The present work deals with the optimization of a liquid chromatography–tandem mass spectrometry method (LC-MS/MS) for the simultaneous determination of different classes of algal toxins in seawater. Since there is no method able to detect palytoxin together with other classes of biotoxins, we developed a LC-MS/MS procedure for the qualitative and quantitative determination of palytoxin and domoic acid, okadaic acid, gonyautoxin-1,4 (GTX 1,4), -2,3 (GTX 2,3) and -5 (GTX 5), decarbamoylgonyautoxin-2,3 (dcGTX 2,3), as representative respectively of amnesic shellfish poisoning (ASP), diarrheic shellfish poisoning (DSP) and paralytic shellfish poisoning (PSP) toxins. The development of the method included the optimization of the mass spectrometer (MS/MS) parameters and the choice of the best chromatographic columns in order to efficiently separate all the analytes in a single run. Furthermore, a purification step was carried out by C18 SPE cartridges in order to evaluate matrix effect and to eliminate high levels of salts. The recoveries were calculated from both synthetic and environmental seawater samples spiked with known amounts of our analytes. We preliminarily tested the method on two strains of Ostreopsis cf. ovata grown at 20 and 25°C in laboratory conditions. The method was good enough to assess significant temperature effects on toxins production also in small sample volume (50ml).

**Optimization of instrumental parameters of the mass spectrometer (MS/MS)**

The instrument parameters were optimized by direct continuous infusion of standard solutions of the analytes. The best results were obtained operating in positive ionization mode for the GTXs, domoic acid and palytoxin, while in negative ionization mode for okadaic acid. The additives used were formic acid for toxins analyzed in positive ionization and the ammonium formate for okadaic acid analyzed in negative ionization. As example, figure 1 and 2 show respectively full scan Q1 and MS-MS spectra of GTX 1-4.

**The following desalination and concentration procedure was applied to both synthetic and environmental samples.**

1. **Step 1: Activation of Bond Elut LRC-C18 cartridge**
2. **Step 2: Clean up**
3. **Step 3: Wash**
4. **Step 4: Elution (ACN/H2O=95/5 and H2O/ACN=5/95)**
5. **Step 5: HPLC-MS/MS Analysis**

**Optimization of chromatographic conditions**

Several columns were tested (C18, CB, HiIlc) in order to optimize the chromatographic separation for the simultaneous determination of the analytes in a single run. The Gemini C18 column provided the best results (Fig. 3). Mobile phase was as follows: eluent A: ACN/H2O 95/5 formic acid 2 mM, ammonium formate 12.5 mM; eluent B: formic acid 10mM and ammonium formate 25mM. Tab 1 shows the elution gradient.

**Calibration Curves**

Three calibration curves (A, B and C) were built in order to evaluate both ionic suppression and matrix effects:

- **Type A** consisted of six standard solutions with increasing analyte concentration.
- **Type B**, five aliquots of seawater (50 ml) were previously subjected to the procedure of Fig. 4, and then spiked with the same standard solutions of curve “A” immediately prior to LC injection.
- **Type C**, five aliquots of 50 ml were spiked with standard solutions of increasing concentration before applying the whole procedure of (Fig. 4).

**Environmental Samples**

Two strains (R and M) of Ostreopsis cf. ovata (Fig. 6), isolated from the Adriatic Sea and provided by prof. Honsel (University of Udine), were cultured at 20 and 25°C in laboratory conditions.

Ostreopsis cf. ovata produces both ovatoxins and palytoxin. Since standards of ovatoxin are not available, a HPLC/MSMS study was performed (Fig. 5) in order to verify the ovatoxin production in the R and M strains. The comparison of the spectra obtained with the literature data allowed to identify the ovatoxin-a. Both strains of Ostreopsis cf. ovata produce palytoxin and ovatoxin-a; furthermore temperature greatly influences the production of toxins (Tab. 2, Tab. 3).

**Conclusions**

i. The analytical method, developed in the present paper, allows the simultaneous identification and quantization of algal toxins from four different classes.

ii. The method, preliminarily applied to samples of Ostreopsis of Ovata, is good enough to identify and quantify the biotoxins also in small sample volume (50ml).

iii. The method is able to assess significant temperature effects on toxins production.