

### O3 OMS2

## UNTARGETED LC-HRMS<sup>n</sup> VERSUS TARGETED LC-MS/MS AND PRE-COX-LC-FLD FOR DETERMINATION OF PARALYTIC SHELLFISH POISONING TOXINS AND TETRODOTOXIN IN SEAWATER AND SHELLFISH

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Paralytic shellfish poisoning toxins (PST) are a group of neurotoxins produced by marine dinoflagellate belonging to genus *Alexandrium*, *Gymnodinium* and *Pyrodinium* as well as by freshwater cyanobacteria that may contaminate drinking water supply. PST represent a major concern for humans since a fatal neurological syndrome may occur following ingestion of contaminated seafood. Tetrodotoxin (TTX) is also a naturally-occurring toxin produced by marine bacteria, well known in Japan to cause lethal food poisonings following ingestion of contaminated puffer fish (*fugu*).

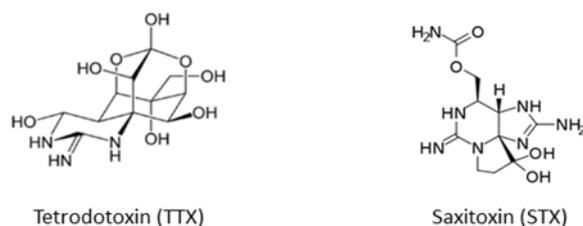


Figure 1. Structures of tetrodotoxin (TTX) and saxitoxin (STX), the parent compound of the PST group of toxins.

Despite presenting different structural features, PST and TTX exert similar toxic effects and, most importantly in an analytical perspective, they are co-extracted under the same conditions; thus availability of a methodological approach for their combined detection is desirable. Whilst PST are regulated and officially monitored in Europe, more data on TTX occurrence in bivalves and gastropods are needed before meaningful regulations can be established.

In this study [1], we used three different analytical methods - based on i) hydrophilic interaction liquid chromatography with high resolution multiple stage mass spectrometry (HILIC-HRMS<sup>n</sup>), ii) ultrahigh performance HILIC-MS/MS, and iii) pre-column oxidation with liquid chromatography and fluorescence detection (Pre-COX-LC-FLD) - to investigate the presence of PST and TTX in seawater and shellfish (mussels, clams) collected in spring/summer 2015 to 2017 in the Mediterranean Sea. Samples were collected at 10 sites

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in the Syracuse Bay (Sicily, Italy) in concomitance with a mixed bloom of *A. minutum* and *A. pacificum*. A very high PST contamination in mussels emerged, unprecedentedly found in Italy, with maximum total concentration of 10850 µg STX eq/kg of shellfish tissue measured in 2016. In addition, for the first time TTX was detected in Italy in most of the analyzed samples in the range 0.8-6.4 µg TTX eq/kg.

The recurring blooms of PST-producing species over the 3-year period, the high PST levels and the first finding of TTX in mussels from the Syracuse bay suggest that human health concerns exist and that monitoring programs of PST and TTX in seafood should be activated in this geographical area. Three different instrumental platforms and 3 separate analytical methods were used for analysis of these samples. Each of these may be applicable to the high throughput testing of shellfish tissues in a monitoring framework, although currently Pre-COX-LC-FLD method is the only analytical method allowable within the EU law. Whilst the qualitative results compared well between the three approaches, some significant differences emerged, particularly in comparison with the HILIC-HRMS<sup>n</sup> approach. Further work is on-going to understand the reasons for such differences and to conduct formal validation of the HILIC-HRMS<sup>n</sup> method before the platform can be used routinely.

#### References

[1] Dell'Aversano C., Tartaglione L., Polito G., Dean K., Giacobbe M., Casabianca S., Capellacci S., Penna A., Turner A. D. Chemosphere, 2019, 215, 881-892