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Sensitive MS method for the detection of palytoxins in shellfish using li^+ cationization

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Background

Palytoxins (PlTXs) are produced by marine dinoflagellates (*Ostreopsis spp*), cyanobacteria and certain corals. Palytoxin is a neurotoxic compound that can end-up in shellfish. PlTXs are within the classes of marine biotoxins the largest non-protein molecules with a molecular weight of ~2700Da. PlTX is a polyhydroxylated compound with both lipophilic and hydrophilic parts, partially an unsaturated aliphatic backbone containing cyclic ethers and hydroxyl and amide functional groups (figure 1). To protect shellfish consumers EFSA established an acute reference dose of 0.2 µg/kg b.w. which can be translated to 30 µg PlTX-eq/kg shellfish (400g shellfish by 60kg person). Sensitive methods for analysis of intact palytoxins in shellfish are lacking.

Objective

- Development of a sensitive MS detection method for intact palytoxins
- Validation of the developed method for shellfish monitoring

Method

- 1 g of shellfish, triplicate extracted with 3mL 50v/v% MeOH.
- SPE Strata-X, after conditioning and sample extract applied, cartridge washed with water and subsequently eluted with 100% MeOH containing 0.1v/v% acetic acid.

- LC column: Kinetex C_{18} 100 × 2.1mm, 1.7µm
- Mobile phase: A: H_2O and B: ACN:H_2O (9:1), both containing 0.25mM LiI and 0.00125v/v% formic acid
- Flow: 0.4 ml/min
- Gradient: Linear, total 10 min run time
- Injection volume: 10µL
- MS: Thermo Q-Exactive Orbitrap and Waters Xevo TQ-S

Results

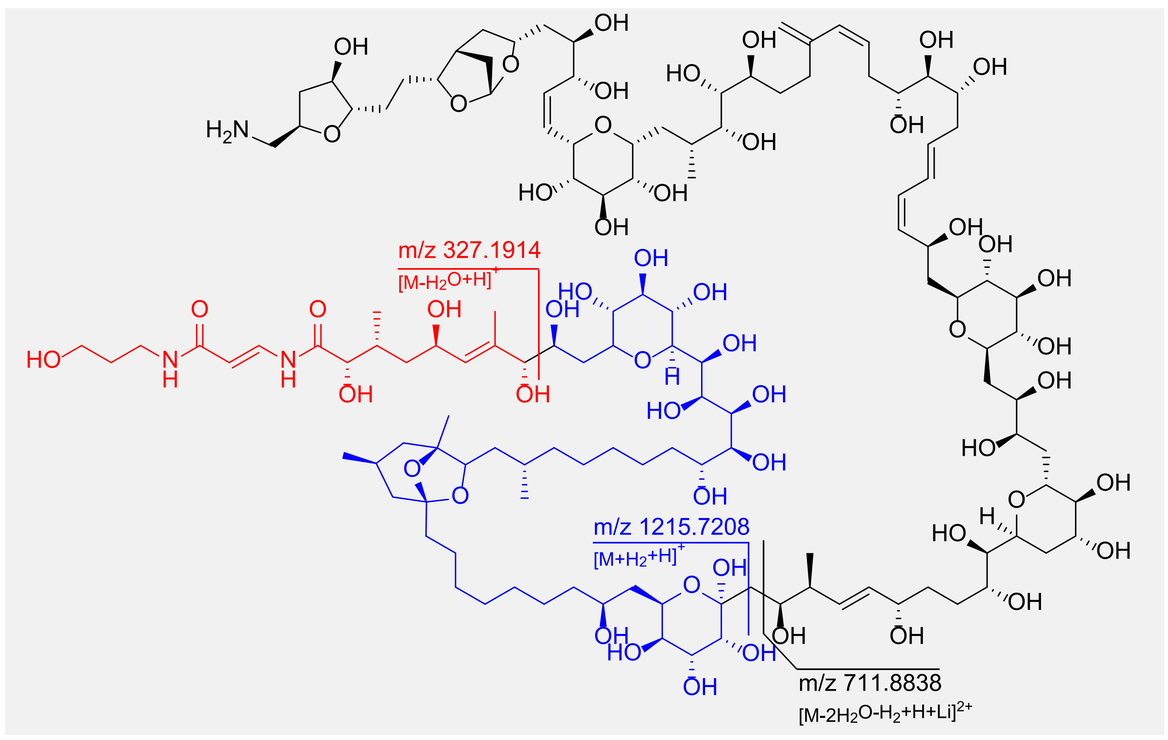

Figure 1. Proposed fragmentation of PlTX under acidic mobile phase conditions with LiI as additive

Results

- LiI (0.25mM) combined with formic acid (0.00125 v/v %) in the mobile phase showed best response in the MS for PlTX.
- Only one abundant ion was formed (m/z 897.8413, $[M+H+2Li]^{3+}$).
- Q-exactive MS^2 spectra revealed both non specific fragments (losses of H_2O) and structural related fragments (figure 1 and 2).
- MRM method developed at Waters Xevo TQS, MRM transitions 898.2>880.2, 898.2>874.2 and 898.2>711.9.
- Sensitivity of the applied methodology on the TQ-S was good with LODs of 8 and 22 µg/kg for respectively mussels and oysters.

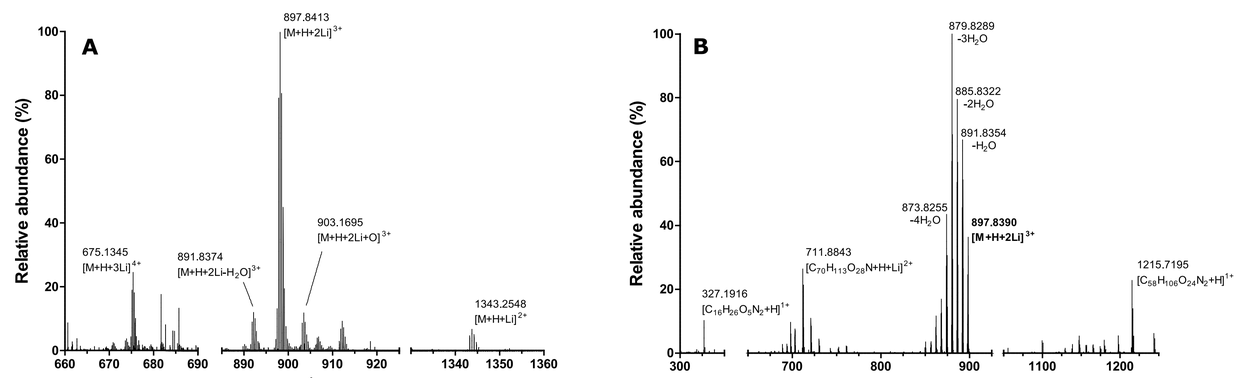

Figure 2. A) MS^1 spectra of palytoxin and **B)** MS^2 spectra of palytoxins with *m/z* 987.8 as precursor mass. Both recorded under acidic mobile phase conditions with LiI as additive.

Validation

- For recovery and repeatability 20 samples (n=5 per matrix), mussels, oysters, cockles and ensis were fortified at 10, 30 and 60 µg PlTX/kg shellfish.
- For selectivity 20 blank samples (n=5 per matrix) were used
- For quantification and linearity matrix matched calibration curves were constructed at concentrations 0, 5, 10, 30, 60 and 100 µg PlTX/kg blank mussel homogenate.

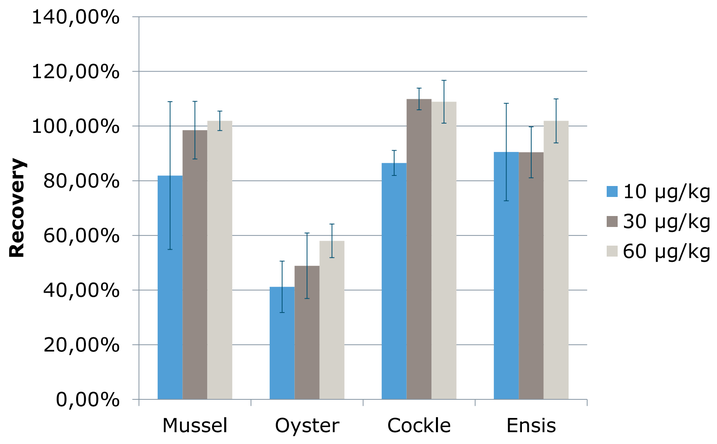

Figure 3. Recovery and repeatability error (RSD n=5) for various shellfish matrices.

Conclusions

- Sensitive LC-MS/MS method for the detection of PlTXs and analogues in shellfish is developed.
- Validation was successful with exception of repeatability at 10 µg/kg which had RSDs>20%, further for oysters recoveries in general were low (40-60%) (figure 3).

Acknowledgements

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