Supplementary Information

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

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Retention Index Measurement using NRC RM-RILC

NRC RM-RILC is a reference material for measurement of liquid chromatography retention indices prepared from a homologous series of *N*-alkylpyridinium-3-sulfonates, which are amenable to LC-MS detection in both positive and negative polarities [1]. RM-RILC was analyzed using positive polarity all-ion-fragmentation experiments (Fig. S1). Using a linear regression of retention index vs. retention time (Fig. S2), the regression equation was applied to the retention times of compounds detected in CRM-FDMT1 (Table 1). Retention time data was collected with two independent experiments on the same LC-HRMS platform, showing < 2.5% variation in calculated retention index values. These retention indices can be used to compare retention of compounds reported in CRM-FDMT1 in this work to suspected similar compounds in CRM-FDMT1 and others samples in separte experiments, or by different users employing similar chromatographic conditions. Work is in progress in our laboratory to evaluate robustness of retention index values. The retention time values for RM-RILC (Fig. S1) and all the toxin analogues detected in this work (Table 1) are provided to assist with determination of retention index values using alternative models.

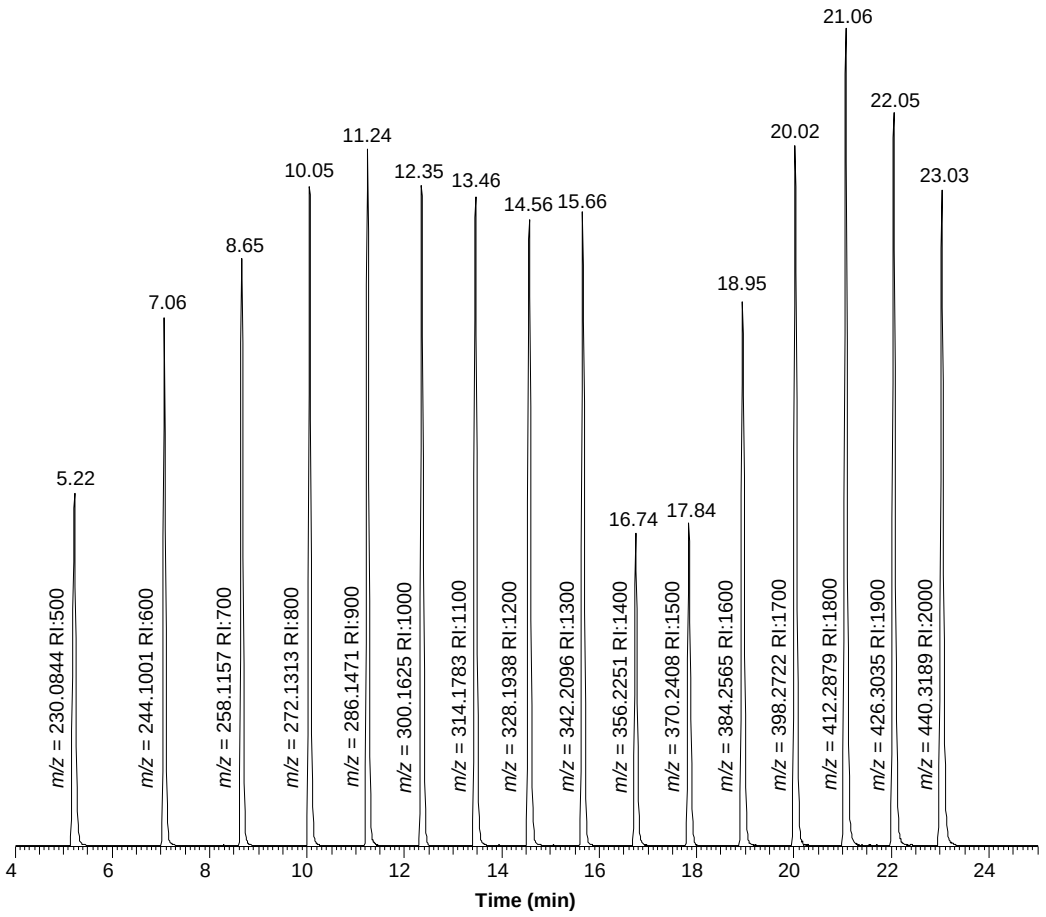

Fig. S1 Positive mode LC-HRMS extracted ion chromatogram of *N*-alkylpyridinium-3-sulfonates (NAPS) in RM-RILC retention index standard.

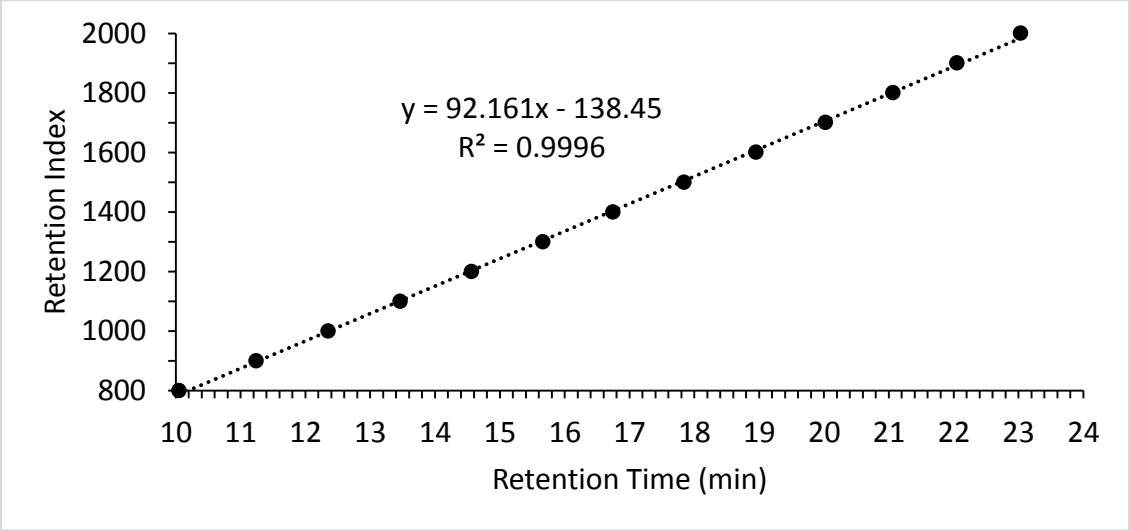

Fig. S2 Least squares regression for retention index value vs. retention time to calculate a linear regression equation for experimental determination of retention indices using RM-RILC.

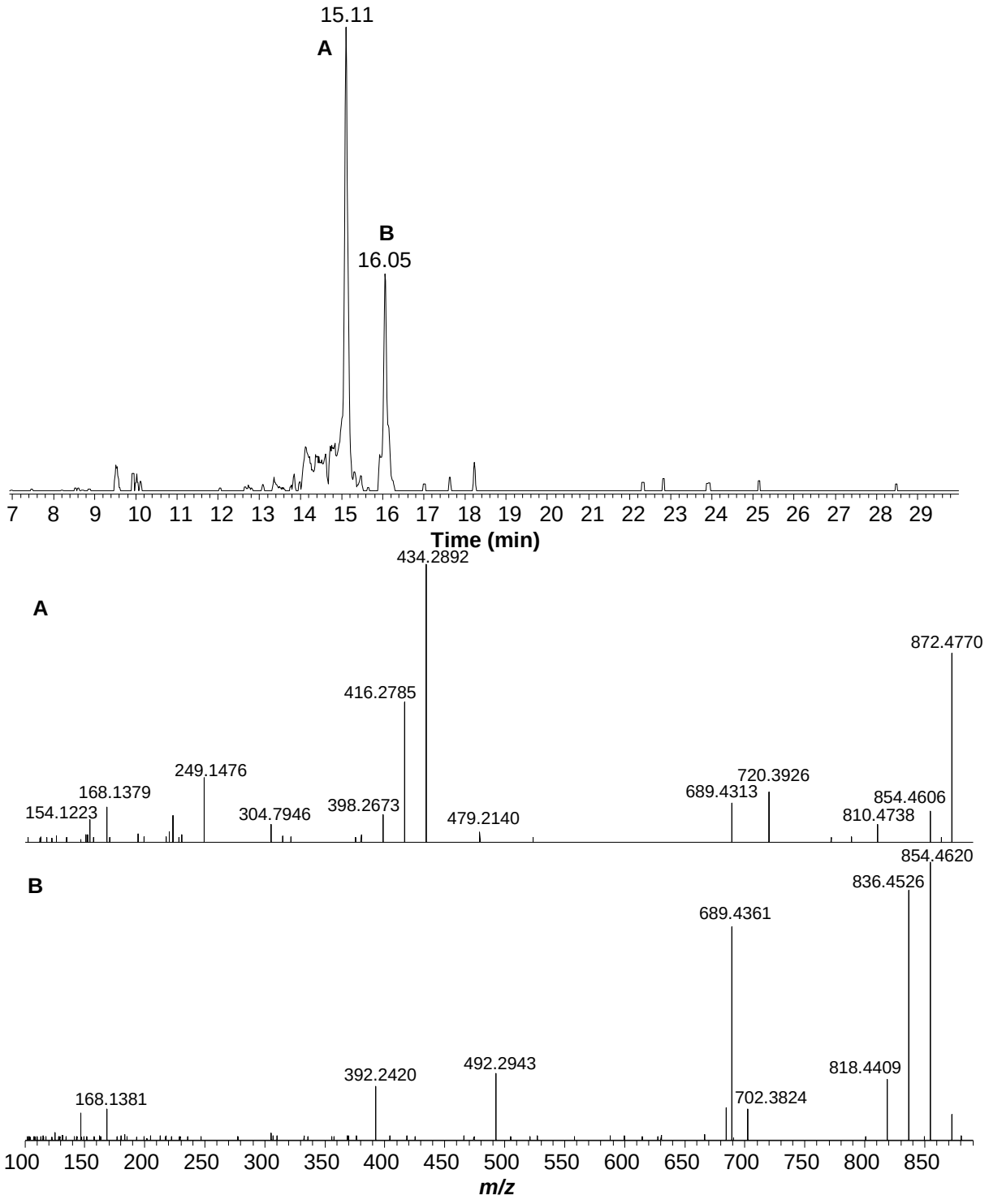

Fig. S3 Extracted ion chromatogram (top) of unknown AZA analogues (proposed formula $C_{47}H_{69}NO_{14}$, *m/z* 872.4791 ± 5 ppm) and corresponding MS/MS spectra (bottom) of *m/z* 872.5 precursor ions isolated with a 0.4 Da window and fragmented with HCD energy at 55 eV.

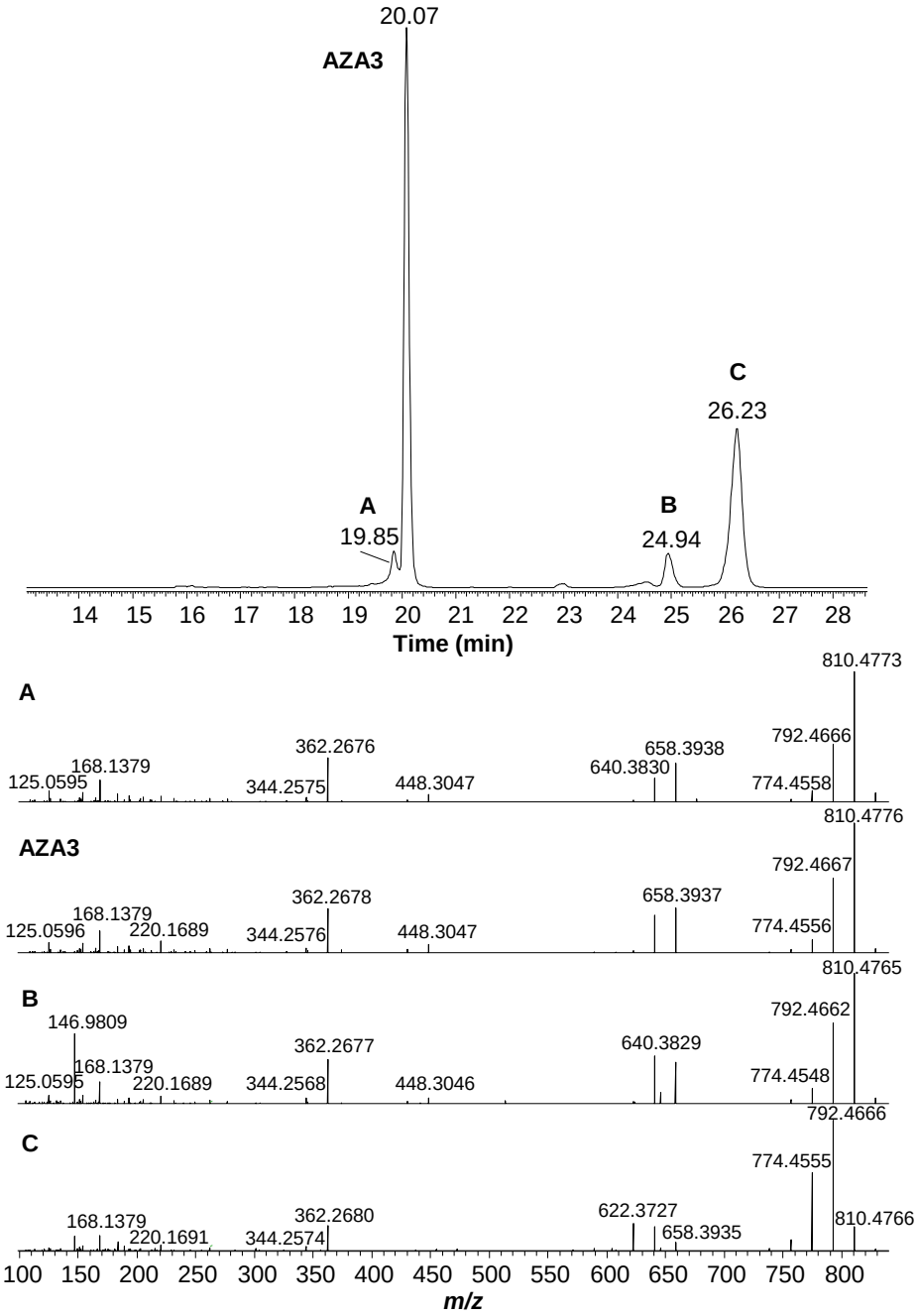

Fig. S4 Extracted ion chromatogram (top) of AZA3 and isomers (*m/z* 828.4893 ± 5 ppm) and corresponding MS/MS spectra using 0.4 Da isolation window and 55 eV HCD collision energy spectra (bottom). Fragmentation at this energy shows the late eluting isomer (C) to have a different water loss intensity profile as well as a water loss at 622 from the 640 ion.

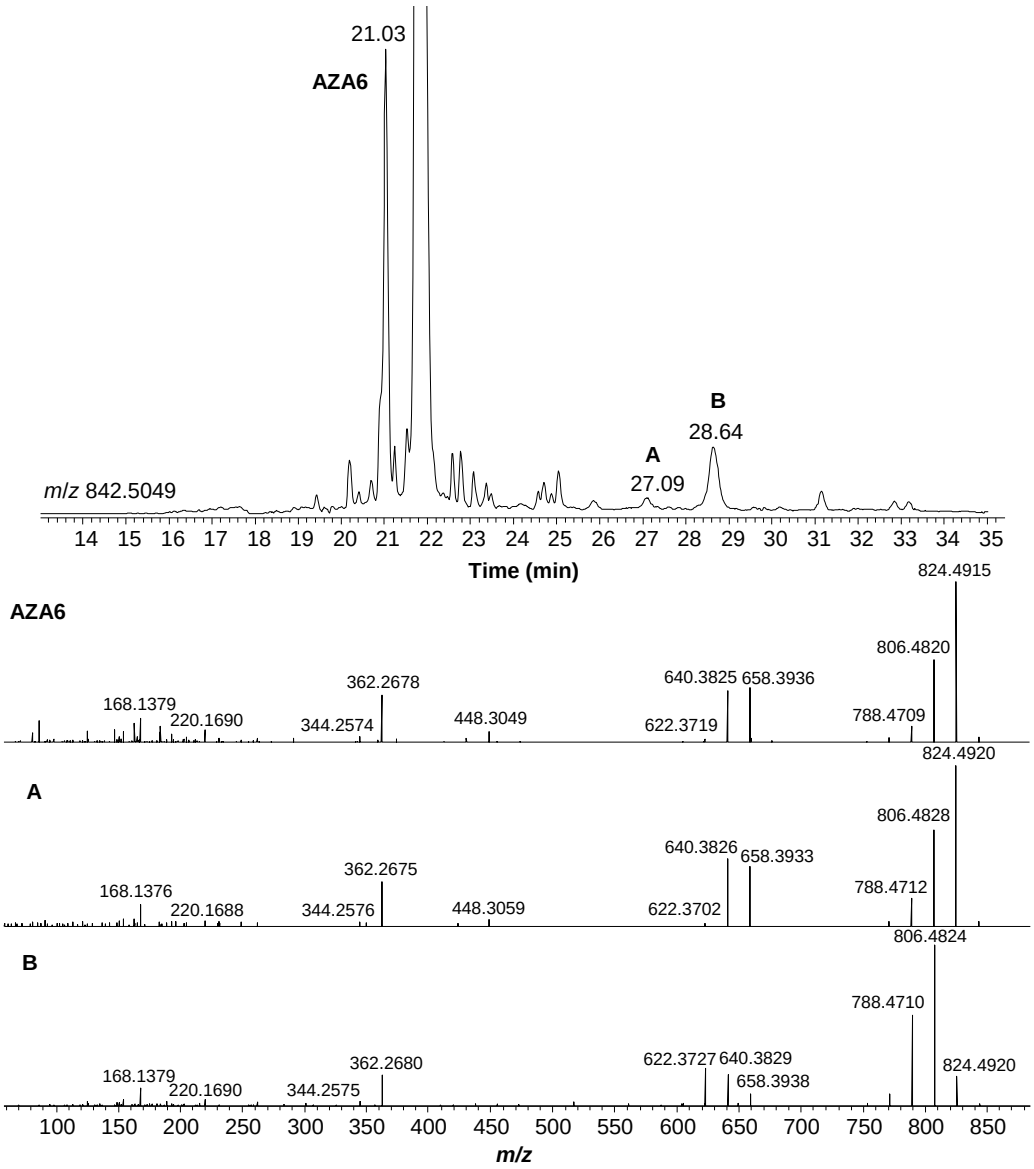

Fig. S5 Extracted ion chromatogram (top) of AZA6 and isomers (*m/z* 842.5049 ± 5 ppm) and corresponding MS/MS spectra using 0.4 Da isolation window and 55 eV HCD collision energy spectra (bottom). Fragmentation at this energy shows the late eluting isomer (B) to have a different water loss intensity profile from the precursor ion as well as a water loss at 622 from the 640 ion.

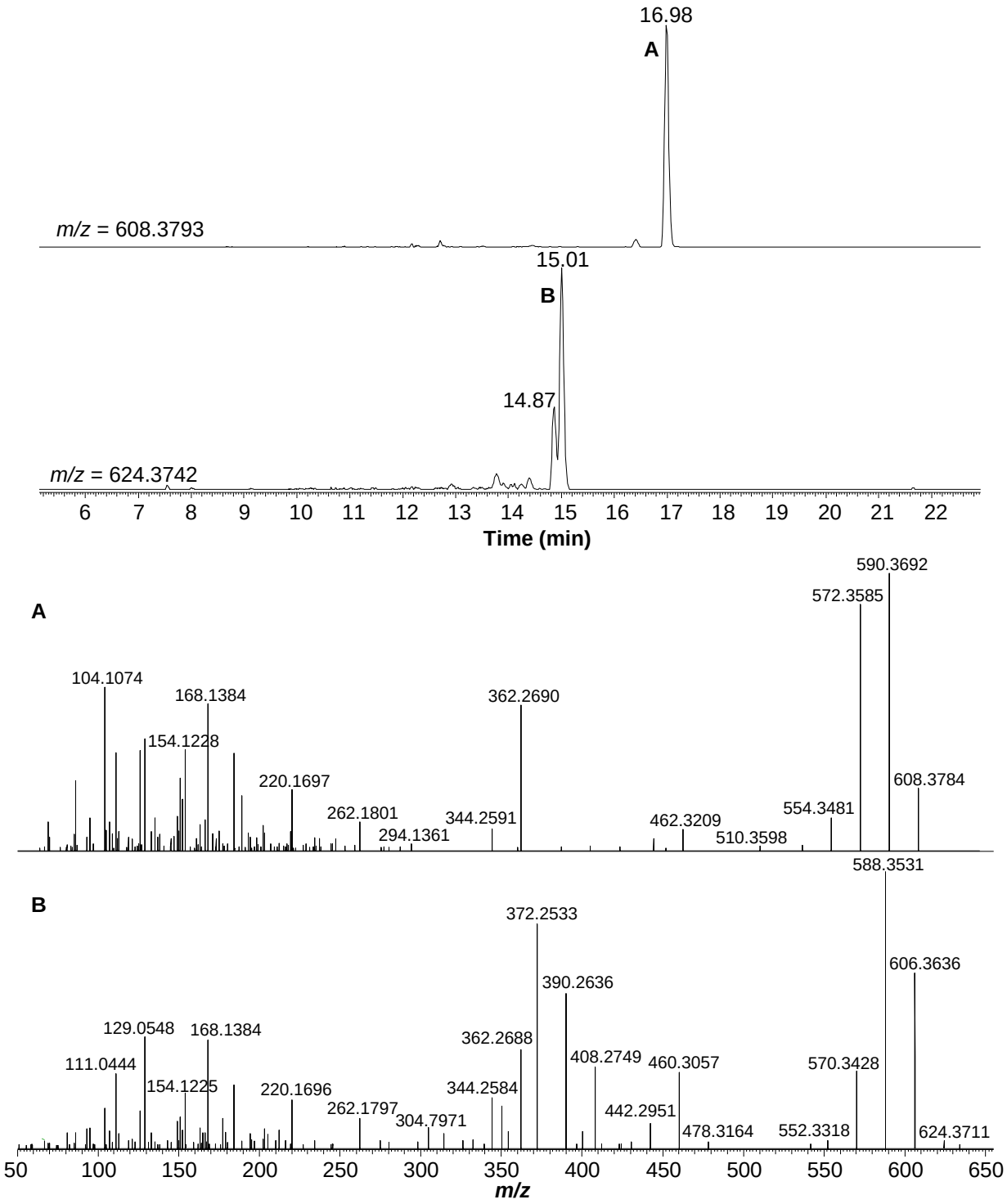

Fig. S6 Extracted ion chromatograms (± 5 ppm) of two AZA-like compounds (top) (A: $C_{33}H_{54}NO_9^+$ *m/z* 608.3793 ± 5 ppm & B: $C_{33}H_{54}NO_{10}^+$ 624.3742 ± 5 ppm) detected in data-dependent acquisition experiments and their corresponding MS/MS spectra (bottom).

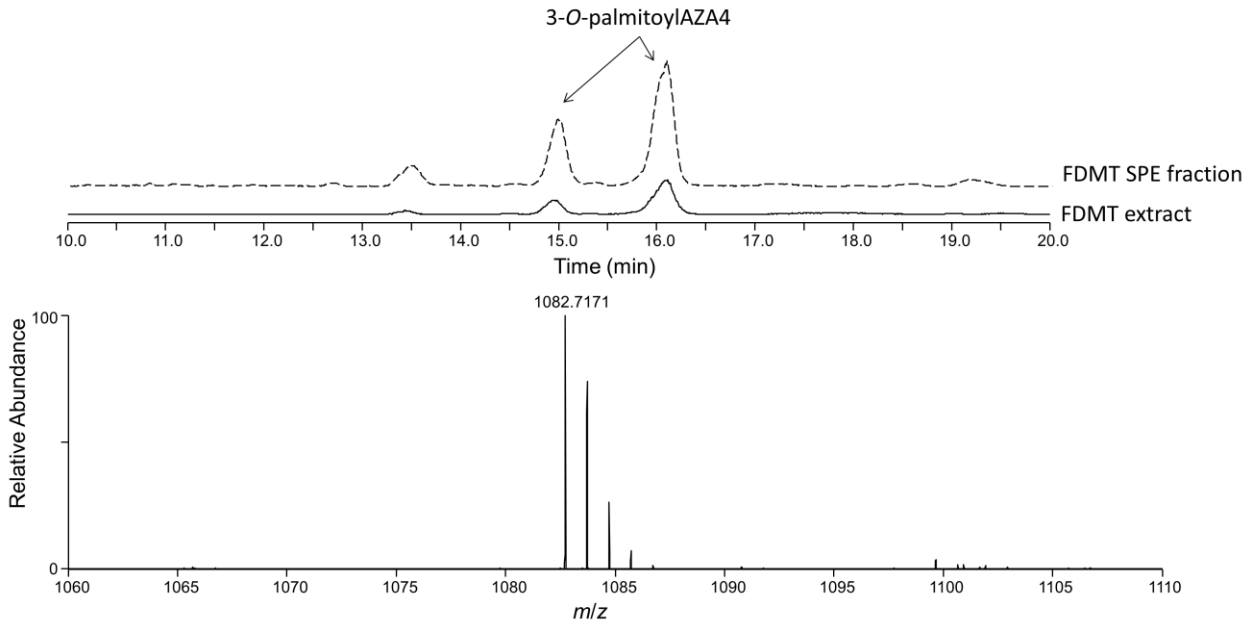

Fig. S7 Extracted ion chromatograms (± 5 ppm) showing presence of 3-O-palmitoylAZA4 in CRM-FDMT1 SPE sample and neat extract. Identity of AZA fatty acid acyl esters and analysis conditions were described previously [2].

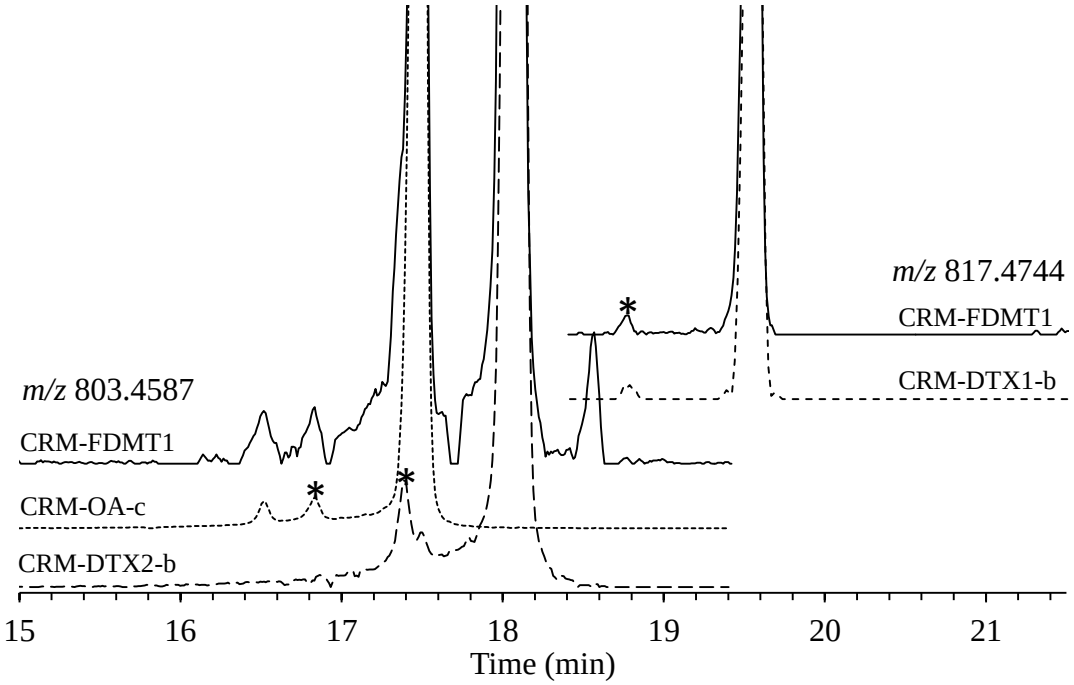

Fig S8 Extracted ion chromatograms (± 5 ppm) for OA/DTX2 (left) and DTX1 (right) comparing isomers of certified toxins in CRM-FDMT1 with isomers present in NRC calibration solution CRMs. Tentative 19-epimer peaks for OA and DTX1 are marked with asterisks for each of the calibration solution based on having the same relative retention time to their main peak as 19-*epi*-DTX2, which was confirmed in CRM-DTX2-b [3].

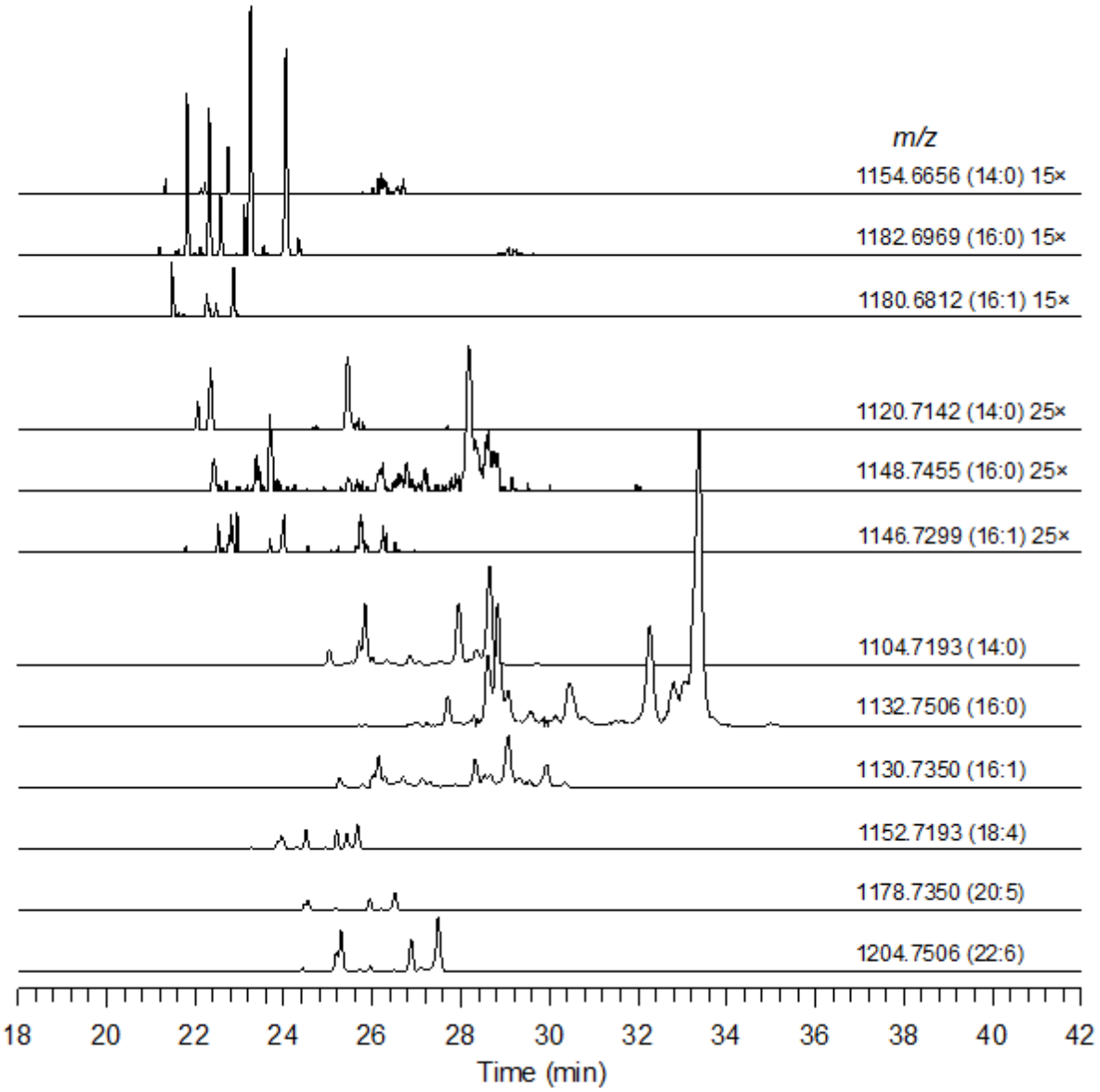

Fig S9 Extracted ion chromatograms (±5 ppm) of select PTX acyl ester accurate masses in data from this study. Select PTX2sa acyl esters are shown without zoom for selected C14 to C22 carbon chain lengths and different levels of unsaturation from 0-6. Unknown PTXsa esters shown at 25× zoom and sulfonated PTX acyl ester analogues shown with 15× zoom.

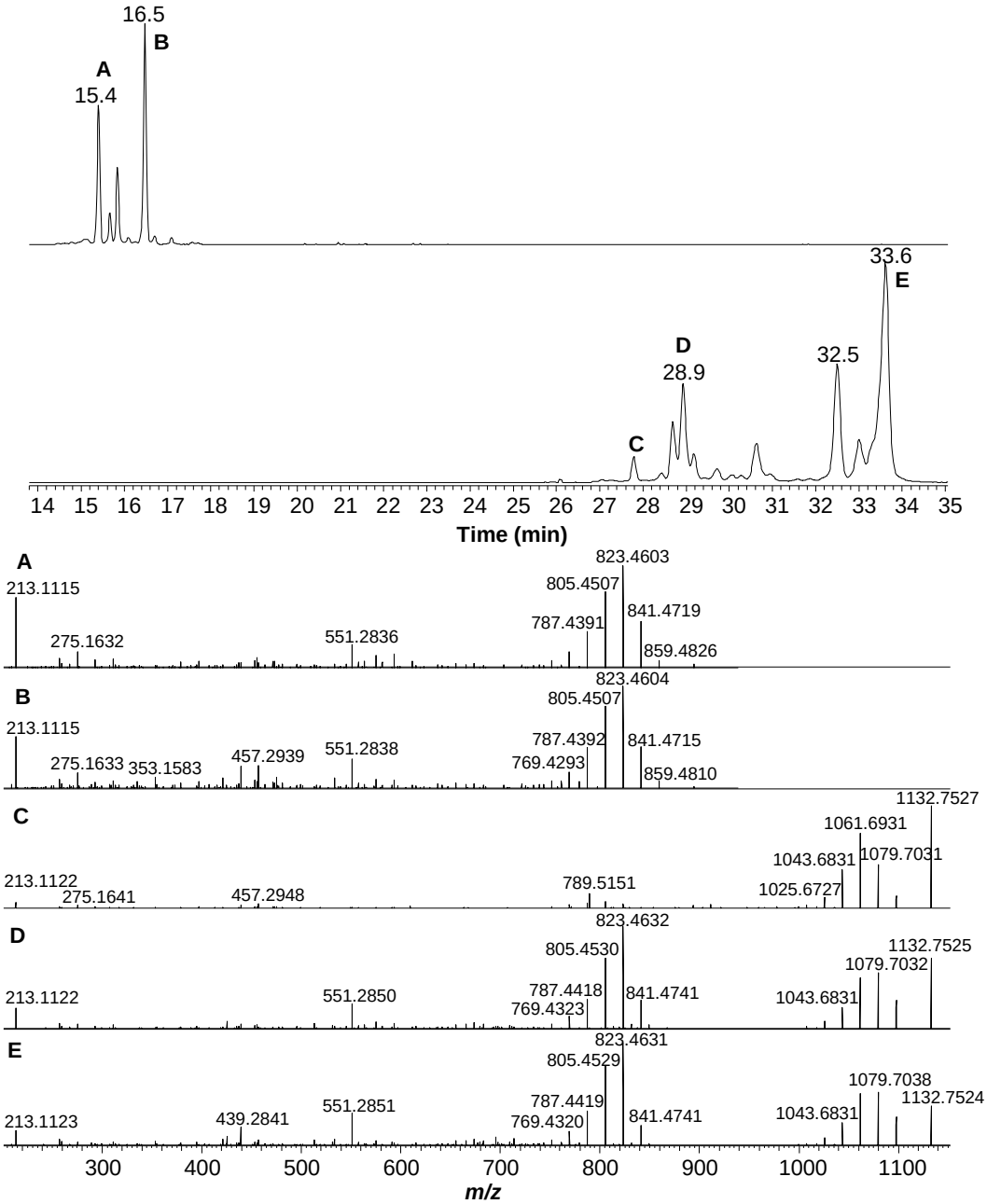

Fig. S10 Extracted ion chromatograms (top) of ammonium adducts of PTX2sa and isomers (*m/z* 894.5209 ± Δ 5 ppm for $C_{47}H_{72}O_{15}$) and (*m/z* 1132.7506 ± Δ 5 ppm for $C_{63}H_{102}O_{16}$) for PTX2sa 16:0 acyl ester. Corresponding MS/MS spectra (bottom panes) using 0.4 Da isolation window and 35 eV HCD collision energy. Compound C shows fragmentation suggesting 11-*O*-acylation, while compounds D & E are more challenging to assign. Note: data shown acquired in separate run from data summarized in Table 1.

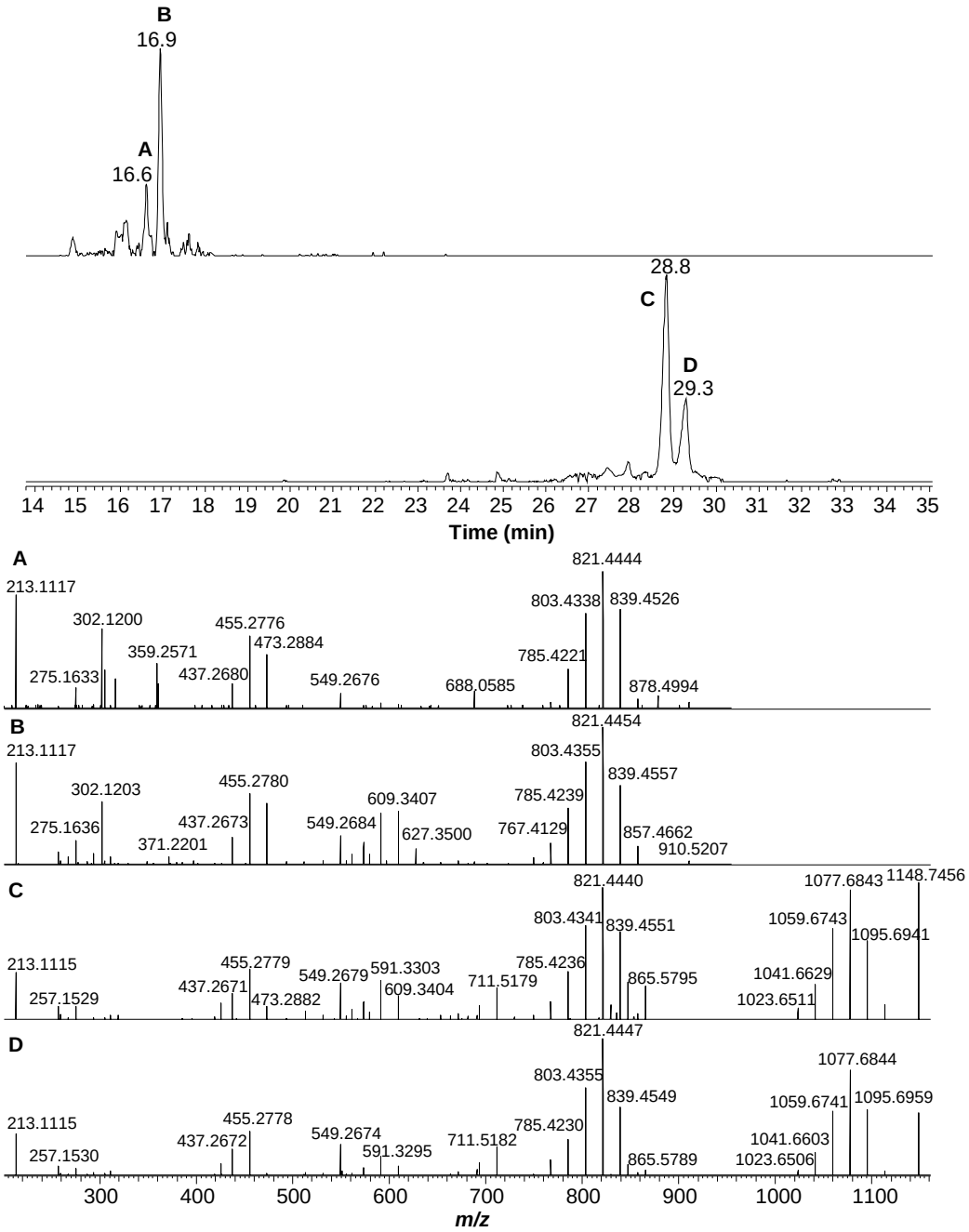

Fig. S11 Extracted ion chromatogram (top) of ammonium adducts of PTX compounds expected to be seco acids (*m/z* 910.5159 ± Δ 5 ppm for $C_{47}H_{72}O_{16}$) and their 16:0 acyl ester (*m/z* 1148.7455 ± 5 ppm for $C_{63}H_{102}O_{17}$) with their corresponding MS/MS spectra (bottom panes) using 0.4 Da isolation window and 35eV HCD collision energy. Note: data shown acquired in separate run from data summarized in Table 1.

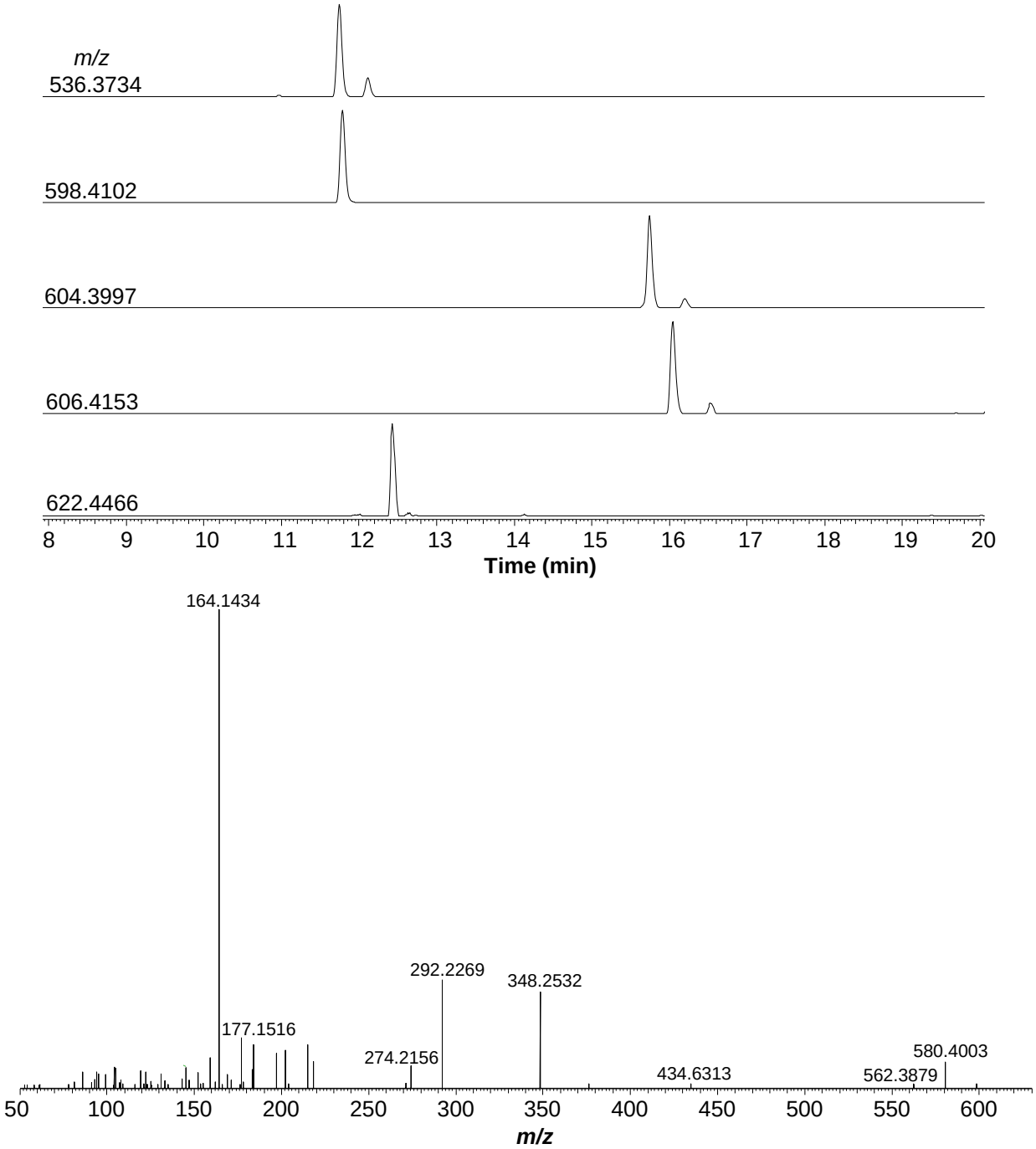

Fig. S12 Extracted ion chromatograms (±5 ppm) of SPX-like compounds present in CRM-FDMT1 (top). The MS/MS spectrum (bottom) for precursor ion *m/z* 598.4102 using 0.4 Da isolation window and 50eV HCD collision energy.is consistent with previously reported data [4].

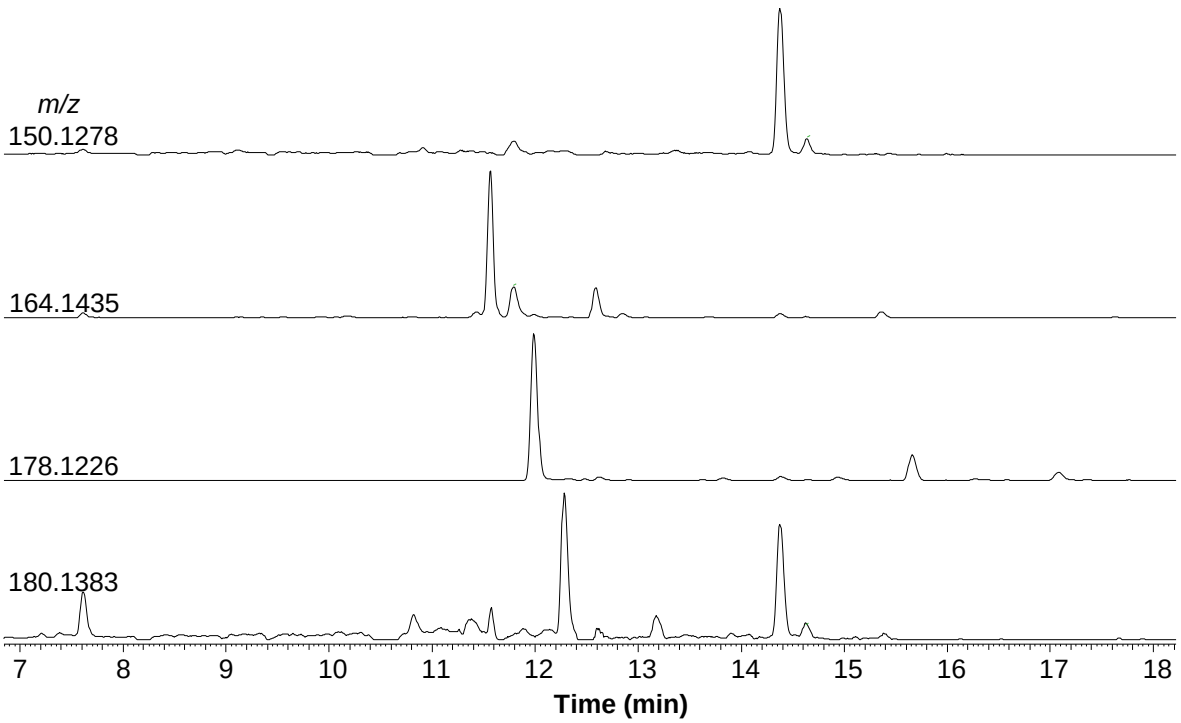

Fig. S13 Extracted product ion chromatograms from AIF data for key product ion exact masses (± 5 ppm) representing structural variations of the SPX imine ring product ion

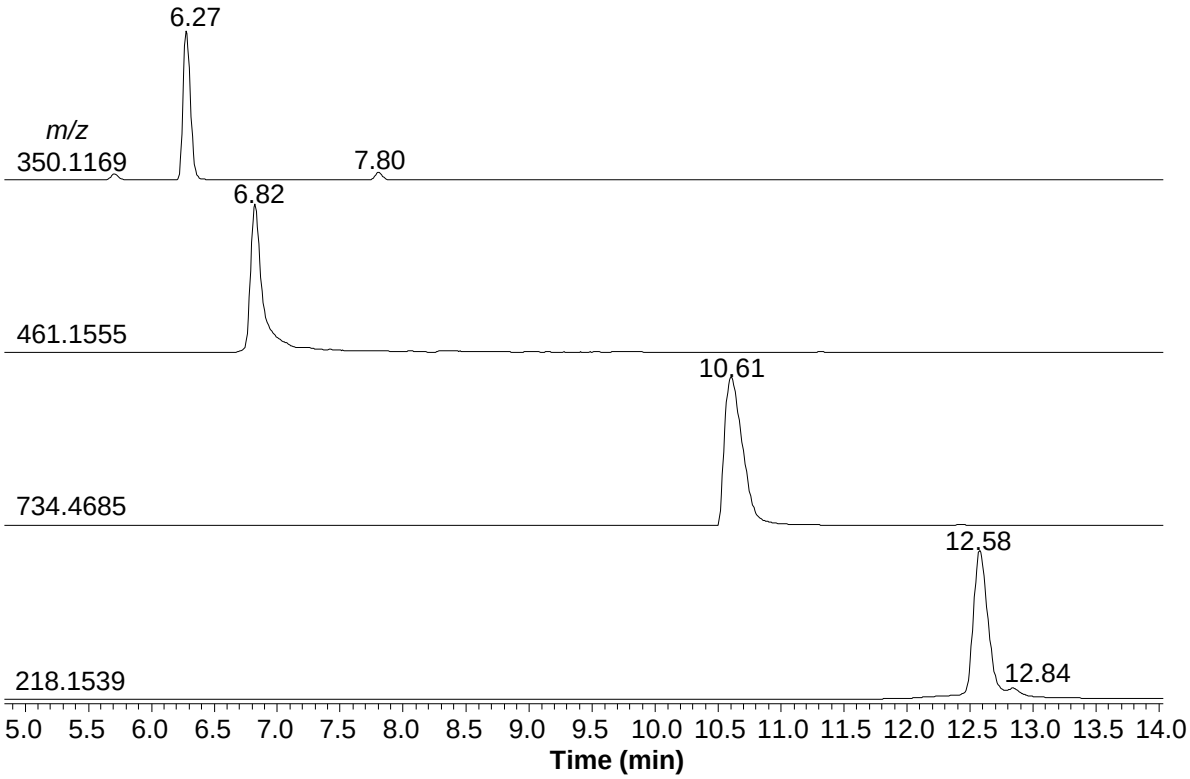

Fig. S14 Extracted ion chromatograms of $[M+H]^+$ accurate masses (± 5 ppm) for additives in CRM-FDMT1: ampicillin ($C_{16}H_{20}N_3O_4S^+$ *m/z* 350.1169); oxytetracycline ($C_{22}H_{25}N_2O_9^+$ *m/z* 461.1555); erythromycin ($C_{37}H_{68}NO_{13}^+$ *m/z* 734.4685; and ethoxyquin ($C_{14}H_{20}NO^+$ *m/z* 218.1539).

References

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