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A mussel tissue certified reference material for multiple phycotoxins. Part 5: profiling by liquid chromatography–high resolution mass spectrometry

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1 ABSTRACT

A freeze-dried mussel tissue certified reference material (CRM-FDMT1) was prepared containing the marine algal toxin classes azaspiracids, okadaic acid and dinophysistoxins, yessotoxins, pectenotoxins, cyclic imines, and domoic acid. Thus far, only a limited number of analogues in CRM-FDMT1 have been assigned certified values, however the complete toxin profile is significantly more complex.

Liquid chromatography–high resolution mass spectrometry was used to profile CRM-FDMT1. Full scan data was searched against a list of previously reported toxin analogues, and characteristic product ions extracted from all-ion-fragmentation data was used to guide the extent of toxin profiling. A series of targeted and untargeted acquisition MS/MS experiments were then used to collect spectra for analogues. A number of toxins previously reported in the literature but not readily available as standards were tentatively identified including dihydroxy and carboxyhydroxyyessotoxin, azaspiracids-33 and -39, sulfonated pectenotoxin analogues, spirolide variants, and fatty acid acyl esters of okadaic acid and pectenotoxins. A number of previously unreported toxins were also observed including analogues from the pectenotoxin, azaspiracid, desulfoyessotoxin and spirolide classes. More than one hundred toxin analogues present in CRM-FDMT1 are summarized along with a demonstration of the major acyl ester conjugates of several toxins. Retention index values were assigned for all confirmed or tentatively identified analogues to help with qualitative identification of the broad range of lipophilic toxins present in the material.

Keywords: CRM-FDMT1, LC-HRMS, yessotoxin, azaspiracid, okadaic acid, dinophysistoxin, pectenotoxin, spirolide

2 INTRODUCTION

Analytical methods based on liquid chromatography with optical or mass-spectrometric detection are now accepted methods for shellfish safety testing [1]. The sensitivity and selectivity of liquid chromatography–mass spectrometry (LC–MS) methods, in particular, has enabled detailed toxin profiling in algae, shellfish and passive samplers [2-8]. Methods with high-resolution mass spectrometry (HRMS) detection are particularly powerful screening tools due to their high analyte capacity and utility for retrospective data analysis [2, 9-11].

Development and implementation of analytical methods for toxin monitoring and screening is facilitated by the availability of fit-for-purpose reference materials (RMs) [12]. A range of calibration solutions [13-15] and a number shellfish tissue certified reference materials (CRMs) are now available for most of the regulated phycotoxin classes [16-18]. There are many challenges to producing individual RMs for the broad range of regulated and unregulated toxins that have been reported to date, and analysts frequently lack positive controls for identity confirmation of unregulated toxins in their samples. In the broader field of analytical chemistry, RM producers occasionally provided information on additional properties of interest in RMs, with examples from the clinical [19], nutritional [20] and environmental fields [21]. Some matrix RMs for phycotoxins have been provided with information values for a limited range of additional toxin analogues [17] but these are by no means comprehensive.

A freeze-dried mussel tissue certified reference material (CRM-FDMT1) containing a range of phycotoxins was prepared from a variety of naturally-incurred tissues, algal biomass, and purified or semi-purified toxins [22] to assist with the development and validation of multi-toxin analysis methods. Analytical methods were refined [23] and accurate measurements performed to assign certified mass fraction values for ten toxin analogues, incorporating data from homogeneity and stability assessments [24] to estimate overall uncertainties for the certified values [25]. The

certified analytes are azaspiracid-1, -2, and-3 (AZA1–3), okadaic acid (OA), dinophysistoxin-1, and -2 (DTX1, -2), yessotoxin (YTX), pectenotoxin-2 (PTX2), 13-desmethylspirolide C (13-desMe-SPX C), and domoic acid (DA). As a result, CRM-FDMT1 has broad utility for method development, validation, and ongoing quality assurance. In addition to the certified values, a number of related toxin analogues were tentatively identified in CRM-FDMT1 using low resolution selected reaction monitoring methods [26].

In this work, LC-HRMS was used to extensively characterize the toxin profile in a concentrated sample of CRM-FDMT1. Data from full scan (FS) experiments were screened for previously-reported toxin analogues, while data from all-ion-fragmentation (AIF) experiments were used to search for characteristic MS/MS product ions from each toxin class. Targeted methods as well as untargeted data-dependent acquisition (DDA) methods were used to collect MS/MS spectra for individual analogues. The approach taken and data acquired for confirmed and tentatively identified analogues is summarized.

3 MATERIALS AND METHODS

3.1 Standards and chemicals

Methanol and acetonitrile (Optima LC-MS grade) were from Fisher Scientific (Whitby, ON, Canada). Formic acid (98%) and LC-MS grade ammonium formate were from Honeywell-Fluka (Oakville, ON, Canada). Distilled water was passed through a Milli-Q purification system (Millipore Corp., Billerica, MA, USA). All RMs were from the National Research Council (Halifax, NS, Canada) including CRM-FDMT1, calibration solutions CRM-OA-c, CRM-DTX1-b, CRM-DTX2-b and a retention index standard for liquid chromatography (RM-RILC). In-house calibration solutions included PTX1, PTX11, PTX2sa and 7-*epi*-PTX2 seco acid (7-*epi*-PTX2sa), 45-hydroxyyessotoxin (45-OH-YTX), AZAs-4–10 and -33 [27, 28], 19-epi-DTX2, spirolide (SPX)

C and 20-Me-SPX G. Standards of the 24-o β-D-glucosides of OA and DTX2 [29]were provided by Alistair L. Wilkins from work performed at the Norwegian Veterinary Institute (Oslo, Norway), and synthetic 7-deoxyOA [30] was provided by Craig J. Forsyth (The Ohio State University, Columbus, OH, USA) from work performed in collaboration with Amy B. Dounay at the University of Minnesota (Minneapolis, MN, USA).

3.2 Sample preparation

CRM-FDMT1 was reconstituted as previously described by weighing 1 g into a 50 mL polypropylene centrifuge tube, adding 5 mL of water, vortex-mixing for 0.5 min, and sonicating for 1 min. The reconstituted tissue was extracted with methanol (20 mL) by vortex-mixing (5 min, 2500 rpm) before centrifuging (10 min at 3950 *g*), and the supernatant decanted into a 50 mL volumetric flask. The pellet was re-extracted with methanol (20 mL), the supernatants combined, and the flask made to volume with methanol. The extract volume was then reduced to approx. 2.5 mL under nitrogen at ambient temperature before adding methanol (1 mL) and water (5 mL). This was loaded onto a solid phase extraction (SPE) cartridge (Waters Oasis HLB, 1 g, 20 mL; Milford, MA, USA) preconditioned with methanol (10 mL) and 20% methanol (10 mL) respectively. Consecutive washes of 20% and 50% methanol were performed before elution with 90% methanol (10 mL) and 100% methanol (20 mL). The 90 and 100% methanol fractions were combined and evaporated under nitrogen at ambient temperature. The residue was reconstituted with methanol (1 mL), sonicated, and filtered (0.45 µm PTFE spin filter; Merck Millipore Ltd., Cork, Ireland). To assign retention index values, a 10 µL aliquot of RM-RILC was mixed with 220 µL of sample.

3.3 Liquid chromatography-high resolution mass spectrometry

Liquid chromatography separations were on an Agilent 1200 system (Agilent Inc., Palo Alto, CA, USA) equipped with a binary pump, degasser, column compartment, and temperature controlled autosampler. An Agilent Poroshell 120 SB-C_{18} column (2.1 × 150 mm, 2.7 µm) was held at 40 °C.

Mobile phase was 50 mM formic acid and 2 mM ammonium formate in both water (A) and 95% acetonitrile (B). The flow rate was 0.275 mL min^{-1} with a 20 min gradient from 5% to 100% B, and held for 25 min before re-equilibration. Injection volumes were 1 µL with the exception of 2 µL injections used for AIF experiments. Mobile phase was diverted to waste for the first 6 min of the run. The LC was connected to a Q Exactive HF Hybrid Quadrupole-Orbitrap mass spectrometer equipped with a heated electrospray ionization probe (HESI-II) (Thermo Fisher Scientific, Bremen, Germany). Source conditions were (+/- settings); spray voltage 3.0/2.7 kV, capillary temperature 350 °C, sheath gas 35/40, auxillary gas 10/15 (arbitrary units) with probe heater temperature set to 300 °C, and S-Lens RF Level 50. The MS was calibrated according to the manufacturer's specification using positive and negative Pierce calibration mixes (Thermo-Fisher Scientific). Customized low-mass calibration, with a collision-induced dissociation of 45 eV, used the n-butyl ammonium ion (*m*/*z* 74.0964) and caffeine (*m*/*z* = 110.0713, 138.0662) product ions in positive mode, and product ions of CF_3^- (*m*/*z* 68.9958) from trifluoroacetic acid and bisulfate (*m*/*z* 96.9601) were used in negative mode. *Full scan (FS)***:** both polarities used the 240 000 resolution setting, 3×10^6 AGC target, max injection time of 200 ms, *m*/*z* range of 500–1600; *FS with AIF***:** FS used the 120 000 resolution setting, 3×10^6 AGC target, max injection time 200 ms, *m*/*z* range of 200–2000 for positive mode and 600–1600 for negative; AIF data was acquired using the 60 000 resolution setting, 3×10^6 AGC target, max injection time 200 ms, and CE set at 45 eV/-70 eV with an *m*/*z* range of 133–1250 for positive mode and *m*/*z* 106.7–1600 for negative; *FS with parallel reaction monitoring (PRM)***:** FS settings were the same as FS with AIF experiments, and PRM was used for targeted MS/MS collection with an isolation window of 0.4 Da on precursor masses and spectra were acquired using the 30 000 resolution setting, AGC target 2×10^5, max injection time 100 ms and CE values of 35, 45, 55, −70, −90 for PTXs, SPXs, AZAs, OA/DTXs, and YTXs, respectively; *DDA*: FS settings were the same as the FS with AIF experiments. For MS/MS, the

precursor isolation windows was 0.4 Da, with the 30 000 resolution setting, AGC target of 1×10^5, max injection time 50 ms for positive and 100 ms for negative, loop count 10 for positive and 15 for negative, and CE of 50 eV/-90 eV with intensity threshold 4×10^5 and 8×10^4 for negative, isotope exclusion on, and dynamic exclusion of 3 s.

4 RESULTS AND DISCUSSION

The extraction and clean-up step was designed to produce a sample concentrated in lipophilic toxins, while avoiding conditions that could lead to formation of toxin degradation or conversion products (e.g., high temperature or pH extremes). Previous work indicated that loading and washing with up to 50% methanol using polymeric SPE cartridges was suitable for concentrating lipophilic toxins on the column and that 90–100% methanol was sufficient for eluting the toxins [31]. The SPE procedure applied in this work provided a gentle clean-up of the lipophilic toxins while concentrating approximately 70-fold over the exhaustive extraction procedure used for certification [25].

LC and MS method considerations were important when establishing the strategy for toxin profiling. A long LC gradient was selected to improve resolution of chromatographic peaks, followed by an extended flush with high percentage organic to ensure complete elution of highly non-polar compounds such as fatty acid acyl esters and late-eluting non-toxin interferences. Full-scan data was analyzed against a list of known toxin variants (adapted from Gerssen *et al.* [10]) to produce a list of ions for targeted MS/MS collection. In parallel, AIF experiments were used to broadly determine the complexity of the profile, beyond the list of known compounds, by extracting characteristic product-ion chromatograms from each toxin class. In situations where previously uncharacterized peaks were observed in the AIF data, a list of candidate precursor ions that aligned with the retention times corresponding to the AIF product ions were selected for exploratory PRM

experiments to obtain clean spectra and identify the precursor ions responsible. Results of DDA experiments were also analyzed to determine whether any precursor ions selected automatically for MS/MS acquisition yielded these key product ions. The MS/MS data for each toxin analogue was then tabulated for tentative identification. Confirmation of identity involved the use of toxin standards when available, or comparison to other previously verified reference materials. Tentative identification was based on comparison to known MS/MS data and consideration of relative retention times. All analogues reported herein were subject to passing a number of criteria: FS data was used to measure accurate masses of the precursor ion and at least two isotopes (data not shown) to within 5 ppm of the exact mass to support the proposed formulae; isotopic ratios were observed and verified to be in agreement with theoretical intensity profiles generated using Xcalibur 4.0; and MS/MS product ions from PRM or DDA experiments were tabulated (< 5 ppm) to demonstrate that the fragmentation was consistent with that of known related toxin analogues.

To provide a reliable basis for comparing retention time data for minor analogues in CRM-FDMT1 between different HPLC platforms using similar chromatographic conditions, retention indices have been provided (Table 1). Retention indices for each compound were calculated by interpolation of a linear regression model of the thirteen latest eluting *N*-alkylpyridinium-3-sulfonate compounds from RM-RILC, assigned ordinate values from 800–2000 (100-unit increments assigned for each sequentially eluting compound) vs. retention time [32]. Further details are provided in the *electronic supplementary material* (*ESM, Fig. S1 & S2*).

The following sections provide summaries of toxin profiling in CRM-FDMT1. Tentative identification of analogues by comparison with known product ion spectra is also discussed, as well as other specific information pertaining to the analysis of each toxin group, including noteworthy examples of previously unreported analogues. Structures of representative toxin analogues in CRM-FDMT1 are given in Fig. 1 for reference in the discussion.

4.1 Azaspiracids

Two of the primary mussel tissues used in the preparation of CRM-FDMT1 contained AZAs [22]. The most highly concentrated of these was from Bruckless, Co. Donegal, Ireland. AZA1–3 are certified analogues, and information values have been provided for AZA4–10 [26].

The FS data was screened for known AZAs and, in parallel, the AIF data was extracted for the H+I ring product ion ($C_{10}H_{18}NO^+$ *m*/*z* 168.1383), which is observed for the majority of AZAs (Fig. 2). The AZA profile was largely dominated by certified toxins, but with significant amounts of AZA4, AZA6, and AZA33 [28]. Peaks consistent with AZA11–15 [33], AZA39 [34], 21,22-dehydroAZA3 (AZA25) [35] and 21,22-dehydro-23-oxoAZA3 (AZA26) [35] were also observed along with other potential dehydro-analogues (Table 1, Fig. 2). As expected due to their thermal lability [36, 37], no 22-carboxylated AZAs were confirmed, although compounds within ± 5 ppm of *m*/*z* 872.4791 ($C_{47}H_{70}NO_{14}^+$) were detected at trace levels, with the latter of the two giving a product ion spectrum with some similarities to carboxylated analogues previously reported in shellfish [38] (*ESM, Fig. S3*). Late-eluting peaks observed in the extracted-ion chromatograms for AZA3 and AZA6 were previously noted as degradation products [24] resulting from thermal stabilization steps conducted on source tissues used to prepare FDMT1 [22]. Similar isomers were observed here in the extracted-ion chromatograms for AZA4–5 and AZA7–9 (Fig 2). The latest eluting of these isomers showed A-ring product-ion patterns with more pronounced water loss at *m*/*z* 622.3738 ($C_{37}H_{52}NO_7^+$) observed at higher intensity than in their earlier-eluting isomeric counterparts, while product ions from fragmentation between the D- and E-rings typically yielding *m*/z 448.3057 ($C_{26}H_{42}NO_5^+$) were not observed (*ESM, Fig. S4 & S5*).

Extracting the DDA positive mode data for the product ion at *m*/*z* 168.1383 revealed the presence of compounds showing AZA-like MS/MS spectra with previously unreported low

precursor ion accurate masses at *m/z* 608.3792 and 624.3752 observed in FS data consistent with $C_{33}H_{54}NO_9^+$ (Δ 0.7 ppm) and $C_{33}H_{54}NO_{10}^+$ (Δ −1.3 ppm), respectively (Table 1, *ESM Fig. S6*). These would be one of the lowest molecular weight AZAs reported to date and identification will require further characterization. This work did not extensively consider C-37 epimers that have been reported for AZAs, [39] as they are better resolved under neutral pH conditions and have nearly identical spectra to their epimeric counterparts. These epimers have been reported for AZA1–3 and AZA6 in CRM-FDMT1 [23], but can be expected for all AZAs that are present in the sample. All-ion fragmentation data also showed trace levels of additional compounds potentially related to AZAs, however tentative identification of these minor components was not pursued further.

Fatty acid acyl esters of AZAs have recently been reported in mussel tissue and in CRM-FDMT1 [40]. These highly non-polar AZA metabolites required alternative chromatographic conditions for elution and detection (*ESM Fig. S7*).

4.2 Okadaic acid and dinophysistoxins

Mussel tissue harvested in Norway was the major source of DTX1 in CRM-FDMT1, while tissue from Ireland was the source of OA and DTX2 [22]. Small isomeric peaks for OA, DTX2 and DTX1 were present in CRM-FDMT1 (Fig. 3, [26]). Elution of some of these compounds aligned with those present in calibration solutions CRMs [14], where 19-*epi*-DTX2 eluted as a shoulder on the OA peak, and isomeric compounds bearing similar relative retention times to their main peak could be tentatively assigned as 19-*epi*-OA and 19-*epi*-DTX1 (*ESM Fig. S8*). An isomer peak eluting after DTX2 also had a product ion spectrum similar to those of OA and DTX2. 7-DeoxyOA [41] was confirmed by matching retention time and spectrum with a standard, while a later eluting 7-deoxyDTX2 analogue was tentatively identified. The presence of the 24-*O*-β-D-glucosyl

conjugates of OA and DTX2 were confirmed by comparison with authentic standards [29] (A. L. Wilkins and C. O. Miles. unpublished information), with the corresponding DTX1 glucoside conjugate also tentatively identified by its MS/MS spectra (Table 1, [29]).

Fatty acid acyl esters of OA, DTX1 and DTX2 have been indirectly measured in CRM-FDMT1 using the base hydrolysis procedure, showing increases of approximately 60, 20, and 35%, respectively [25]. The AIF data clearly shows the provenance of the acyl ester profile, dominated by OA/DTX2 conjugates, by extracting ion chromatograms for OA/DTX2 after neutral loss of the carboxylic acid moiety of the acyl ester yielding *m/z* 785.4482 ($C_{44}H_{65}O_{12}^{-}$) (Fig. 3). Direct analysis of 7-*O*-acyl esters showed a profile of OA and DTX2 esters consistent with previous reports, where the 14:0, 16:0, 16:1, 18:4, and 22:6 acyl esters were dominant [42-44]. Direct analysis of DTX1 acyl esters showed only trace levels of 18:4 DTX1 (tentative) despite seeing an approximate 20% increase in free toxin after base hydrolysis [25]. The increase may be due to various trace level acyl esters and related additional compounds that hydrolyze under base conditions [45]. Data-dependent acquisition and AIF data did not reveal significant levels of additional analogues related to OA and DTXs.

4.3 Yessotoxins

YTX was added to CRM-FDMT1 as a purified disodium salt isolated from algae [22]. However, the mussel tissue sourced from Norway for DTX1 also contained low levels of various YTX analogues. Information values were previously assigned for 45-hydroxyyessotoxin (45-OH-YTX) and carboxyyessotoxin (COOH-YTX) in CRM-FDMT1 [26].

Evaluation of FS data for previously reported compounds and extracting the AIF data for the masses of common YTX product ions ($C_{42}H_{63}O_{16}S^{-}$, *m/z* 855.3842; and $C_{42}H_{63}O_{17}S^{-}$, *m/z* 871.3791) showed the presence of additional putative YTXs (Table 1 and Fig. 4). Several known

YTX analogues were tentatively identified in CRM-FDMT1 based on their MS/MS fragmentation and retention times relative to analogues such as dihydroxyyessotoxin (diOH-YTX) and carboxyhydroxyyessotoxin (COOH-OH-YTX), with the latter showing a tendency to produce multiply charged FS ions consistent with carboxylated YTXs. The product ion spectra of the tentative COOH-OH-YTX peaks indicate variable positions of the hydroxy group (Table 1). Beyond tentative identification of the YTXs mentioned above, several significant signals in the AIF trace were still unexplained. Analyzing DDA spectra for these showed the absence of a common YTX neutral loss (SO_3, *m*/*z* 79.9568) suggesting that these were monodesulfonated counterparts of the disulfated toxins [46, 47]. The response of each of these later-eluting singly desulfonated compounds relative to their disulfated counterpart was approximately equal in full scan intensity (Fig. 4), with the exception of YTX which was the only YTX analogue fortified in CRM-FDMT1.

4.4 Pectenotoxins

Pectenotoxin-2 was added to CRM-FDMT1 from an ethereal extract of *Dinophysis* spp. [22], while mussel tissues from the Southwest of Ireland were also a source of PTXs. An isomer of PTX2 in CRM-FDMT1 was observed as a degradation product in stability studies [24]. PTX2sa and its epimer 7-*epi*-PTX2sa, have been assigned information values [26], while several additional PTX2sa isomeric analogues giving nearly identical MS/MS spectra were also present (Fig. 5 and Table 1).

Extracting an AIF product ion chromatogram for *m*/*z* 213.1121 ($C_{11}H_{17}O_4^+$) indicates a notable PTX profile with a prominent acyl esters, as well as several peaks eluting earlier than PTX2 and PTX2sa (Fig. 5). Direct analysis of PTX acyl esters in this work (*ESM Fig. S9*) showed an ester profile and MS/MS spectra consistent with previous reports [48] [49]. The complex PTX2sa

acyl ester profile is due to the number of PTX2sa isomers present and their potential to be acylated at three known sites, giving 11-, 33-, and 37-*O*-acyl esters [48]. 11-*O*-acyl esters are easily identified in positive ionization mode using the 11-*O*-acyl fragment composed of A–E rings attached to the ester ($C_{45}H_{73}O_{11}^{+}$, *m*/*z* 789.5147) [48], however 33-, and 37-*O*-acyl esters (*ESM Fig. S10*) were more challenging to assign. For simplicity, data for the most intense peak is listed for PTX2sa esters in Table 1.

Pectenotoxin-12 and -12sa have been reported at low levels in samples containing PTX2sa and its esters [50]. Two peaks of *m*/*z* 874.4947 in an extracted FS chromatogram showed MS/MS spectra and relative retention times consistent with those reported for 36*S*-PTX12 and 36*R*-PTX12 (Table 1) [51]. Chromatographic peaks isobaric with PTX1/11 ($[C_{47}H_{70}O_{15}+NH_4]^{+}$, *m*/*z* 892.5053) eluted earlier (13.4 and 14.3 min) than in-house standards of PTX1/11 [52]. Sulfonated PTXs were detected along with corresponding desulfonated counterparts. These compounds gave product ion spectra consistent with previous data [29], and major acyl esters of these were verified in negative mode PRM experiments by showing product ions within ± 5 ppm for HSO_4^{-} (*m*/*z* 96.9601), ester fragments (e.g. $C_{16}H_{31}O_2^{-}$, *m*/*z* 255.2330), product ions representing loss of carboxylic acid moiety of esters, and a product ion from fragmentation of the unconjugated toxin analog upon loss of the ester group ($C_{29}H_{41}SO_9^{-}$, *m*/*z* 565.2477).

Previously unreported compounds consistent with a PTX seco acid were detected as ammonium adduct ions ($[C_{47}H_{72}O_{16}+NH_4]^{+}$, *m*/*z* 910.5159) with MS/MS data showing several fragments typical of PTXs (Table 1). Extracting theoretical *m*/*z* values for common acyl esters of these seco acid-like compounds (*m*/z 1148.7455 for 16:0 acyl ester) showed an acyl ester profile with individual peaks having product ion spectra bearing similarities to those observed for PTX2sa esters (Table 1, *ESM Fig. S11*).

4.5 Cyclic Imines

Alexandrium ostenfeldii biomass was used as the 13-desMe-SPX C source for CRM-FDMT1 [22]. The culture used (NRC AOSH1) produces a range of SPXs, some of which have been assigned structures [53], while others have only been tentatively identified [54]. Several SPXs were assigned information values in CRM-FDMT including 20-methylspirolide G (20-Me-SPX G), SPX C, 13-desMe-SPX D, and a small amount of a 13-desMe-SPX C isomer. Esterified 20-Me-SPX G [7] was not observed. An information value was assigned for pinnatoxin G (PnTX G) previously [25], however no PnTX G esters [8] were observed.

Examining the FS data for known cyclic imines and extracting the positive mode AIF product ion chromatogram for the characteristic cyclic imine fragment *m*/*z* 164.1434 (Fig. 6) shows that the major cyclic imines in CRM-FDMT1 have been identified and assigned information values prior to this study [26], although many minor analogues were nonetheless present. Compounds with the precursors with *m*/*z* 706.4677 included SPX C, 20-Me-SPX G, and an unidentified compound previously labelled "SPX C3" [53]. Minor peaks of this mass eluted later, also bearing SPX-like product ion spectra (Table 1). A similar chromatographic profile was observed for the precursor *m*/*z* 708.4834, and product ion spectra support tentative assignments of SPX D, a peak analogous to 20-Me-SPX G which is likely saturated on C-2–C-3 [7], and a compound which fragments similarly to SPX D (Table 1). Additional SPX-like peaks were observed, sharing the precursor *m*/*z* 708.4470, one showing a product ion spectrum consistent with 27-OH-13-desMe-SPX C, and other potentially oxidized SPXs yielding an *m*/*z* 180.1383 product ion indicative of hydroxylation on or near the imine ring (Table 1). Also detected were several low mass compounds previously [55] postulated as being spirolides (*ESM Fig. S12*).

Other reported structural variants of the imine ring [56, 57] produce product ions at *m*/*z* 150.1278 ($C_{10}H_{16}N^+$) and 178.1226 ($C_{11}H_{16}NO^+$). AIF chromatograms for these product ions showed chromatographic peaks (*ESM Fig. S13*), however definitive SPX-precursor ions producing these SPX-like product ions were not pursued further as part of this work.

4.6 Additional Compounds

The sample preparation and LC conditions used for this profiling work were selected to accommodate analysis of a broad range of primarily lipophilic algal toxins. However, the experiments conducted in this study are amenable to analysis of other chemicals present in CRM-FDMT1. One example of this is the detection of additive stabilizers (*ESM Fig. S14*) used during preparation of CRM-FDMT1 [22].

Domoic acid (DA) and its analogues are an important class of polar toxins present in CRM-FDMT1. The concentrations of DA and 5′-*epi*-DA are certified, while information values have been provided for several other DA-isomers [25]. Using analytical conditions more suited to detailed analysis these polar toxins, a range of additional DA analogues and isomers have been observed in CRM-FDMT1 (M. Quilliam, personal communication). β-*N*-methylamino-L-alanine (BMAA), a non-proteinogenic diamino acid, has also been confirmed in CRM-FDMT1 in separate work [58].

5 CONCLUSION

This work summarizes the complex profile of lipophilic toxin analogues in CRM-FDMT1. This detailed profiling was intended to add utility to CRM-FDMT1 beyond the certified values. With the increasing scope of analytical methods for toxin measurement, and continuously growing list of phycotoxin structural variants being described, CRM-FDMT1 is suited to play a role in

developing next-generation analytical methods, particularly those based on LC–MS. Retention indices are provided to help analysts cross-reference compounds present in CRM-FDMT1 with compounds present in their own samples. Further work is required to confirm the structures of novel toxin analogues and isomers highlighted.

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DECLARATIONS

The authors declare that they have no conflicts of interest.

7 REFERENCES

1. Anonymous. Commission Regulation (EU) No 15/2011 of 10 January 2011 amending Regulation (EC) No 2074/2005 as regards recognised testing methods for detecting marine biotoxins in live bivalve molluscs. Off J Eur Union. 2011;L006 of 11 January 2011: 3-6.

2. Zendong Z, McCarron P, Herrenknecht C, Sibat M, Amzil Z, Cole RB, et al. High resolution mass spectrometry for quantitative analysis and untargeted screening of algal toxins in mussels and passive samplers. J Chromatogr A. 2015;1416:10-21.

3. McCarron P, Wright E, Quilliam MA. Liquid chromatography–mass spectrometry of domoic acid and lipophilic shellfish toxins with selected reaction monitoring and optional confirmation by library searching of product ion spectra. J AOAC Int. 2014;97(2):316-24.

4. Turner AD, Goya AB. Occurrence and profiles of lipophilic toxins in shellfish harvested from Argentina. Toxicon. 2015;102:32-42.

5. Harju K, Koskela H, Kremp A, Suikkanen S, de la Iglesia P, Miles CO, et al. Identification of gymnodimine D and presence of gymnodimine variants in the dinoflagellate *Alexandrium ostenfeldii* from the Baltic Sea. Toxicon. 2016;112:68-76.

6. Kim JH, Tillmann U, Adams NG, Krock B, Stutts WL, Deeds JR, et al. Identification of *Azadinium* species and a new azaspiracid from *Azadinium poporum* in Puget Sound, Washington State, USA. Harmful Algae. 2017;68:152-67.

7. Aasen JAB, Hardstaff W, Aune T, Quilliam MA. Discovery of fatty acid ester metabolites of spirolide toxins in mussels from Norway using liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom. 2006;20(10):1531-7.

8. McCarron P, Rourke WA, Hardstaff W, Pooley B, Quilliam MA. Identification of pinnatoxins and discovery of their fatty acid ester metabolites in mussels (*Mytilus edulis*) from Eastern Canada. J Agric Food Chem. 2012;60(6):1437-46.

9. Blay P, Hui JPM, Chang J, Melanson JE. Screening for multiple classes of marine biotoxins by liquid chromatography–high-resolution mass spectrometry. Anal Bioanal Chem. 2011;400(2):577-85.

10. Gerssen A, Mulder PPJ, de Boer J. Screening of lipophilic marine toxins in shellfish and algae: Development of a library using liquid chromatography coupled to orbitrap mass spectrometry. Anal Chim Acta. 2011;685(2):176-85.

11. Domenech A, Cortes-Francisco N, Palacios O, Franco JM, Riobo P, Llerena JJ, et al. Determination of lipophilic marine toxins in mussels. Quantification and confirmation criteria using high resolution mass spectrometry. J Chromatogr A. 2014;1328:16-25.

12. Hess P, McCarron P, Quilliam MA. Fit-for-purpose shellfish reference materials for internal and external quality control in the analysis of phycotoxins. Anal Bioanal Chem. 2007;387(7):2463-74.

13. Perez RA, Rehmann N, Crain S, LeBlanc P, Craft C, MacKinnon S, et al. The preparation of certified calibration solutions for azaspiracid-1, -2, and -3, potent marine biotoxins found in shellfish. Anal Bioanal Chem. 2010;398:2243-52.

14. Beach DG, Crain S, Lewis NI, LeBlanc P, Hardstaff WR, Perez RA, et al. Development of certified reference materials for diarrhetic shellfish poisoning toxins, part 1: Calibration solutions. J AOAC Int. 2016;99(5):1151-62.

15. National Research Council Canada. Certified Reference Materials. https://nrc.canada.ca/en/certifications-evaluations-standards/certified-reference-materials. Accessed on 30 September 2020

16. Hardstaff WR, Jamieson WD, Milley JE, Quilliam MA, Sim PG. Reference materials for domoic acid, a marine neurotoxin. Fresenius J Anal Chem. 1990;338:520-5.

17. McCarron P, Giddings SD, Reeves KL, Hess P, Quilliam MA. A mussel (*Mytilus edulis*) tissue certified reference material for the marine biotoxins azaspiracids. Anal Bioanal Chem. 2015;407(11):2985-96.

18. McCarron P, Reeves KL, Giddings SD, Beach DG, Quilliam MA. Development of certified reference materials for diarrhetic shellfish poisoning toxins, part 2: shellfish matrix materials. J AOAC Int. 2016;99(5):1163-72.

19. Schantz MM, Benner BA, Jr., Heckert NA, Sander LC, Sharpless KE, Vander Pol SS, et al. Development of urine standard reference materials for metabolites of organic chemicals including polycyclic aromatic hydrocarbons, phthalates, phenols, parabens, and volatile organic compounds. Anal Bioanal Chem. 2015;407(11):2945-54.

20. Sharpless KE, Lindstrom RM, Nelson BC, Phinney KW, Rimmer CA, Sander LC, et al. Preparation and characterization of standard reference material 1849 infant/adult nutritional formula. J AOAC Int. 2010;93(4):1262-74.

21. Kucklick JR, Schantz MM, Pugh RS, Porter BJ, Poster DL, Becker PR, et al. Marine mammal blubber reference and control materials for use in the determination of halogenated organic compounds and fatty acids. Anal Bioanal Chem. 2010;397(2):423-32.

22. McCarron P, Emteborg H, Nulty C, Rundberget T, Loader JI, Teipel K, et al. A mussel tissue certified reference material for multiple phycotoxins. Part 1: design and preparation. Anal Bioanal Chem. 2011;400(3):821-33.

23. McCarron P, Giddings SD, Quilliam MA. A mussel tissue certified reference material for multiple phycotoxins. Part 2: liquid chromatography–mass spectrometry, sample extraction and quantitation procedures. Anal Bioanal Chem. 2011;400(3):835-46.

24. McCarron P, Emteborg H, Giddings SD, Wright E, Quilliam MA. A mussel tissue certified reference material for multiple phycotoxins. Part 3: homogeneity and stability. Anal Bioanal Chem. 2011;400(3):847-58.

25. McCarron P, Wright E, Emteborg H, Quilliam MA. A mussel tissue certified reference material for multiple phycotoxins. Part 4: certification. Anal Bioanal Chem. 2017;409(1):95-106.

26. Wright E, Reeves KL, Giddings SD, Quilliam MA, McCarron P. NRC CRM-FDMT1: mussel tissue reference material for multiple phycotoxins. 2016. Biotoxin Metrology Technical Report CRM-FDMT1 20070717 https://nrc.canada.ca/en/certifications-evaluations-standards/certified-reference-materials/list/39/html, Accessed September 30th, 2020.

27. Kilcoyne J, Twiner MJ, McCarron P, Crain S, Giddings SD, Foley B, et al. Structure elucidation, relative LC–MS response and *in vitro* toxicity of azaspiracids 7–10 isolated from mussels (*Mytilus edulis*). J Agric Food Chem. 2015;63(20):5083-91.

28. Kilcoyne J, Nulty C, Jauffrais T, McCarron P, Herve F, Foley B, et al. Isolation, structure elucidation, relative LC–MS response, and in vitro toxicity of azaspiracids from the dinoflagellate *Azadinium spinosum*. J Nat Prod. 2014;77(11):2465-74.

29. Knudsen BK. Mass spectral characterisation of three new groups of algal toxins. Master of Science (MSc) in Chemistry. Hamilton, New Zealand: The University of Waikato; 2009.

30. Dounay AB, Urbanek RA, Sabes SF, Forsyth CJ. Total synthesis of the marine natural product 7-deoxy-okadaic acid: a potent inhibitor of serine/threonine-specific protein phosphatases. Angewandte Chemie International Edition. 1999;38(15):2258-62.

31. These A, Scholz J, Preiss-Weigert A. Sensitive method for the determination of lipophilic marine biotoxins in extracts of mussels and processed shellfish by high-performance liquid chromatography–tandem mass spectrometry based on enrichment by solid-phase extraction. J Chromatogr A. 2009;1216(21):4529-38.

32. Quilliam MA, Giddings SD, McNamara C, Bekri K. RM-RILC, a reference material for measurement of liquid chromatography retention indices. 2020. Biotoxin Metrology Technical Report RM RILC 20140827 https://nrc.canada.ca/en/certifications-evaluations-standards/certified-reference-materials/list/115/html, Accessed September 30th, 2020.

33. Hess P, McCarron P, Krock B, Kilcoyne J, Miles CO. Azaspiracids: chemistry, biosynthesis, metabolism, and detection. In: Botana LM, editor. Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection, 3rd Edition. 3rd ed. Boca Raton, FL, USA: CRC Press; 2014. p. 799-823.

34. Krock B, Tillmann U, Voss D, Koch BP, Salas R, Witt M, et al. New azaspiracids in Amphidomataceae (Dinophyceae). Toxicon. 2012;60(5):830-9.

35. Kilcoyne J, McCarron P, Twiner MJ, Rise F, Hess P, Wilkins AL, et al. Identification of 21,22-dehydroazaspiracids in mussels (*Mytilus edulis*) and *in vitro* toxicity of azaspiracid-26. J Nat Prod. 2018;81(4):885-93.

36. McCarron P, Kilcoyne J, Miles CO, Hess P. Formation of azaspiracids-3, -4, -6, and -9 via decarboxylation of carboxyazaspiracid metabolites from shellfish. J Agric Food Chem. 2009;57(1):160-9.

37. Kilcoyne J, McCarron P, Hess P, Miles CO. Effects of heating on proportions of azaspiracids 1–10 in mussels (*Mytilus edulis*) and identification of carboxylated precursors for azaspiracids 5, 10, 13, and 15. J Agric Food Chem. 2015;63(51):10980-7.

38. Ji Y, Qiu J, Xie T, McCarron P, Li A. Accumulation and transformation of azaspiracids in scallops (*Chlamys farreri*) and mussels (*Mytilus galloprovincialis*) fed with *Azadinium poporum*, and response of antioxidant enzymes. Toxicon. 2017;143:20-8.

39. Kilcoyne J, McCarron P, Twiner MJ, Nulty C, Crain S, Quilliam MA, et al. Epimers of azaspiracids: isolation, structural elucidation, relative LC-MS response, and in vitro toxicity of 37-*epi*-azaspiracid-1. Chem Res Toxicol. 2014;27(4):587-600.

40. Mudge EM, Miles CO, Hardstaff WR, McCarron P. Fatty acid esters of azaspiracids identified in mussels (M*ytilus edulis*) using liquid chromatography-high resolution mass spectrometry. Toxicon: X. 2020;8:100059.

41. Holmes MJ, Lee FC, Khoo HW, Teo SLM. Production of 7-deoxy-okadaic acid by a New Caledonian strain of *prorocentrum lima* (Dinophyceae). J Phycol. 2001;37(2):280-8.

42. Torgersen T, Wilkins AL, Rundberget T, Miles CO. Characterization of fatty acid esters of okadaic acid and related toxins in blue mussels (*Mytilus edulis*) from Norway. Rapid Commun Mass Spectrom. 2008;22(8):1127-36.

43. Li A, Ma J, Cao J, McCarron P. Toxins in mussels (*Mytilus galloprovincialis*) associated with diarrhetic shellfish poisoning episodes in China. Toxicon. 2012;60:420-5.

44. Holland PT, MacKenzie AL, McNabb P, Selwood AI, editors. Esterified forms of okadaic acid and dinophysistoxins in New Zealand shellfish. 6th International Conference on Molluscan Shellfish Safety; 2007; Nantes, France.

45. Torgersen T, Miles CO, Rundberget T, Wilkins AL. New esters of okadaic acid in seawater and blue mussels (Mytilus edulis). J Agric Food Chem. 2008;56(20):9628-35.

46. Ciminiello P, Dell'Aversano C, Fattorusso E, Forino M, Grauso L, Magno GS, et al. Desulfoyessotoxins from Adriatic mussels: a new problem for seafood safety control. Chem Res Toxicol. 1997;20:95-8.

47. Daiguji M, Satake M, Ramstad H, Aune T, Naoki H, Yasumoto T. Structure and fluorometric HPLC determination of 1-desulfoyessotoxin, a new yessotoxin analog isolated from mussels from Norway. Nat Toxins. 1998;6:235-9.

48. Wilkins AL, Rehmann N, Torgersen T, Rundberget T, Keogh M, Petersen D, et al. Identification of fatty acid esters of pectenotoxin-2 seco acid in blue mussels (*Mytilus edulis*) from Ireland. J Agric Food Chem. 2006;54:5672-8.

49. Torgersen T, Sandvik M, Lundve B, Lindegarth S. Profiles and levels of fatty acid esters of okadaic acid group toxins and pectenotoxins during toxin depuration. Part II: blue mussels (*Mytilus edulis*) and flat oyster (*Ostrea edulis*). Toxicon. 2008;52(3):418-27.

50. Torgersen T, Lindegarth S, Ungfors A, Sandvik M. Profiles and levels of fatty acid esters of okadaic acid group toxins and pectenotoxins during toxin depuration. Part I: brown crab (*Cancer pagurus*). Toxicon. 2008;52(3):407-17.

51. Miles CO, Wilkins AL, Samdal IA, Sandvik M, Petersen D, Quilliam MA, et al. A novel pectenotoxin, PTX-12, in *Dinophysis* spp. and shellfish from Norway. Chem Res Toxicol. 2004;17(11):1423-33.

52. Suzuki T, Walter JA, LeBlanc P, MacKinnon S, Miles CO, Wilkins AL, et al. Identification of pectenotoxin-11 as 34*S*-hydroxypectenotoxin-2, a new pectenotoxin analogue in the toxic dinoflagellate *Dinophysis acut*a from New Zealand. Chem Res Toxicol. 2006;19(2):310-8.

53. Cembella A, Lewis N, Quilliam MA. The marine dinoflagellate *Alexandrium ostenfeldii* (Dinophyceae) as the causative organism of spirolides shellfish toxins. Phycologia. 2000;39(1):67-74.

54. Qiu J, Rafuse C, Lewis NI, Li A, Meng F, Beach DG, et al. Screening of cyclic imine and paralytic shellfish toxins in isolates of the genus *Alexandrium* (Dinophyceae) from Atlantic Canada. Harmful Algae. 2018;77:108-18.

55. Sleno L, Chalmers MJ, Volmer DA. Structural study of spirolide marine toxins by mass spectrometry. Part II. Mass spectrometric characterization of unknown spirolides and related compounds in a cultured phytoplankton extract. Anal Bioanal Chem. 2004;378(4):977-86.

56. Ciminiello P, Dell'aversano C, Iacovo ED, Fattorusso E, Forino M, Grauso L, et al. Characterization of 27-hydroxy-13-desmethyl spirolide C and 27-oxo-13,19-didesmethyl spirolide C. Further insights into the complex Adriatic *Alexandrium ostenfeldii* toxin profile. Toxicon. 2010;56(8):1327-33.

57. Garcia-Altares M, Casanova A, Bane V, Diogene J, Furey A, de la Iglesia P. Confirmation of pinnatoxins and spirolides in shellfish and passive samplers from Catalonia (Spain) by liquid chromatography coupled with triple quadrupole and high-resolution hybrid tandem mass spectrometry. Mar Drugs. 2014;12(6):3706-32.

58. Beach DG, Kerrin ES, Giddings SD, Quilliam MA, McCarron P. Differential mobility-mass spectrometry double spike isotope dilution study of release of β-methylaminoalanine and proteinogenic amino acids during biological sample hydrolysis. Sci Rep. 2018;8(1):117.

8 TABLES AND FIGURES

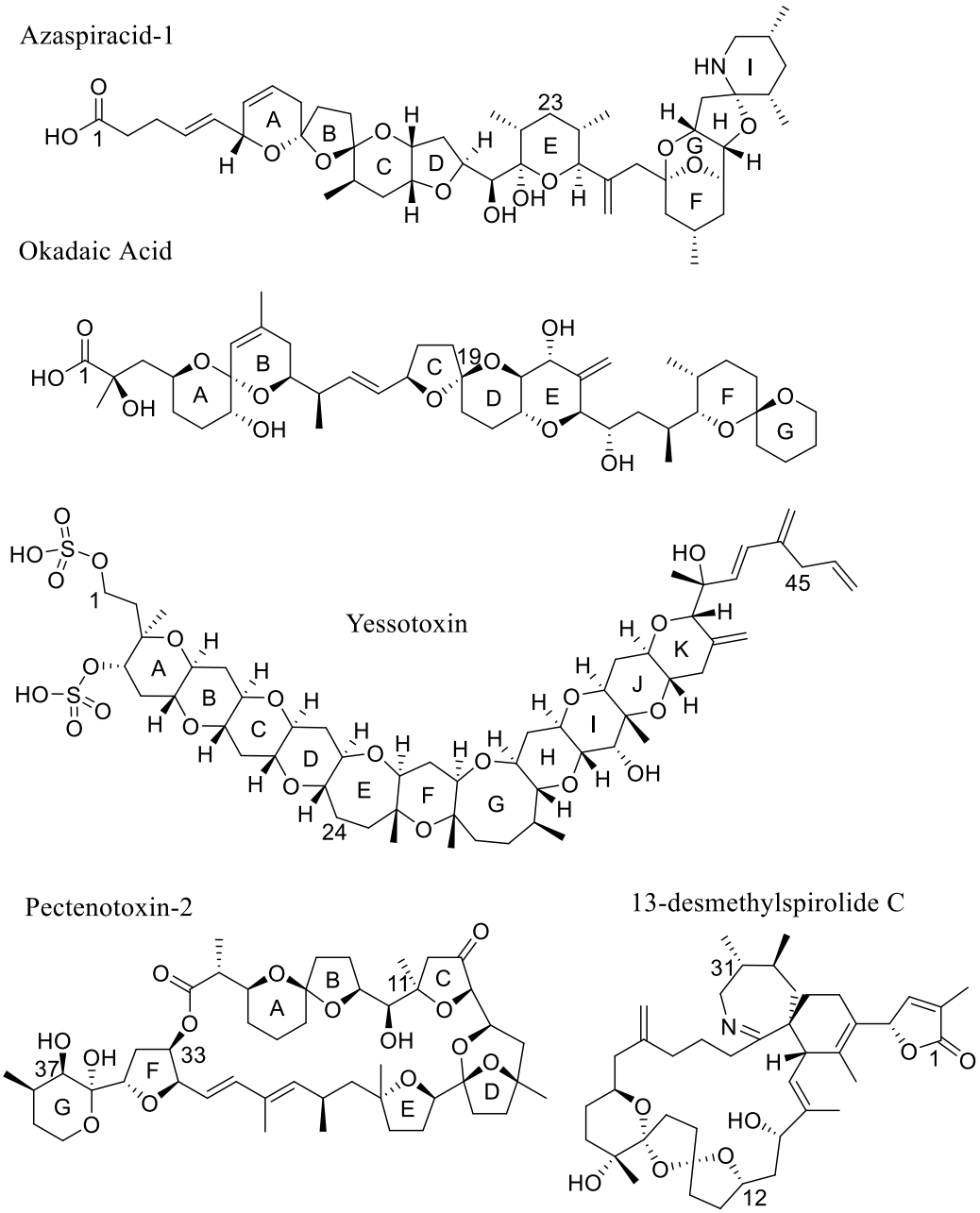

Fig. 1 Structures of selected toxin analogues in CRM-FDMT1 with selected carbon positions and rings labelled for reference with text discussions.

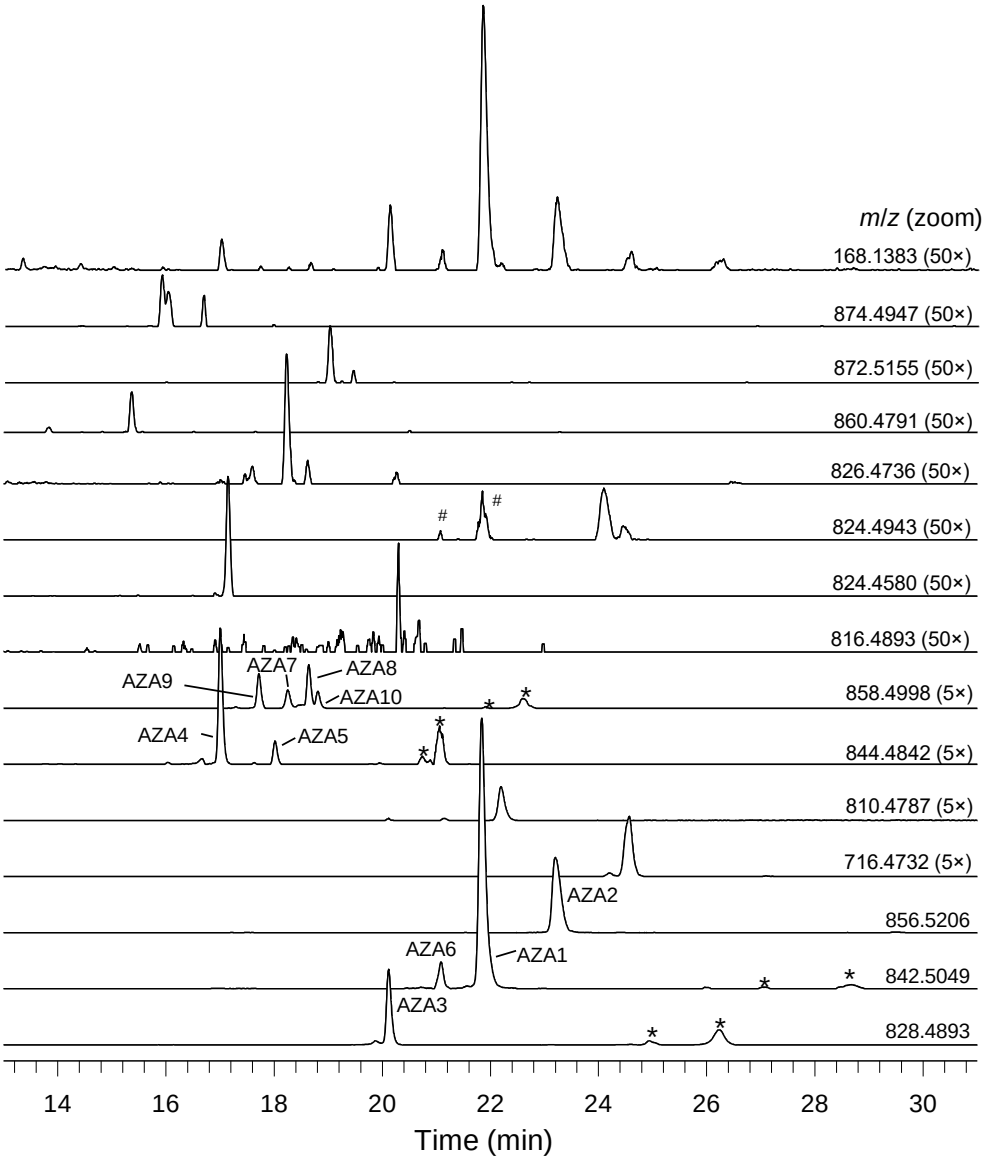

Fig. 2 Extracted positive ion chromatograms (± 5 ppm) of selected AZAs identified in CRM-FDMT1 and (top) AIF chromatogram extracted at *m*/*z* 168.1383 showing the overall AZA profile. Isomers resulting from thermal sterilization are marked with an asterisks. Peaks marked with an # are products of in-source collision-induced water losses. Refer to Table 1 for MS/MS information on peaks shown.

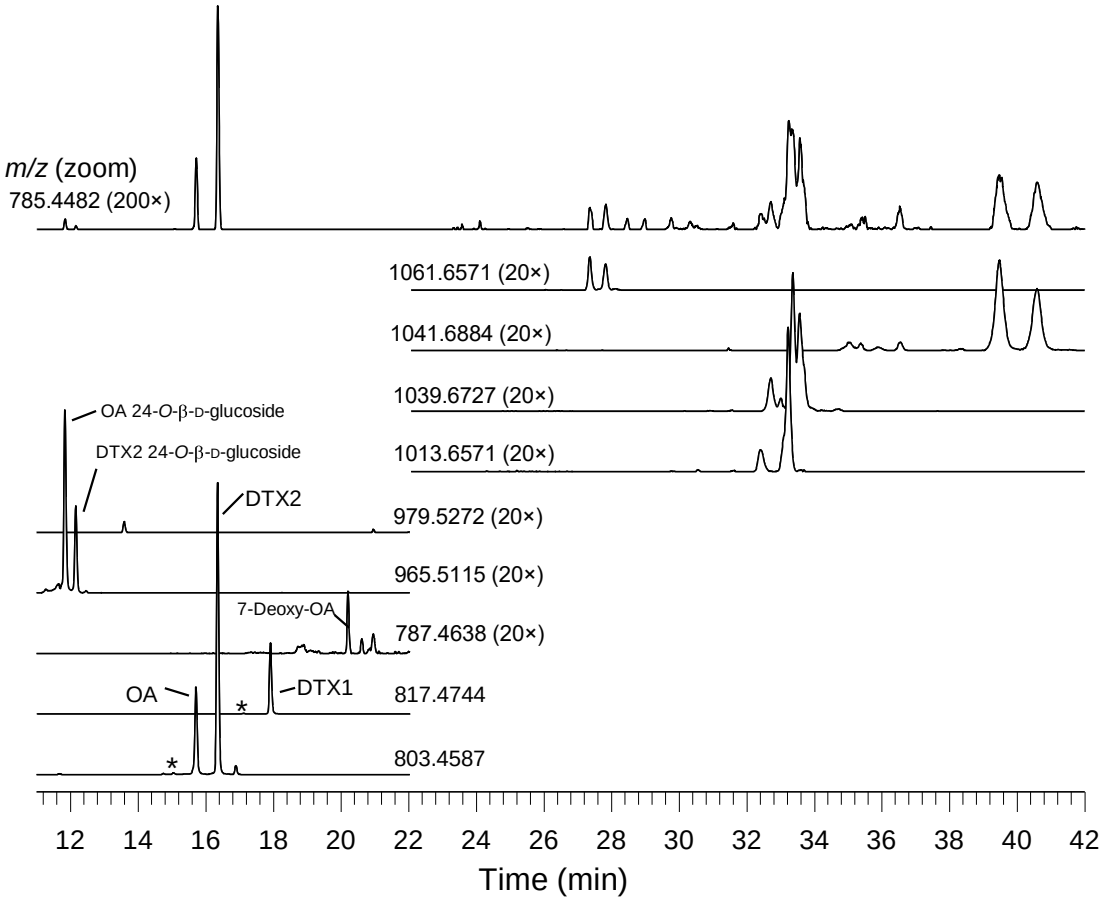

Fig. 3 Extracted negative ion chromatograms (± 5 ppm) of selected OA/DTXs identified in CRM-FDMT1 and (top) AIF chromatogram extracted at *m*/*z* 785.4482 showing the major OA/DTX2 profile respectively. 19-*epi*-analogues elute at times marked with an asterisk. Refer to Table 1 for MS/MS information on peaks shown.

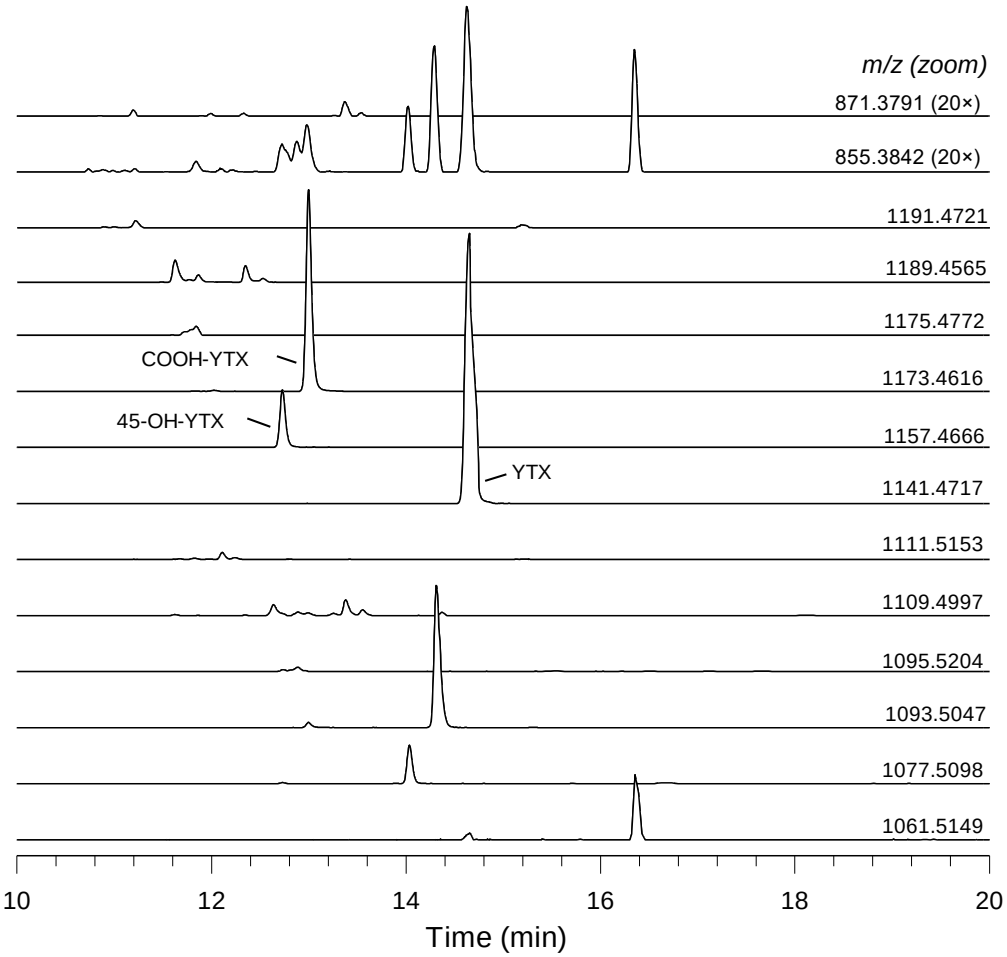

Fig. 4 Extracted negative ion chromatograms (± 5 ppm) of selected YTXs identified in CRM-FDMT1 and (top) two AIF chromatograms extracted at *m*/*z* 871.3791 and 855.3842 showing the major YTX profile. Refer to Table 1 for MS/MS information on peaks shown.

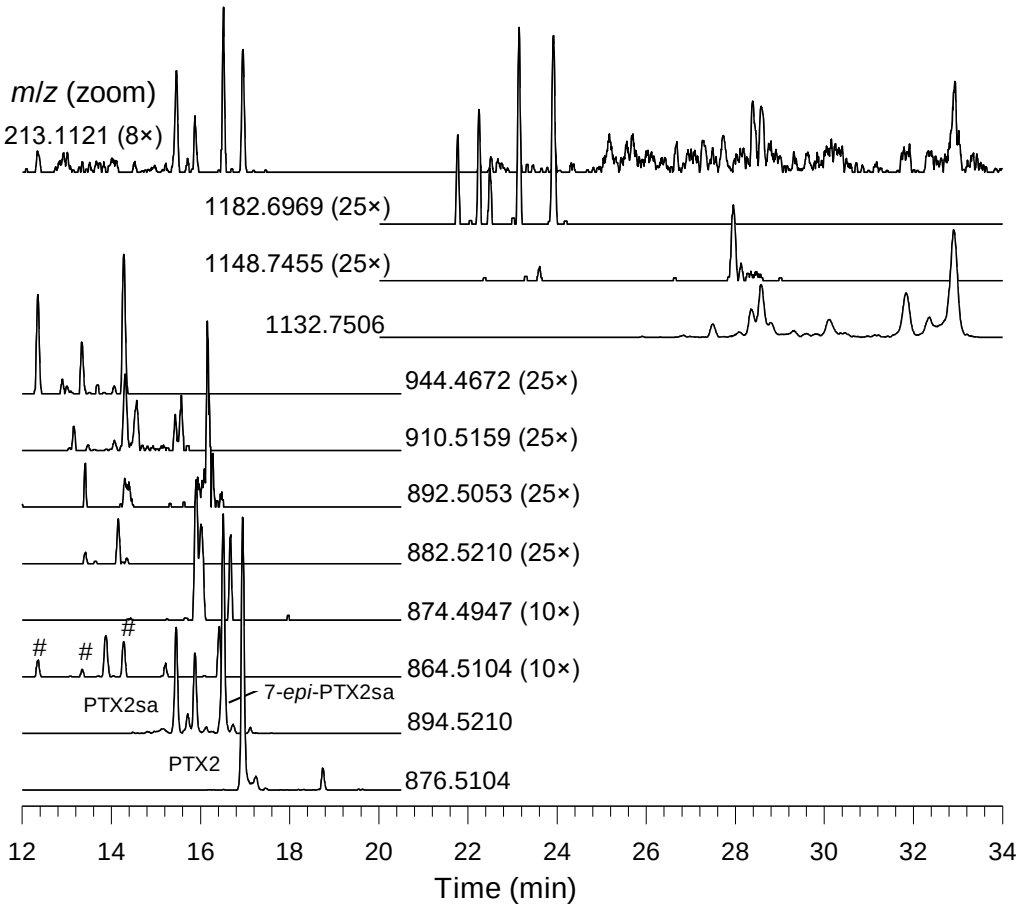

Fig. 5 Extracted positive ion chromatograms (± 5 ppm) of selected PTXs identified in CRM-FDMT1 and (top) AIF chromatogram extracted at *m*/*z* 213.1121 showing the PTX profile in the tissue. Peaks marked with # are from in-source collision-induced SO_3 losses. Refer to Table 1 for MS/MS information on peaks shown.

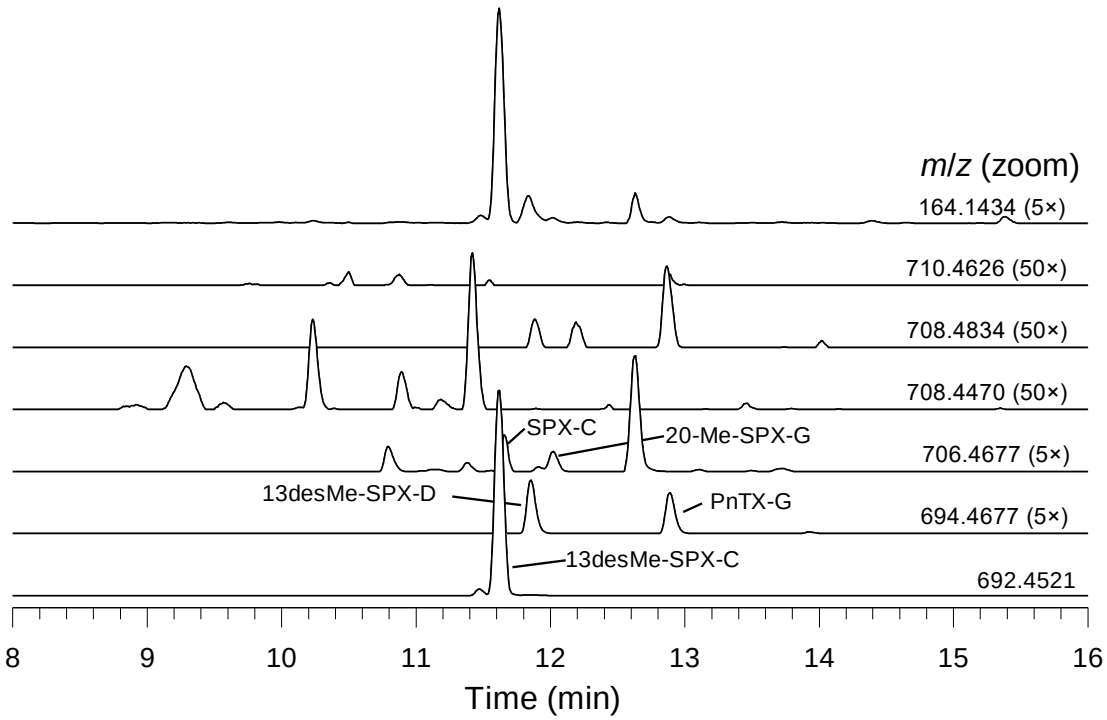

Fig. 6 Extracted positive ion chromatograms (± 5 ppm) of selected cyclic imines identified in CRM-FDMT1 and (top) AIF chromatogram extracted at *m*/*z* 164.1434 included to demonstrate major cyclic imine analogues in the material. Refer to Table 1 for MS/MS information on peaks shown.

Table 1. Summary of toxin analogues detected in CRM-FDMT1. Accurate mass values for adducts specified were verified with two additional isotopic peaks (Δ < 5 ppm). Product ion formulae are shown with accurate masses (Δ < 5 ppm from the exact mass). Identities in bold are for toxins confirmed with external standards while those in italics are tentative based on fragmentation and relative retention times. Unknowns were not assigned tentative IDs.

Entry	Identity	Neutral Formula	Adduct	*m/z*	Retention Time (min)	Retention Index	Product ion formula	*m/z*	Product ion formula	*m/z*	Product ion formula	*m/z*	Product ion formula	*m/z*
1		$C_{33}H_{53}NO_9$	$[M+H]^+$	608.3792	16.98	1426			$C_{27}H_{44}NO_5^+$	462.3209	$C_{22}H_{36}NO_3^+$	362.2690	$C_{10}H_{18}NO^+$	168.1384
2		$C_{33}H_{53}NO_{10}$	$[M+H]^+$	624.3752	15.00	1244			$C_{27}H_{44}NO_6^+$	478.3164	$C_{22}H_{36}NO_3^+$	362.2688	$C_{10}H_{18}NO^+$	168.1385
3	*AZA33 isomer*	$C_{41}H_{65}NO_9$	$[M+H]^+$	716.4747	24.20	>2000			$C_{27}H_{44}NO_5^+$	462.3203	$C_{22}H_{36}NO_3^+$	362.2681	$C_{10}H_{18}NO^+$	168.1379
4	**AZA33**	$C_{41}H_{65}NO_9$	$[M+H]^+$	716.4744	24.56	>2000			$C_{27}H_{44}NO_5^+$	462.3204	$C_{22}H_{36}NO_3^+$	362.2681	$C_{10}H_{18}NO^+$	168.1379
5	*AZA25 isomer*	$C_{46}H_{67}NO_{11}$	$[M+H]^+$	810.4802	21.14	1810	$C_{37}H_{56}NO_9^+$	658.3928	$C_{26}H_{42}NO_5^+$	448.3049	$C_{22}H_{36}NO_3^+$	362.2681	$C_{10}H_{18}NO^+$	168.1377
6	*AZA25*	$C_{46}H_{67}NO_{11}$	$[M+H]^+$	810.4807	22.19	1907	$C_{37}H_{56}NO_9^+$	658.3930	$C_{26}H_{42}NO_5^+$	448.3047	$C_{22}H_{36}NO_3^+$	362.2679	$C_{10}H_{18}NO^+$	168.1379
7	**AZA39**	$C_{45}H_{69}NO_{12}$	$[M+H]^+$	816.4906	20.28	1731			$C_{26}H_{42}NO_5^+$	448.3047	$C_{21}H_{34}NO_3^+$	348.2524	$C_9H_{16}NO^+$	154.1223
8	**AZA26**	$C_{46}H_{65}NO_{12}$	$[M+H]^+$	824.4584	17.15	1442	$C_{37}H_{54}NO_{10}^+$	672.3723	$C_{26}H_{38}NO_5^+$	444.2749	$C_{22}H_{36}NO_3^+$	362.2680	$C_{10}H_{18}NO^+$	168.1380
9	*dehydroAZA6*	$C_{47}H_{69}NO_{11}$	$[M+H]^+$	824.4972	24.10	>2000	$C_{37}H_{56}NO_9^+$	658.3935	$C_{26}H_{42}NO_5^+$	448.3052	$C_{22}H_{36}NO_3^+$	362.2679	$C_{10}H_{18}NO^+$	168.1379
10	*dehydroAZA1*	$C_{47}H_{69}NO_{11}$	$[M+H]^+$	824.4973	24.47	>2000	$C_{38}H_{58}NO_9^+$	672.4090	$C_{27}H_{44}NO_5^+$	462.3213	$C_{22}H_{36}NO_3^+$	362.2678	$C_{10}H_{18}NO^+$	168.1378
11	*AZA48*	$C_{46}H_{67}NO_{12}$	$[M+H]^+$	826.4740	18.23	1542	$C_{37}H_{56}NO_9^+$	658.3953	$C_{26}H_{42}NO_5^+$	448.3057	$C_{22}H_{36}NO_3^+$	362.2689	$C_{10}H_{18}NO^+$	168.1386
12	*early AZA3 isomer*	$C_{46}H_{69}NO_{12}$	$[M+H]^+$	828.4907	19.87	1693	$C_{37}H_{56}NO_9^+$	658.3928	$C_{26}H_{42}NO_5^+$	448.3046	$C_{22}H_{36}NO_3^+$	362.2679	$C_{10}H_{18}NO^+$	168.1378
13	**AZA3**	$C_{46}H_{69}NO_{12}$	$[M+H]^+$	828.4907	20.11	1715	$C_{37}H_{56}NO_9^+$	658.3930	$C_{26}H_{42}NO_5^+$	448.3045	$C_{22}H_{36}NO_3^+$	362.2679	$C_{10}H_{18}NO^+$	168.1379
14	*AZA3 isomer*	$C_{46}H_{69}NO_{12}$	$[M+H]^+$	828.4917	23.08	1989	$C_{37}H_{56}NO_9^+$	658.3933	$C_{26}H_{42}NO_5^+$	448.3042	$C_{22}H_{36}NO_3^+$	362.2681	$C_{10}H_{18}NO^+$	168.1378
15	*AZA3 isomer*	$C_{46}H_{69}NO_{12}$	$[M+H]^+$	828.4917	24.61	>2000	$C_{37}H_{56}NO_9^+$	658.3931	$C_{26}H_{42}NO_5^+$		$C_{22}H_{36}NO_3^+$	362.2678	$C_{10}H_{18}NO^+$	168.1380
16	*AZA3 isomer*	$C_{46}H_{69}NO_{12}$	$[M+H]^+$	828.4913	24.94	>2000	$C_{37}H_{56}NO_9^+$	658.3937	$C_{26}H_{42}NO_5^+$	448.3050	$C_{22}H_{36}NO_3^+$	362.2678	$C_{10}H_{18}NO^+$	168.1379

17	*AZA3 isomer*	$C_{46}H_{69}NO_{12}$	$[M+H]^+$	828.4911	26.24	>2000	$C_{37}H_{56}NO_9^+$	658.3935	$C_{26}H_{42}NO_5^+$		$C_{22}H_{36}NO_3^+$	362.2680	$C_{10}H_{18}NO^+$	168.1379
18	*AZA6 isomer*	$C_{47}H_{71}NO_{12}$	$[M+H]^+$	842.5068	20.71	1770	$C_{37}H_{56}NO_9^+$	658.3935	$C_{26}H_{42}NO_5^+$	448.3064	$C_{22}H_{36}NO_3^+$	362.2680	$C_{10}H_{18}NO^+$	168.1379
19	**AZA6**	$C_{47}H_{71}NO_{12}$	$[M+H]^+$	842.5068	21.09	1805	$C_{37}H_{56}NO_9^+$	658.3933	$C_{26}H_{42}NO_5^+$	448.3046	$C_{22}H_{36}NO_3^+$	362.2678	$C_{10}H_{18}NO^+$	168.1379
20	**AZA1**	$C_{47}H_{71}NO_{12}$	$[M+H]^+$	842.5060	21.83	1873	$C_{38}H_{58}NO_9^+$	672.4092	$C_{27}H_{44}NO_5^+$	462.3201	$C_{22}H_{36}NO_3^+$	362.2680	$C_{10}H_{18}NO^+$	168.1379
21	*AZA1 isomer*	$C_{47}H_{71}NO_{12}$	$[M+H]^+$	842.5071	25.95	>2000	$C_{38}H_{58}NO_9^+$	672.4098	$C_{27}H_{44}NO_5^+$	462.3210	$C_{22}H_{36}NO_3^+$	362.2678	$C_{10}H_{18}NO^+$	168.1381
22	*AZA6 isomer*	$C_{47}H_{71}NO_{12}$	$[M+H]^+$	842.5063	27.08	>2000	$C_{37}H_{56}NO_9^+$	658.3942	$C_{26}H_{42}NO_5^+$	448.3046	$C_{22}H_{36}NO_3^+$	362.2678	$C_{10}H_{18}NO^+$	168.1379
23	*AZA6 isomer*	$C_{47}H_{71}NO_{12}$	$[M+H]^+$	842.5068	28.67	>2000	$C_{37}H_{56}NO_9^+$	658.3937	$C_{26}H_{42}NO_5^+$		$C_{22}H_{36}NO_3^+$	362.2678	$C_{10}H_{18}NO^+$	168.1379
24	*AZA4 isomer*	$C_{46}H_{69}NO_{13}$	$[M+H]^+$	844.4858	16.66	1397	$C_{37}H_{56}NO_9^+$	658.3944	$C_{26}H_{42}NO_5^+$	448.3044	$C_{22}H_{36}NO_3^+$	362.2679	$C_{10}H_{18}NO^+$	168.1380
25	**AZA4**	$C_{46}H_{69}NO_{13}$	$[M+H]^+$	844.4845	17.00	1428	$C_{37}H_{56}NO_9^+$	658.3932	$C_{26}H_{42}NO_5^+$	448.3048	$C_{22}H_{36}NO_3^+$	362.2680	$C_{10}H_{18}NO^+$	168.1379
26[a]	*AZA5 isomer*	$C_{46}H_{69}NO_{13}$	$[M+H]^+$	844.4864	17.63	1486	$C_{37}H_{56}NO_{10}^+$	674.3862	$C26H42NO_6^+$	464.2965	$C_{22}H_{36}NO_3^+$	362.2678	$C_{10}H_{18}NO^+$	168.1380
27[a]	**AZA5**	$C_{46}H_{69}NO_{13}$	$[M+H]^+$	844.4860	18.03	1523	$C_{37}H_{56}NO_{10}^+$	674.3881	$C26H42NO_6^+$	464.2998	$C_{22}H_{36}NO_3^+$	362.2679	$C_{10}H_{18}NO^+$	168.1379
28	*AZA4 isomer*	$C_{46}H_{69}NO_{13}$	$[M+H]^+$	844.4859	20.74	1773	$C_{37}H_{56}NO_9^+$	658.3933	$C_{26}H_{42}NO_5^+$	448.3009	$C_{22}H_{36}NO_3^+$	362.2680	$C_{10}H_{18}NO^+$	168.1379
29	*AZA4 isomer*	$C_{46}H_{69}NO_{13}$	$[M+H]^+$	844.4863	21.07	1803	$C_{37}H_{56}NO_9^+$	658.3917	$C26H42NO_6^+$		$C_{22}H_{36}NO_3^+$	362.2679	$C_{10}H_{18}NO^+$	168.1378
30	**AZA2**	$C_{48}H_{73}NO_{12}$	$[M+H]^+$	856.5223	23.19	1999	$C_{38}H_{58}NO_9^+$	672.4111	$C_{27}H_{44}NO_5^+$	462.3217	$C_{22}H_{36}NO_3^+$	362.2689	$C_{10}H_{18}NO^+$	168.1383
31	*AZA2 isomer*	$C_{48}H_{73}NO_{12}$	$[M+H]^+$	856.5236	24.41	>2000	$C_{38}H_{58}NO_9^+$	672.4111	$C_{27}H_{44}NO_5^+$	462.3213	$C_{22}H_{36}NO_3^+$	362.2687	$C_{10}H_{18}NO^+$	168.1382
32	*AZA2 isomer*	$C_{48}H_{73}NO_{12}$	$[M+H]^+$	856.5233	29.52	>2000	$C_{38}H_{58}NO_9^+$	672.4107	$C_{27}H_{44}NO_5^+$	462.3212	$C_{22}H_{36}NO_3^+$	362.2687	$C_{10}H_{18}NO^+$	168.1382
33	*AZA9 isomer*	$C_{47}H_{71}NO_{13}$	$[M+H]^+$	858.5007	17.28	1454	$C_{37}H_{56}NO_9^+$	658.3930	$C_{26}H_{42}NO_5^+$	448.3035	$C_{22}H_{36}NO_3^+$	362.2683	$C_{10}H_{18}NO^+$	168.1380
34	**AZA9**	$C_{47}H_{71}NO_{13}$	$[M+H]^+$	858.5012	17.72	1495	$C_{37}H_{56}NO_9^+$	658.3939	$C_{26}H_{42}NO_5^+$	448.3049	$C_{22}H_{36}NO_3^+$	362.2681	$C_{10}H_{18}NO^+$	168.1380
35	**AZA7**	$C_{47}H_{71}NO_{13}$	$[M+H]^+$	858.5001	18.26	1544	$C_{38}H_{58}NO_9^+$	672.4089	$C_{27}H_{44}NO_5^+$	462.3203	$C_{22}H_{36}NO_3^+$	362.2679	$C_{10}H_{18}NO^+$	168.1379
36		$C_{47}H_{71}NO_{13}$	$[M+H]^+$	858.5011	18.50	1567	$C_{37}H_{56}NO_9^+$				$C_{22}H_{36}NO_3^+$	362.2680	$C_{10}H_{18}NO^+$	168.1379
37[a]	**AZA8**	$C_{47}H_{71}NO_{13}$	$[M+H]^+$	858.5012	18.63	1579	$C_{38}H_{58}NO_{10}^+$	688.4035	$C_{27}H_{44}NO_6^+$	478.3152	$C_{22}H_{36}NO_3^+$	362.2680	$C_{10}H_{18}NO^+$	168.1381
38[a]	**AZA10**	$C_{47}H_{71}NO_{13}$	$[M+H]^+$	858.5012	18.80	1594	$C_{37}H_{56}NO_{10}^+$	674.3879	$C_{26}H_{40}NO_5^+$	446.2892	$C_{22}H_{36}NO_3^+$	362.2681	$C_{10}H_{18}NO^+$	168.1380
39	*AZA9 isomer*	$C_{47}H_{71}NO_{13}$	$[M+H]^+$	858.4998	21.92	1882	$C_{37}H_{56}NO_9^+$	658.3931	$C_{26}H_{42}NO_5^+$	448.3050	$C_{22}H_{36}NO_3^+$	362.2683	$C_{10}H_{18}NO^+$	168.1380
40	*AZA9 isomer*	$C_{47}H_{71}NO_{13}$	$[M+H]^+$	858.5028	22.61	1945	$C_{37}H_{56}NO_9^+$	658.3938	$C_{26}H_{42}NO_5^+$		$C_{22}H_{36}NO_3^+$	362.2679	$C_{10}H_{18}NO^+$	168.1379
41[a]	*AZA13*	$C_{46}H_{69}NO_{14}$	$[M+H]^+$	860.4816	15.37	1278	$C_{37}H_{56}NO_{10}^+$	674.3878	$C_{26}H_{40}NO_5^+$	446.2892	$C_{22}H_{36}NO_3^+$	362.2678	$C_{10}H_{18}NO^+$	168.1380
42		$C_{47}H_{69}NO_{14}$	$[M+H]^+$	872.4807	14.83	1253			$C_{25}H_{40}NO_5^+$	434.2892			$C_{10}H_{18}NO^+$	168.1379
43[b]		$C_{47}H_{69}NO_{14}$	$[M+H]^+$	872.4799	15.73	1342			$C_{27}H_{42}NO_7^+$	492.2943	$C_{22}H_{34}NO_5^+$	392.2420	$C_{10}H_{18}NO^+$	168.1381

44	*AZA11*	$C_{48}H_{73}NO_{13}$	$[M+H]^+$	872.5164	19.00	1613	$C_{38}H_{58}NO_9^+$	672.4093	$C_{27}H_{44}NO_5^+$	462.3202	$C_{22}H_{36}NO_3^+$	362.2683	$C_{10}H_{18}NO^+$	168.1379
45[a]	*AZA12*	$C_{48}H_{73}NO_{13}$	$[M+H]^+$	872.5178	19.44	1653	$C_{38}H_{58}NO_{10}^+$	688.4040	$C_{27}H_{44}NO_6^+$	478.3159	$C_{22}H_{36}NO_3^+$	362.2679	$C_{10}H_{18}NO^+$	168.1380
46[a]	*AZA14*	$C_{47}H_{71}NO_{14}$	$[M+H]^+$	874.4965	15.89	1326	$C_{38}H_{58}NO_{10}^+$	688.4043	$C_{27}H_{44}NO_6^+$	478.3154	$C_{22}H_{36}NO_3^+$	362.2680	$C_{10}H_{18}NO^+$	168.1382
47	*AZA15*	$C_{47}H_{71}NO_{14}$	$[M+H]^+$	874.4952	16.01	1337	$C_{37}H_{56}NO_{10}^+$	674.3884	$C_{26}H_{42}NO_6^+$	464.2989	$C_{22}H_{36}NO_3^+$	362.2683	$C_{10}H_{18}NO^+$	168.1380
48[a]		$C_{47}H_{71}NO_{14}$	$[M+H]^+$	874.4971	16.67	1398	$C_{38}H_{58}NO_{11}^+$	704.3976	$C_{26}H_{42}NO_6^+$	446.2882	$C_{22}H_{36}NO_3^+$	362.2676	$C_{10}H_{18}NO^+$	168.1380
49	**7-Deoxy-OA**	$C_{44}H_{68}O_{12}$	$[M-H]^-$	787.4649	20.17	1720	$C_{30}H_{43}O_9^-$	547.2932	$C_{19}H_{29}O_4^-$	321.2062	$C_{13}H_{19}O_4^-$	239.1291	$C_6H_9O^-$	97.0659
50	*7-Deoxy-DTX2*	$C_{44}H_{68}O_{12}$	$[M-H]^-$	787.4648	20.59	1759			$C_{19}H_{29}O_4^-$	321.2062	$C_{13}H_{19}O_4^-$	239.1291	$C_6H_9O^-$	97.0658
51	*OA/DTX2 isomer*	$C_{44}H_{68}O_{13}$	$[M-H]^-$	803.4592	14.78	1224	$C_{30}H_{43}O_{10}^-$	563.2844			$C_{13}H_{19}O_5^-$	255.1238	$C_6H_9O_2^-$	113.0608
52	*19-epi-OA*	$C_{44}H_{68}O_{13}$	$[M-H]^-$	803.4591	15.07	1250			$C_{19}H_{29}O_4^-$	321.2066	$C_{13}H_{19}O_5^-$	255.1239	$C_6H_9O_2^-$	113.0608
53	**OA**	$C_{44}H_{68}O_{13}$	$[M-H]^-$	803.4585	15.73	1311	$C_{30}H_{43}O_{10}^-$	563.2873	$C_{19}H_{29}O_4^-$	321.2069	$C_{13}H_{19}O_5^-$	255.1239	$C_6H_9O_2^-$	113.0609
54	**DTX2**	$C_{44}H_{68}O_{13}$	$[M-H]^-$	803.4587	16.36	1369	$C_{30}H_{43}O_{10}^-$	563.2863	$C_{19}H_{29}O_4^-$	321.2075	$C_{13}H_{19}O_5^-$	255.1239	$C_6H_9O_2^-$	113.0608
55	*OA/DTX2 isomer*	$C_{44}H_{68}O_{13}$	$[M-H]^-$	803.4589	16.92	1421	$C_{30}H_{43}O_{10}^-$	563.2877	$C_{19}H_{29}O_4^-$	321.2072	$C_{13}H_{19}O_5^-$	255.1239	$C_6H_9O_2^-$	113.0608
56[b]	*19-epi-DTX1*	$C_{45}H_{70}O_{13}$	$[M-H]^-$	817.4738	17.19	1446					$C_{13}H_{19}O_5^-$	255.1243		
57	**DTX1**	$C_{45}H_{70}O_{13}$	$[M-H]^-$	817.4746	17.91	1512	$C_{30}H_{43}O_{10}^-$	563.2863	$C_{20}H_{32}O_4^-$	335.2228	$C_{13}H_{19}O_5^-$	255.1238	$C_6H_9O_2^-$	113.0608
58	**OA 24-*O*-β-D-glucoside**	$C_{50}H_{78}O_{18}$	$[M-H]^-$	965.5130	11.84	953	$C_{36}H_{53}O_{15}^-$	725.3401	$C_{19}H_{29}O_4^-$	321.2074	$C_{13}H_{19}O_5^-$	255.1240	$C_6H_9O_2^-$	113.0608
59	**DTX2 24-*O*-β-D-glucoside**	$C_{50}H_{78}O_{18}$	$[M-H]^-$	965.5134	12.16	982	$C_{36}H_{53}O_{15}^-$	725.3388	$C_{19}H_{29}O_4^-$	321.2074	$C_{13}H_{19}O_5^-$	255.1239	$C_6H_9O_2^-$	113.0608
60	*DTX1 24-O-β-D-glucoside*	$C_{51}H_{80}O_{18}$	$[M-H]^-$	979.5273	13.60	1115					$C_{13}H_{19}O_5^-$	255.1246	$C_6H_9O_2^-$	113.0609
61[c]	**7-*O*-palmitoyl OA**	$C_{60}H_{98}O_{14}$	$[M-H]^-$	1041.6887	38.92	>2000	$C_{44}H_{65}O_{12}^-$ [d]	785.4488[d]	$C_{29}H_{49}O_6^-$	493.3531	$C_{13}H_{19}O_5^-$	255.1235	$C_{16}H_{31}O_2^-$	255.2327
62	*desulfoYTX*	$C_{55}H_{82}O_{18}S$	$[M-H]^-$	1061.5147	16.35	1368			$C_{46}H_{69}O_{17}S^-$	925.4249	$C_{42}H_{63}O_{16}S^-$	855.3833	$C_{35}H_{53}O_{13}S^-$	713.3203
63	*OH-desulfoYTX*	$C_{55}H_{82}O_{19}S$	$[M-H]^-$	1077.5101	14.03	1155			$C_{46}H_{69}O_{17}S^-$	925.4257	$C_{42}H_{63}O_{16}S^-$	855.3850	$C_{35}H_{53}O_{13}S^-$	713.3221
64	*COOH-desulfoYTX*	$C_{55}H_{82}O_{20}S$	$[M-H]^-$	1093.5049	14.31	1180			$C_{46}H_{69}O_{17}S^-$	925.4276	$C_{42}H_{63}O_{16}S^-$	855.3846	$C_{35}H_{53}O_{13}S^-$	713.3217
65	*diOH-desulfoYTX*	$C_{55}H_{84}O_{20}S$	$[M-H]^-$	1095.5215	12.89	1050			$C_{46}H_{69}O_{17}S^-$	925.4284	$C_{42}H_{63}O_{16}S^-$	855.3848	$C_{35}H_{53}O_{13}S^-$	713.3243
66	*COOH-OH-desulfoYTX*	$C_{55}H_{82}O_{21}S$	$[M-H]^-$	1109.5016	12.63	1026			$C_{46}H_{69}O_{17}S^-$	925.4271	$C_{42}H_{63}O_{16}S^-$	855.3868	$C_{35}H_{53}O_{13}S^-$	713.3234
67	*COOH-OH-desulfoYTX*	$C_{55}H_{82}O_{21}S$	$[M-H]^-$	1109.5009	12.89	1050			$C_{46}H_{69}O_{17}S^-$	925.4247	$C_{42}H_{63}O_{16}S^-$	855.3857	$C_{35}H_{53}O_{13}S^-$	713.3179
68	*COOH-OH-desulfoYTX*	$C_{55}H_{82}O_{21}S$	$[M-H]^-$	1109.5010	12.99	1059			$C_{46}H_{69}O_{17}S^-$	925.4295	$C_{42}H_{63}O_{16}S^-$	855.3866		
69	*COOH-OH-desulfoYTX*	$C_{55}H_{82}O_{21}S$	$[M-H]^-$	1109.4995	13.25	1083					$C_{42}H_{63}O_{17}S^-$	871.3785	$C_{35}H_{53}O_{14}S^-$	729.3169
70	*COOH-OH-desulfoYTX*	$C_{55}H_{82}O_{21}S$	$[M-H]^-$	1109.5012	13.38	1095			$C_{46}H_{69}O_{18}S^-$	941.4202	$C_{42}H_{63}O_{17}S^-$	871.3790	$C_{35}H_{53}O_{14}S^-$	729.3172
71	*COOH-OH-desulfoYTX*	$C_{55}H_{82}O_{21}S$	$[M-H]^-$	1109.5001	13.55	1110			$C_{46}H_{69}O_{18}S^-$	941.4231	$C_{42}H_{63}O_{17}S^-$	871.3789	$C_{35}H_{53}O_{14}S^-$	729.3178

72		$C_{55}H_{84}O_{21}S$	$[M-H]^-$	1111.5175	12.11	978			$C_{46}H_{69}O_{17}S^-$	925.4225	$C_{42}H_{63}O_{16}S^-$	855.3862	$C_{35}H_{53}O_{13}S^-$	713.3225
73		$C_{55}H_{84}O_{21}S$	$[M-H]^-$	1111.5158	12.24	990					$C_{42}H_{63}O_{16}S^-$	855.3853	$C_{35}H_{53}O_{13}S^-$	713.3224
74	**Yessotoxin**	$C_{55}H_{82}O_{21}S_2$	$[M-H]^-$	1141.4710	14.65	1212	$C_{55}H_{81}O_{18}S^-$	1061.5119	$C_{46}H_{69}O_{17}S^-$	925.4246	$C_{42}H_{63}O_{16}S^-$	855.3831	$C_{35}H_{53}O_{13}S^-$	713.3202
75	**45-OH-YTX**	$C_{55}H_{82}O_{22}S_2$	$[M-H]^-$	1157.4679	12.73	1035	$C_{55}H_{81}O_{19}S^-$	1077.5088	$C_{46}H_{69}O_{17}S^-$	925.4251	$C_{42}H_{63}O_{16}S^-$	855.3830	$C_{35}H_{53}O_{13}S^-$	713.3207
76	*COOH-YTX*	$C_{55}H_{82}O_{23}S_2$	$[M-H]^-$	1173.4625	13.00	1060	$C_{55}H_{81}O_{20}S^-$	1093.5031	$C_{46}H_{69}O_{17}S^-$	925.4245	$C_{42}H_{63}O_{16}S^-$	855.3832	$C_{35}H_{53}O_{13}S^-$	713.3206
77	*diOH-YTX*	$C_{55}H_{84}O_{23}S_2$	$[M-H]^-$	1175.4783	11.84	953	$C_{55}H_{83}O_{20}S^-$	1095.5163	$C_{46}H_{69}O_{17}S^-$	925.4245	$C_{42}H_{63}O_{16}S^-$	855.3830	$C_{35}H_{53}O_{13}S^-$	713.3206
78	*COOH-OH-YTX*	$C_{55}H_{82}O_{24}S_2$	$[M-H]^-$	1189.4579	11.62	932	$C_{55}H_{81}O_{21}S^-$	1109.4977	$C_{46}H_{69}O_{17}S^-$	925.4244	$C_{42}H_{63}O_{16}S^-$	855.3823	$C_{35}H_{53}O_{13}S^-$	713.3192
79	*COOH-OH-YTX*	$C_{55}H_{82}O_{24}S_2$	$[M-H]^-$	1189.4574	11.77	946	$C_{55}H_{81}O_{21}S^-$	1109.5010	$C_{46}H_{69}O_{17}S^-$	925.4200	$C_{42}H_{63}O_{16}S^-$	855.3827	$C_{35}H_{53}O_{13}S^-$	713.3209
80	*COOH-OH-YTX*	$C_{55}H_{82}O_{24}S_2$	$[M-H]^-$	1189.4570	11.86	955	$C_{55}H_{81}O_{21}S^-$	1109.4985	$C_{46}H_{69}O_{17}S^-$	925.4253	$C_{42}H_{63}O_{16}S^-$	855.3819	$C_{35}H_{53}O_{13}S^-$	713.3213
81	*COOH-OH-YTX*	$C_{55}H_{82}O_{24}S_2$	$[M-H]^-$	1189.4576	12.34	999	$C_{55}H_{81}O_{21}S^-$	1109.4977	$C_{46}H_{69}O_{18}S^-$	941.4194	$C_{42}H_{63}O_{17}S^-$	871.3787	$C_{35}H_{53}O_{14}S^-$	729.3151
82	*COOH-OH-YTX*	$C_{55}H_{82}O_{24}S_2$	$[M-H]^-$	1189.4565	12.53	1016	$C_{55}H_{81}O_{21}S^-$	1109.4971	$C_{46}H_{69}O_{18}S^-$	941.4190	$C_{42}H_{63}O_{17}S^-$	871.3783	$C_{35}H_{53}O_{14}S^-$	729.3149
83		$C_{55}H_{84}O_{24}S_2$	$[M-H]^-$	1191.4734	11.22	896	$C_{55}H_{84}O_{21}S^-$	1111.5138	$C_{46}H_{69}O_{17}S^-$	925.4263	$C_{42}H_{63}O_{16}S^-$	855.3856	$C_{35}H_{53}O_{13}S^-$	713.3239
84[e, f]	*PTX-i*	$C_{46}H_{70}O_{14}$	$[M+NH_4]^+$	864.5131	13.87	1140							$C_{11}H_{17}O_4^+$	213.1118
85[e, f]	*PTX-i*	$C_{46}H_{70}O_{14}$	$[M+NH_4]^+$	864.5120	16.42	1375								
86	*36*S-*PTX12*	$C_{47}H_{68}O_{14}$	$[M+NH_4]^+$	874.4952	16.02	1338	$C_{29}H_{43}O_{10}^+$	551.2834	$C_{28}H_{37}O_4^+$	437.2681	$C_{17}H_{21}O_3^+$	273.1494	$C_{11}H_{17}O_4^+$	213.1116
87	*36*R-*PTX12*	$C_{47}H_{68}O_{14}$	$[M+NH_4]^+$	874.4957	17.97	1518	$C_{29}H_{43}O_{10}^+$	551.2836	$C_{28}H_{39}O_5^+$	455.2778	$C_{17}H_{21}O_3^+$	273.1487	$C_{11}H_{17}O_4^+$	213.1116
88	**PTX2**	$C_{47}H_{70}O_{14}$	$[M+NH_4]^+$	876.5114	16.95	1423	$C_{29}H_{43}O_{10}^+$	551.2837	$C_{28}H_{39}O_4^+$	439.2832	$C_{17}H_{23}O_3^+$	275.1636	$C_{11}H_{17}O_4^+$	213.1117
89	*PTX2 isomer*	$C_{47}H_{70}O_{14}$	$[M+NH_4]^+$	876.5120	17.26	1452	$C_{29}H_{43}O_{10}^+$	551.2843	$C_{28}H_{39}O_4^+$	439.2827	$C_{17}H_{23}O_3^+$	275.1635	$C_{11}H_{17}O_4^+$	213.1115
90	*PTX2 isomer*	$C_{47}H_{70}O_{14}$	$[M+NH_4]^+$	876.5114	18.75	1590	$C_{29}H_{43}O_{10}^+$	551.2840	$C_{28}H_{39}O_4^+$	439.2840	$C_{17}H_{23}O_3^+$	275.1636	$C_{11}H_{17}O_4^+$	213.1116
91		$C_{46}H_{72}O_{15}$	$[M+NH_4]^+$	882.5244	14.15	1166							$C_{11}H_{17}O_4^+$	213.1118
92		$C_{47}H_{70}O_{15}$	$[M+NH_4]^+$	892.5090	13.41	1097	$C_{29}H_{43}O_{10}^+$	551.2828			$C_{17}H_{21}O_3^+$	273.1476	$C_{11}H_{17}O_4^+$	213.1116
93		$C_{47}H_{70}O_{15}$	$[M+NH_4]^+$	892.5082	14.30	1179	$C_{29}H_{43}O_{11}^+$	567.2821			$C_{17}H_{23}O_3^+$	257.1538	$C_{11}H_{17}O_4^+$	213.1112
94	PTX2sa & isomers	$C_{47}H_{72}O_{15}$	$[M+NH_4]^+$	894.5223	15.18	1261	$C_{29}H_{43}O_{10}^+$	551.2840			$C_{17}H_{23}O_3^+$	275.1633	$C_{11}H_{17}O_4^+$	213.1116
95	PTX2sa & isomers	$C_{47}H_{72}O_{15}$	$[M+NH_4]^+$	894.5222	15.45	1285	$C_{29}H_{43}O_{10}^+$	551.2836	$C_{28}H_{39}O_5^+$	455.2779	$C_{17}H_{23}O_3^+$	275.1633	$C_{11}H_{17}O_4^+$	213.1115
96	PTX2sa & isomers	$C_{47}H_{72}O_{15}$	$[M+NH_4]^+$	894.5226	15.71	1309	$C_{29}H_{43}O_{10}^+$	551.2838	$C_{28}H_{39}O_5^+$	455.2785	$C_{17}H_{23}O_3^+$	275.1633	$C_{11}H_{17}O_4^+$	213.1115
97	PTX2sa & isomers	$C_{47}H_{72}O_{15}$	$[M+NH_4]^+$	894.5213	15.87	1324	$C_{29}H_{43}O_{10}^+$	551.2838	$C_{28}H_{39}O_5^+$	455.2782	$C_{17}H_{23}O_3^+$	275.1632	$C_{11}H_{17}O_4^+$	213.1115
98	PTX2sa & isomers	$C_{47}H_{72}O_{15}$	$[M+NH_4]^+$	894.5230	16.11	1346	$C_{29}H_{43}O_{10}^+$	551.2833					$C_{11}H_{17}O_4^+$	213.1118
99	7-*epi*-PTX2sa	$C_{47}H_{72}O_{15}$	$[M+NH_4]^+$	894.5234	16.50	1382	$C_{29}H_{43}O_{10}^+$	551.2839	$C_{28}H_{39}O_5^+$	455.2779	$C_{17}H_{23}O_3^+$	275.1633	$C_{11}H_{17}O_4^+$	213.1116
100	PTX2sa & isomers	$C_{47}H_{72}O_{15}$	$[M+NH_4]^+$	894.5222	16.72	1402	$C_{29}H_{43}O_{10}^+$	551.2849	$C_{28}H_{39}O_5^+$	455.2767	$C_{17}H_{23}O_3^+$	275.1635	$C_{11}H_{17}O_4^+$	213.1117

101	PTX2sa & isomers	$C_{47}H_{72}O_{15}$	$[M+NH_4]^+$	894.5225	17.12	1439	$C_{29}H_{43}O_{10}^+$	551.2840	$C_{28}H_{39}O_5^+$	455.2785	$C_{17}H_{23}O_3$	275.1635	$C_{11}H_{17}O_4^+$	213.1116
102		$C_{47}H_{72}O_{16}$	$[M+NH_4]^+$	910.5194	13.15	1073							$C_{11}H_{17}O_4^+$	213.1115
103		$C_{47}H_{72}O_{16}$	$[M+NH_4]^+$	910.5192	14.30	1179	$C_{29}H_{43}O_{10}^+$	551.2834	$C_{28}H_{39}O_5^+$	455.2775	$C_{17}H_{23}O_4$	291.1586	$C_{11}H_{17}O_4^+$	213.1117
104		$C_{47}H_{72}O_{16}$	$[M+NH_4]^+$	910.5192	14.55	1202	$C_{29}H_{43}O_{10}^+$	551.2833	$C_{28}H_{39}O_5^+$	455.2781	$C_{17}H_{23}O_4$	291.1587	$C_{11}H_{17}O_4^+$	213.1116
105		$C_{47}H_{72}O_{16}$	$[M+NH_4]^+$	910.5171	15.43	1284			$C_{28}H_{39}O_5^+$	455.2771	$C_{17}H_{23}O_4$	291.1576	$C_{11}H_{17}O_4^+$	213.1118
106		$C_{47}H_{72}O_{16}$	$[M+NH_4]^+$	910.5180	15.56	1296			$C_{28}H_{39}O_5^+$	455.2773	$C_{17}H_{23}O_4$	291.1585	$C_{11}H_{17}O_4^+$	213.1115
107[f]	*PTX-i sulfate*	$C_{46}H_{70}O_{17}S$	$[M+NH_4]^+$	944.4680	12.34	999	$C_{29}H_{43}O_{10}S^-$	583.2579					HSO_4^-	96.9601[f]
108[f]	*PTX-i sulfate*	$C_{46}H_{70}O_{17}S$	$[M+NH_4]^+$	944.4705	14.28	1178	$C_{29}H_{43}O_{10}S^-$	583.2592					HSO_4^-	96.9601[f]
109[c]	*O*-palmitoyl *PTX2sa*	$C_{63}H_{102}O_{16}$	$[M+NH_4]^+$	1132.7527	32.91	>2000	$C_{29}H_{43}O_{10}^+$	551.2852	$C_{47}H_{67}O_{12}^{+}$ [d]	823.4628[d]	$C_{16}H_{31}O_2^-$	255.2329	$C_{11}H_{17}O_4^+$	213.1121
110[c]		$C_{63}H_{102}O_{17}$	$[M+NH_4]^+$	1148.7496	27.93	>2000			$C_{47}H_{67}O_{13}^{+}$ [d]	839.4549[d]			$C_{11}H_{17}O_4^+$	213.1116
111[c, f,]	*O*-palmitoyl *PTX-i sulfate*	$C_{62}H_{100}O_{18}S$	$[M+NH_4]^+$	1182.7006	23.92	>2000	$C_{29}H_{43}O_{10}^+$	551.2848	$C_{46}H_{69}O_{13}^{+}$ [d]	829.4731[d]	$C_{16}H_{31}O_2^-$	255.2330	$C_{11}H_{17}O_4^+$	213.1121
112	**13-desMe-SPX C**	$C_{42}H_{61}NO_7$	$[M+H]^+$	692.4523	11.61	932	$C_{27}H_{42}NO_4^+$	444.3108	$C_{23}H_{36}NO^+$	342.2787	$C_{16}H_{24}N^+$	230.1903	$C_{11}H_{18}N^+$	164.1435
113	*13-desMe-SPXC isomer*	$C_{42}H_{61}NO_7$	$[M+H]^+$	692.4528	11.47	919	$C_{27}H_{42}NO_4^+$	444.3109	$C_{23}H_{36}NO^+$	342.2797	$C_{16}H_{24}N^+$	230.1904	$C_{11}H_{18}N^+$	164.1435
114	*13-desMe-SPX D*	$C_{42}H_{63}NO_7$	$[M+H]^+$	694.4681	11.85	954	$C_{27}H_{42}NO_4^+$	444.3108	$C_{23}H_{36}NO^+$	342.2790	$C_{16}H_{24}N^+$	230.1905	$C_{11}H_{18}N^+$	164.1435
115	**PnTX G**	$C_{42}H_{63}NO_7$	$[M+H]^+$	694.4699	12.88	1049	$C_{28}H_{44}NO_4^+$	458.3264	$C_{23}H_{36}NO^+$	342.2800	$C_{16}H_{24}N^+$	230.1904	$C_{11}H_{18}N^+$	164.1435
116	**SPX C**	$C_{43}H_{63}NO_7$	$[M+H]^+$	706.4684	11.66	936	$C_{28}H_{44}NO_4^+$	458.3263	$C_{23}H_{36}NO^+$	342.2791	$C_{16}H_{24}N^+$	230.1903	$C_{11}H_{18}N^+$	164.1434
117	**20-Me-SPX G**	$C_{43}H_{63}NO_7$	$[M+H]^+$	706.4697	12.01	968	$C_{23}H_{38}NO_4^+$	392.2796	$C_{22}H_{36}NO_2^+$	346.2737	$C_{16}H_{24}N^+$	230.1905	$C_{11}H_{18}N^+$	164.1435
118	*SPX C3*	$C_{43}H_{63}NO_7$	$[M+H]^+$	706.4688	12.62	1025	$C_{28}H_{44}NO_4^+$	458.3264	$C_{23}H_{36}NO^+$	342.2791	$C_{16}H_{24}N^+$	230.1903	$C_{11}H_{18}N^+$	164.1435
119		$C_{43}H_{63}NO_7$	$[M+H]^+$	706.4699	13.10	1069	$C_{28}H_{44}NO_4^+$	458.3259					$C_{11}H_{18}N^+$	164.1434
120		$C_{43}H_{63}NO_7$	$[M+H]^+$	706.4697	13.50	1106	$C_{28}H_{44}NO_4^+$	458.3259					$C_{11}H_{18}N^+$	164.1434
121		$C_{43}H_{63}NO_7$	$[M+H]^+$	706.4683	13.72	1126	$C_{28}H_{44}NO_4^+$	458.3265					$C_{11}H_{18}N^+$	164.1435
122		$C_{42}H_{61}NO_8$	$[M+H]^+$	708.4478	10.22	803	$C_{27}H_{42}NO_4^+$	444.3111	$C_{23}H_{36}NO^+$	342.2795	$C_{16}H_{24}N^+$	230.1903	$C_{11}H_{18}N^+$	164.1435
123	*27-hydroxy-13-desMe-SPX C*	$C_{42}H_{61}NO_8$	$[M+H]^+$	708.4480	11.43	915	$C_{27}H_{44}NO_6^+$	478.3166	$C_{23}H_{36}NO_2^+$	358.2740	$C_{16}H_{24}NO^+$	246.1852	$C_{11}H_{18}NO^+$	180.1384
124	*SPX D*	$C_{43}H_{65}NO_7$	$[M+H]^+$	708.4845	11.87	956	$C_{28}H_{44}NO_4^+$	458.3265					$C_{11}H_{18}N^+$	164.1434
125	*20-Me-SPX G (saturated C2-C3)*	$C_{43}H_{65}NO_7$	$[M+H]^+$	708.4840	12.17	983	$C_{23}H_{38}NO_4^+$	392.2793	$C_{22}H_{36}NO_2^+$	346.2741			$C_{11}H_{18}N^+$	164.1434
126	*SPX D3*	$C_{43}H_{65}NO_7$	$[M+H]^+$	708.4863	12.87	1048	$C_{28}H_{44}NO_4^+$	458.3264			$C_{16}H_{24}N^+$	230.1898	$C_{11}H_{18}N^+$	164.1434
127		$C_{42}H_{63}NO_8$	$[M+H]^+$	710.4638	10.89	865	$C_{27}H_{42}NO_4^+$	444.3107					$C_{11}H_{18}N^+$	164.1434
128	*27-hydroxy-13-desMe-SPX D*	$C_{42}H_{63}NO_8$	$[M+H]^+$	710.4638	11.55	926	$C_{27}H_{42}NO_5^+$	460.3056	$C_{23}H_{36}NO_2^+$	358.2734			$C_{11}H_{18}NO^+$	180.1383

Table Footnotes:

a) AZAs confirmed to have *m*/*z* 408.2744 typical of C23-hydroxyAZA analogues.
b) < 3 isotopes observed on main peak but was verified with standards or MS/MS spectra provided (ESM)
c) Most intense *O*-palmitoyl ester shown to demonstrate key fragments observed. Several isomers are observed giving similar fragmentation spectra. Position of esterification not verified unless indicated.
d) Product ion from the unconjugated toxin after ester removal involving one or more water losses, or hydrogen sulfite, ammonia or other neutral loss from precursor toxin analogue
e) MS/MS data either not collected as part of this study, or of insufficient quality to summarize fragmentation
f) Previously reported and referred to as PTX-i analogues [29]