Development and application of LC/MS based analysis for marine algal toxins in Hood Canal

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Development and application of LC/MS based analysis for marine algal toxins in Hood Canal

Sang Seon Yun, Seth Book, Ron Figlar-Barnes, Lisa Belleveau, Aaron Bentson-Royal, and Kenneth Collins

Department of Natural Resources, Skokomish Indian Tribe
Overview

• Background
• Methods
• Results
• Discussion
• Future studies
• Acknowledgements
Background: Marine algal toxins

- Hydrophilic toxins
  - Domoic acid (ASP)+
  - Saxitoxin (PSP)+
  - Gonyautoxin (PSP)+
  - Neosaxitoxins (PSP)+

- Lipophilic toxins
  - Okadaic acid (DSP)-
  - Dinophysistoxins (DSP)-
  - Pectenotoxins (DSP)+
  - Yessotoxin-
Background: Marine algal toxin analysis

• Climate change and harmful algal blooms in Hood Canal?
  • Increasing surface water temperature
  • Increasing weather events
  • Increasing nutrient input
• Skokomish Tribe’s initiative to establish an early warning system for marine algal toxins in Hood Canal
  • Focusing on water and phytoplankton samples
• BIA funded algal toxin monitoring program launched

• Analytical methods
  • Mouse bioassay (MBA)
  • ELISA
  • Receptor binding assay
  • Chemical analysis
    • HPLC
    • LC/MS
Background: LC/MS based analysis

• Combines LC separation with mass spectrometric detection
• Individual compounds can be identified and quantified
• Offers very sensitive detection
• Enable detection and quantitation of multiple compounds in one run
• Requires costly equipment
Methods: Solid Phase extraction

**Lipophilic Toxins**
- Oasis HLB
- Methanol
- 250 ml seawater
- Oasis HLB
- Methanol

**Hydrophilic Toxins**
- Envi Carb-graphitized carbon
- 20% acetonitrile
- Up to 12 ml
- DI water
- 40% acetonitrile
Methods: LC/MS analysis

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<tr>
<th>LC parameters</th>
<th>Mass spectrometric parameters</th>
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<td><strong>Column</strong></td>
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<tr>
<td><strong>Solvents</strong></td>
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<tr>
<td>• A: DI water with 2 mM ammonium formate + 50 mM formic acid</td>
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<tr>
<td>• B: 95% Acetonitrile with 2 mM ammonium formate + 50 mM formic acid</td>
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<td><strong>Gradient</strong></td>
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<td>• Negative mode: 10 min gradient</td>
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<td>• Positive mode: 9 min gradient</td>
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Analytical toxin standards were obtained from NRC CRM (Canada) and used for method development and quantitation.

References: McCarron et al., 2014; Silver et al., 2010; Wang et al., 2007
Methods: Hood Canal monitoring sites

• Sampling sites
  • Sequim Bay (SB)-a reference point
  • Hood Canal Bridge (HC)
  • Quilcene (QC)
  • Pointe Whitney (PW)
  • Triton State Park (TS)
  • Ayock (AY)
  • Glen Ayr (GA)
  • Lilliwaup (LW)
  • Dewatto Beach (DB)
  • Rensland (RS)
  • Union Dock (UD)
  • Hood Sport (HS)
  • Port of Allyn (PA)

• Sampling and monitoring period
  • June 1 – September 30, 2017
Results: Separation and Detection of toxins

Negative mode detection
OA eluting at 4.29 min Detection limits: 4 ng/L
DTX-2 eluting at 4.50 min Detection limits: 4 ng/L
DTX-1 eluting at 5.04 min Detection limits: 4 ng/L

Positive mode detection
DA eluting at 3.41 min Detection limits: 40 ng/L
PTX-2 eluting at 5.19 min Detection limits: 4 ng/L
Results: Toxin monitoring in Hood Canal

Domoic acid

• DA is present throughout the monitoring period in HC
• DA surged at the 13th week (Aug 25th) peaking at the 14th week (Sept 1)
• Some sites observed over 2 µg/L DA concentrations - NOAA’s recommended risk limits
• DA levels subsided after the 15th week
• No phytoplankton and toxicity data available
Results: Toxin monitoring in Hood Canal
Dinophysistoxin-1

- Low levels of DTX-1 was observed in Hood Canal, while higher concentrations in Sequim Bay
- Slight variation in DTX-1 concentrations over sampling sites and sampling period in Hood Canal
- Sequim Bay maintained higher concentrations over time—although toxicity is not known
Results: Toxin monitoring in Hood Canal
Pectenotoxin-2

- Low levels of PTX-2 were present during the monitoring period (below 100 ng/L) in Hood Canal
- Sequim Bay exhibited fluctuating concentrations over the sampling period
- In Sequim Bay, PTX-2 reached a peak over 2.5 µg/L
- No toxicity information arising from this toxin is known
Results: Method development for saxitoxin

- Porous graphitized carbon (PGC) cartridges tested
- Sigma’s Envi carb cartridge works best
- zHILIC column found most reliable
- A 10 min gradient program developed
- Due to the limitation of volume that can be extracted, the detection limits of this method will be over 1 µg/L
- Further refinement may need

References: Amstrong, 2017; Brag et al., 2015; Halme et al., 2012
Conclusions

• Sensitive analytical methods for 5 toxins using LC/MS were developed
• Pilot monitoring study conducted on 13 sampling sites demonstrated that the LC/MS analysis can provide reliable measurements of 5 toxins in water and phytoplankton samples
• SPE and LC/MS methods for hydrophilic toxins have been worked out and will be deployed to monitor in 2018
• Current chemistry data need to be combined with phytoplankton abundance and shellfish toxicity data
• Further collaborations with DOH and Sound Toxins
• Further refinement of analytical protocols
Further Studies

- Deployment of LC/MS analysis for hydrophilic toxins (STX, GTX)
- Phytoplankton analysis: identification and abundance
- Environmental fate studies for algal toxins
- Dynamics of toxins in foodwebs
- Preparation for future accreditation
- Open to collaborations
Acknowledgements

• Skokomish Indian Tribe Council for their general support
• EPA for their support for the Skokomish Tribe’s water quality lab.
• BIA for funding this project

Thanks for listening