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Transition metal's doping for improving the fluorescence response of paralytic shellfish poisoning toxins upon pre-chromatographic oxidation

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INTRODUCTION & AIM

Paralytic shellfish toxins (PSTs) can be quantified in bivalves by the AOAC-2005 method. This method is sensitive for several toxins, but other toxins suffer from strong matrix effects or fluorescence partitioning by multiple oxidation products, reducing their sensitivity.

Metals were assessed as candidate catalysts to improve the oxidation reactions instead of the 'oyster matrix modifier' preconized in this method.

METHOD

Toxins were analysed following the AOAC-2005 method. The HPLC system was from Agilent Technologies 1260 Infinity II series, controlled with OpenLab CDS Workstation Software. The separation was performed on a Supelcosil LC-18, 150 x 4.6 mm, 5 µm column (Supelco, USA). 30 µL were injected for peroxide reactions and 60 µL for periodate reactions, and the detection wavelengths set at 340 nm for excitation and 395 nm for emission.

Several metal stocks were prepared in 0.01 M **acetic acid (AcOH):** 1 g/L of iron(II) sulfate heptahydrate ($FeSO_4*7H_2O$) with 201 mg/L or 3.6 mM of iron; 1 g/L of iron(III) chloride hexahydrate ($FeCl_3*6H_2O$) with 3.7 mM of iron; 1.06 g/L of zinc sulphate heptahydrate ($ZnSO_4*7H_2O$) with 3.7 mM of zinc; 0.50 g/L of zinc chloride (ZnCl) with 3.7 mM of zinc; 0.48 g/L of nickel chloride (NiCl) with 3.7 mM of nickel. The metal stocks were kept in plastic containers, and metal dilutions were prepared in 2 mL Eppendorf tubes. For the **peroxide oxidation**, 25 µL of metal solutions were added to the peroxide mix. For the **periodate oxidation**, 100 µL of metal solutions were added instead of the oyster matrix modifier (obtained with C18 cleanup).

RESULTS & DISCUSSION

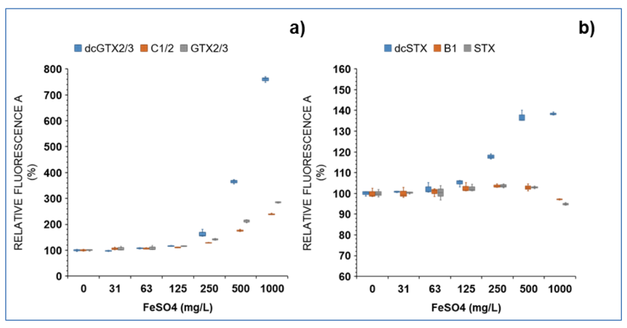

Fig. 1. Relative fluorescence yield of N1-H toxins spiked together in oyster C18-cleaned extract and doped with varying concentrations of $FeSO_4$ prior to peroxide oxidation: a) 11-hydroxysulphated and b) non-11-hydroxysulphated analogues (N=3).

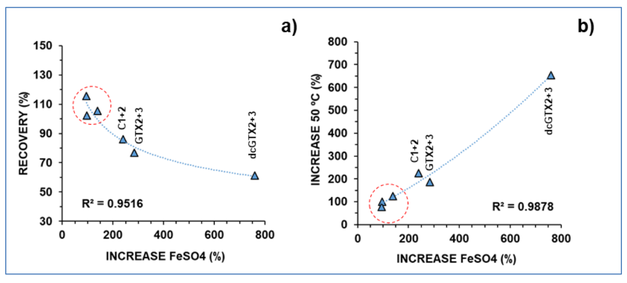

Fig. 2. Increase in fluorescence of all N1-H toxins spiked in oyster after doping with 1000 mg/L FeSO4 compared with: a) toxin recovery in oyster matrix. b) the relative increase in fluorescence after heating at 50 ºC for 4 min. Red circle highlights the non-11-hydroxysulphated analogues (dcSTX, B1, STX).

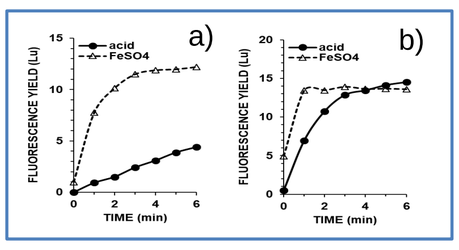

Fig. 3. Progression of the fluorescence yield of a mix of N1-H toxins spiked in oyster C18-cleaned extract and oxidized at room temperature with or without FeSO4 doping at 1000 mg/L: a) dcGTX2+3, b) dcSTX,

The N1-H sub-group of toxins exhibited increased fluorescence upon doping with iron sulphate (Fig. 1). The increase was inversely proportional to the fluorescence reduction caused by matrix suppression (Fig. 2a). Iron acted as a catalyst, lowering the activation energy of the reaction, which otherwise required heating to achieve a similar boost in the fluorescence yield (Fig. 2b-3).

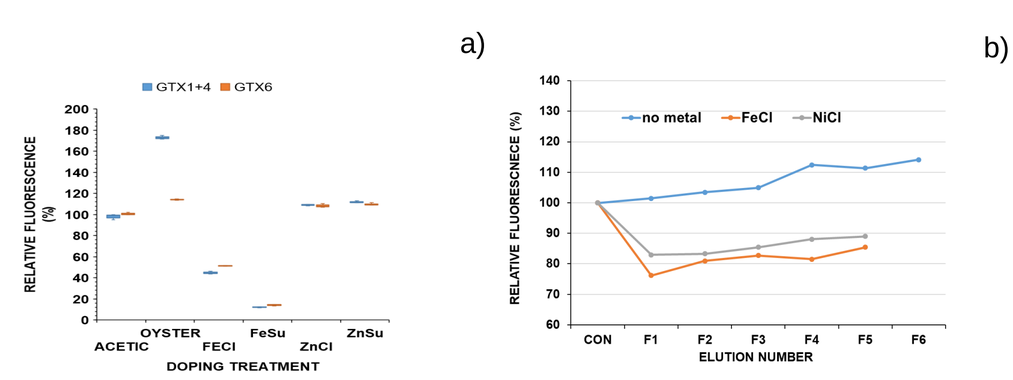

Fig. 4. a) Relative fluorescence yield obtained by doping pure GTX1+4 plus B2 with dilute AcOH (control), C18 oyster matrix, iron and zinc salts. All treatments were significantly different at $p < 0.001$ from control for both toxins. b) Relative fluorescence yield of the quantification peak of GTX1+4 doped with 0.1 mM AcOH (control) or doped with the successive eluates obtained by passing 2.5 mL of 0.05 M NaCl through the COOH cartridge (alternatively, 2 mL of 0.09 mM metal solutions were applied separately to the cartridge before collecting NaCL solutions).

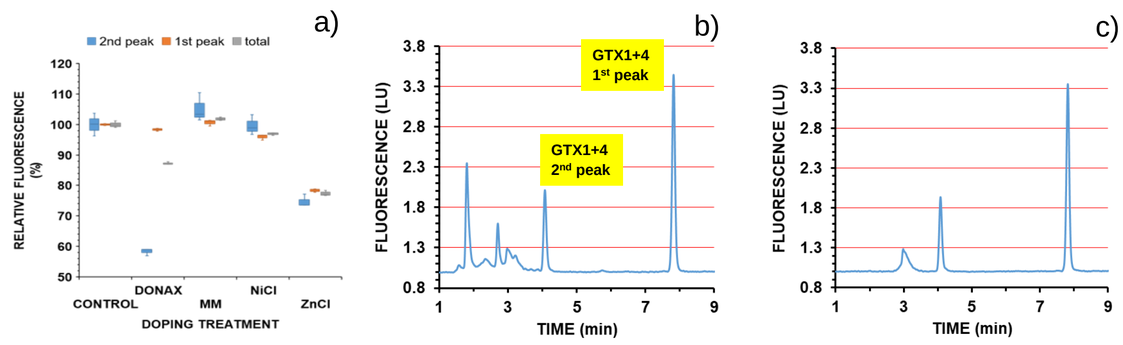

Fig. 5. a) fluorescence yields obtained by doping GTX1+4 spiked in donax clams fraction #2 with: acetic acid (*DONAX*), oyster (MM), NiCl or ZnCl, compared to the control obtained by spiking in AcOH only and doping with AcOH (CONTROL). Chromatograms for doping with b) oyster MM; c) NiCl.

The fluorescence of the N1-hydroxyl sub-group of toxins GTX1+4 and GTX6 increased with zinc but decreased with iron (Fig. 4a). When doped with a metal solution that had passed in fraction-#2 of the COOH-partitioning, transition metals (Fe, Zn, Ni) reduced the fluorescence, while the eluent alone (NaCL 0.05 M) enhanced it (Fig. 4b). As metals are retained by COOH cartridges, sulfonic acid leachables might contribute to this reduction.

The reduction observed for GTX1+4 was primarily due to the decrease in the proportion of the secondary peak (*quantification peak*) over the primary oxidation peak, which can be reversed by doping with an oyster matrix (Fig. 5a). Nickel chloride was able to replace the oyster modifier effectively in all bivalve matrices tested, providing cleaner chromatograms (Figs. 5b—5c). Zinc chloride was not similarly effective.

CONCLUSION

Metal doping can increase the sensitivity of AOAC Official Method nº 2005.06 for several toxins. It is a cheap alternative to using bivalve matrices as doping agents, provides cleaner chromatograms and minimizes the injection of matrix in HPLC guard columns and columns. It can circumvent the intrinsic variabilities of a matrix modifier with an unknown composition, typical of an animal that feeds on variable phytoplankton composition throughout the year and is exposed to variable anthropogenic agents in its habitat.

FUTURE WORK / REFERENCES

- AOAC (2005a) AOAC Official Method 2005.06, Quantitative determination of paralytic shellfish poisoning toxins in shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection. In: *Official Methods of Analysis of AOAC INTERNATIONAL*, 18th ed. (AOAC INTERNATIONAL ed.), AOAC INTERNATIONAL, Gaithersburg. MD, USA.
- Vale, P. (2024) Temperature dependence of the pre-chromatographic 'Lawrence' method for bivalves contaminated with paralytic shellfish poisoning toxins. *Food Anal. Methods*. 17, 1657–1667.