/*z* 510–610 of the HRMS/MS spectra of C-CTX-3/-4 (**3**/**4**) (top) and 56-deutero-C-CTX-3/-4 (**5**/**6**) (bottom).

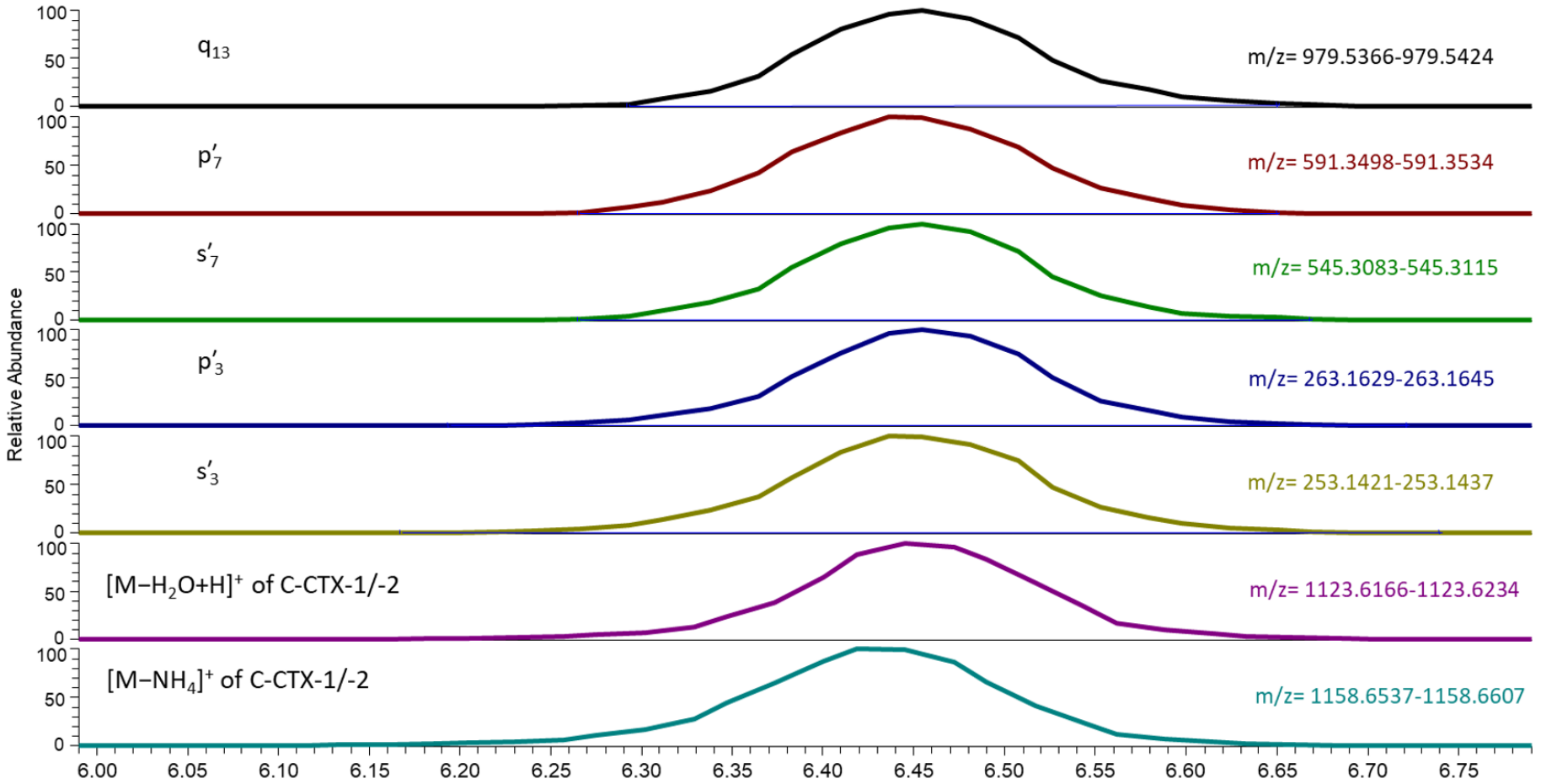

Figure S4. Extracted ion chromatograms of key C-CTX-1/2 (**1**/**2**) product-ions and $[M-H_2O+H]^+$ and $[M-NH_4]^+$ of intact (parent) ions.

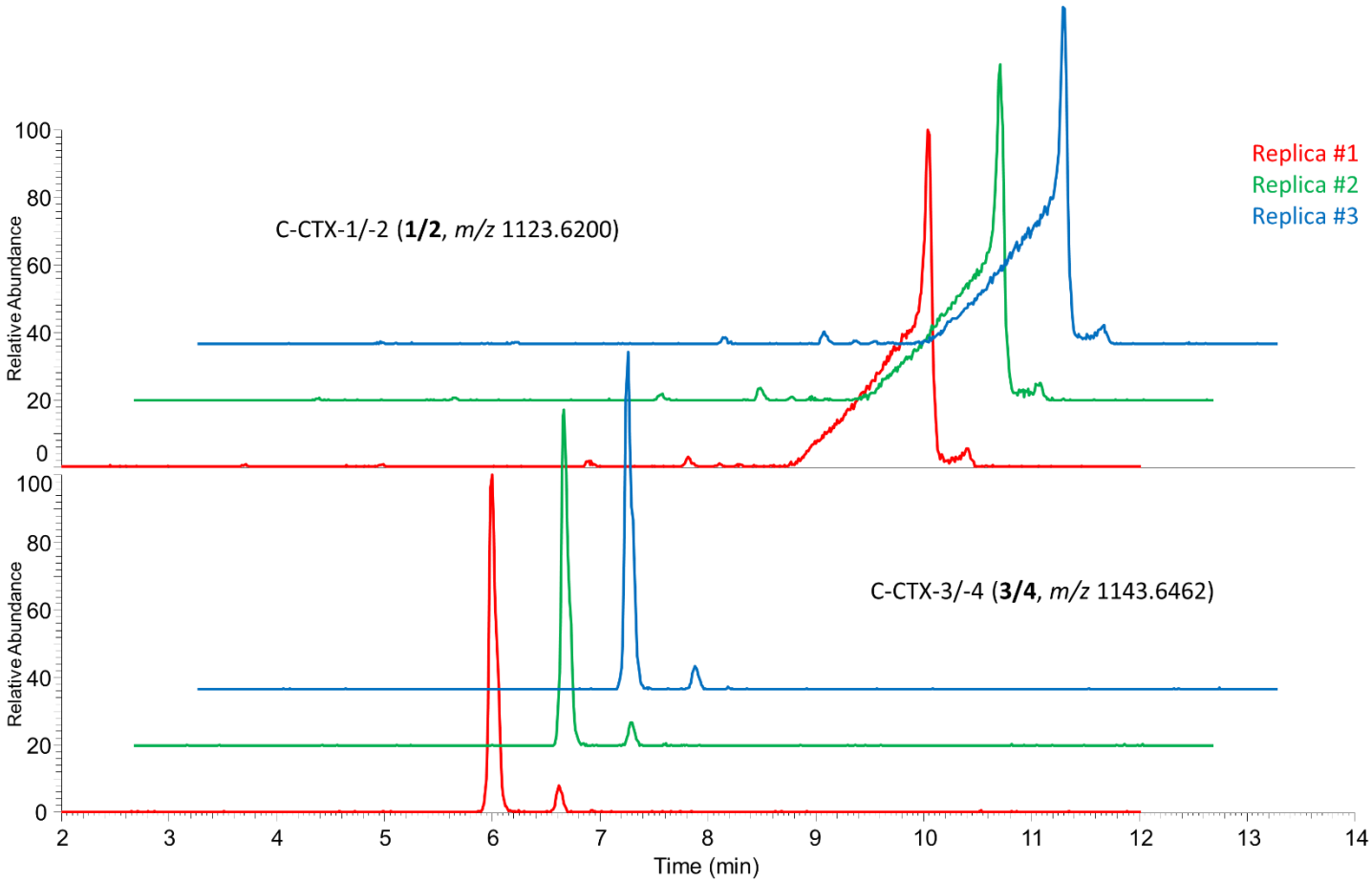

Figure S5. Extracted ion chromatograms (± 5 ppm, triplicate injections) for $[M-H_2O+H]^+$ of C-CTX-1/-2 (**1**/**2**) and $[M+H]^+$ of C-CTX-3/4 (**3**/**4**) in a ciguatoxic *S. barracuda* extract using a Vanquish C18+ UHPLC column and an acidic mobile phase. Ciguatoxicity of the fish extract was determined by MTT-N2A assay, data not shown.

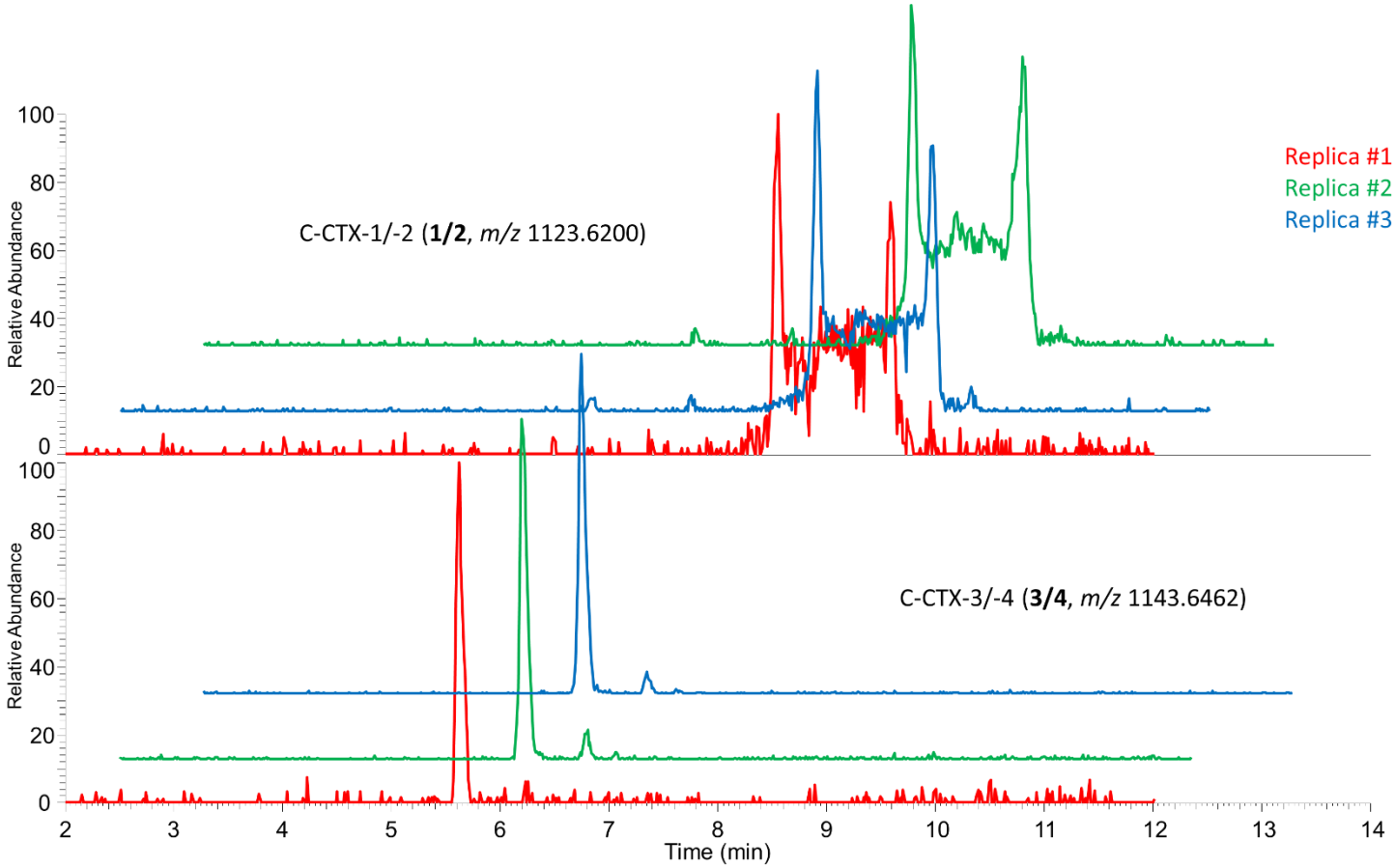

Figure S6. Extracted ion chromatograms (± 5 ppm, triplicate injections) for $[M-H_2O+H]^+$ of C-CTX-1/-2 (**1**/**2**) and $[M+H]^+$ of C-CTX-3/-4 (**3**/**4**) in a ciguatoxic *S. barracuda* using a Vanquish C18+ UHPLC column and a neutral mobile phase. Ciguatoxicity of the fish extract was determined by MTT-N2A assay, data not shown.

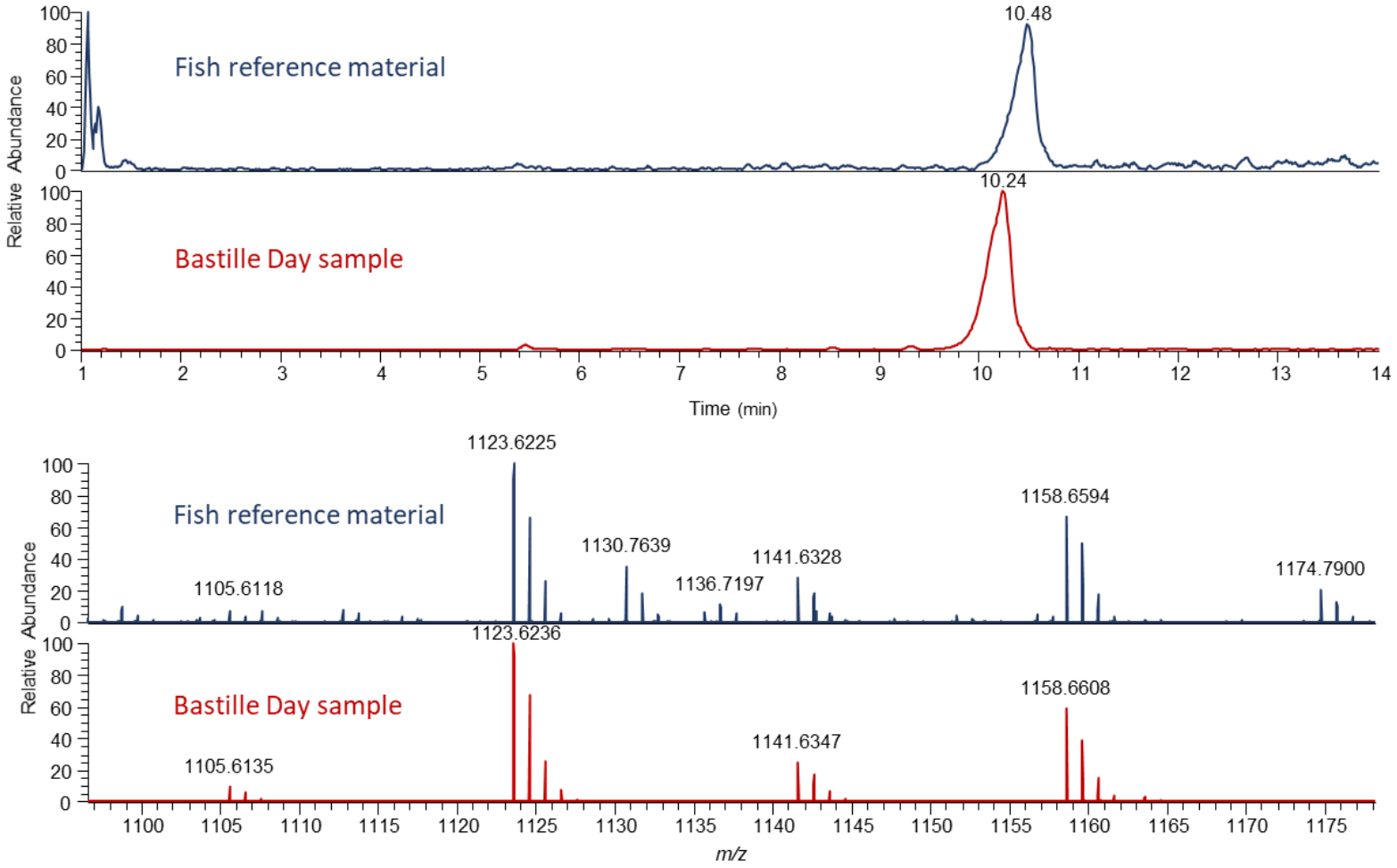

Figure S7. Extracted ion chromatograms (± 5 ppm, upper layers) for $[M-H_2O+H]^+$ of C-CTX-1/-2 (**1**/**2**) and HRMS spectra (shown in bottom) in fish reference material (shown in blue) and in a ciguatoxic *S. barracuda* (shown in red).

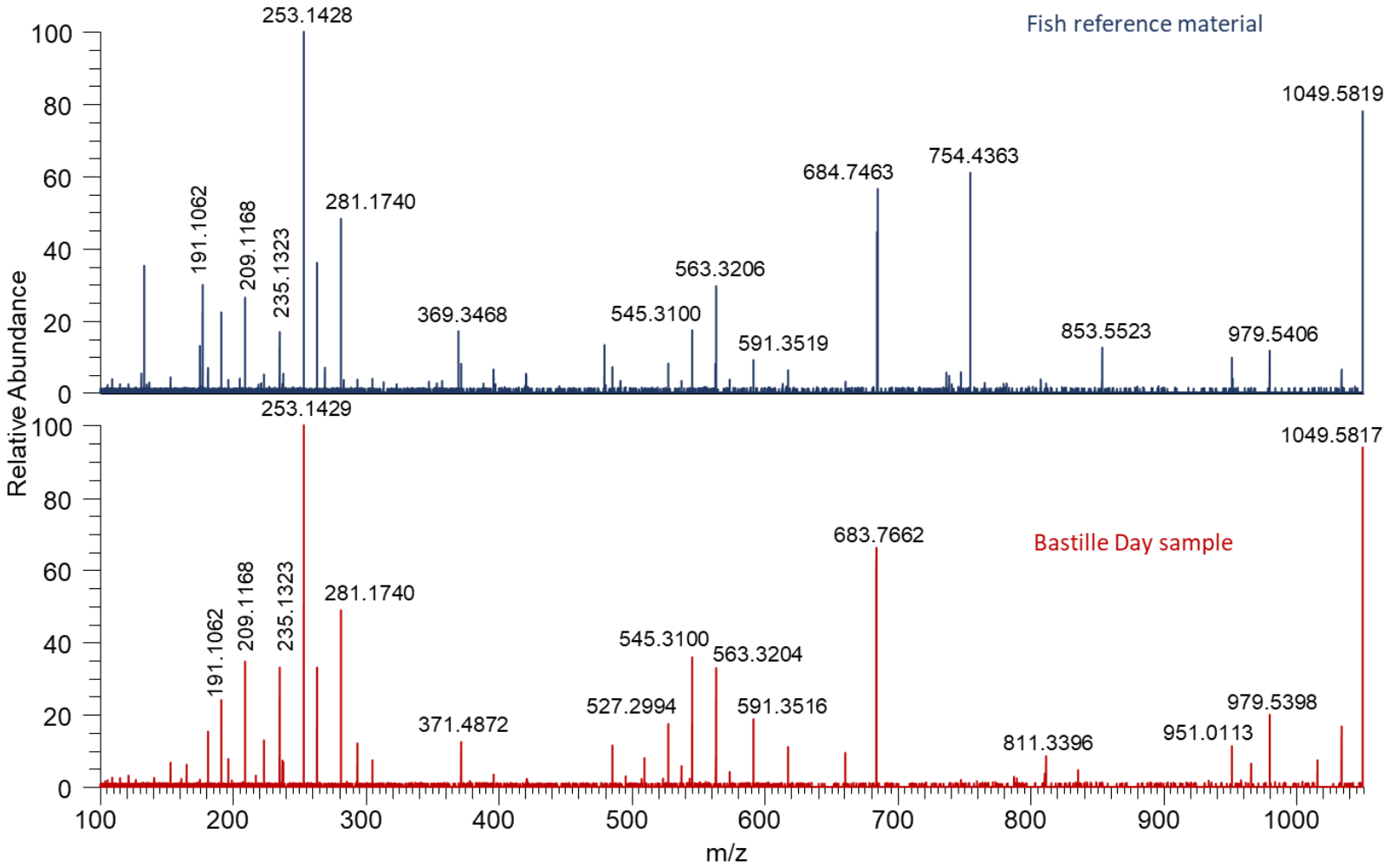

Figure S8. Comparison of the HRMS/MS spectra of C-CTX-1/-2 (**1**/**2**) acquired in fish reference material (shown in blue) and in a ciguatoxic *S. barracuda* (shown in red).